TWO STAGE RECONSTRUCTION PROTOCOL IN MANAGEMENT OF HIGH-ENERGY PROXIMAL TIBIA FRACTURES (SCHATZKER, TYPE IV-VI)

Nikunj Maru, Devang Mandaliya, Rahul Parmar

1. Assistant Professor, Department of Orthopedics, P.D.U. Medical College, Rajkot.
2. Assistant Professor, Department of Orthopedics, P.D.U. Medical College, Rajkot.
3. Resident Doctor, Department of Orthopedics, P.D.U. Medical College, Rajkot.

CORRESPONDING AUTHOR

Dr. Nikunj Maru,
P. D. U. Medical College & Hospital,
Trauma Care Centre, Rajkot,
E-mail: dr.nikunj83@gmail.com,
Ph: 0091 9879681202.

ABSTRACT: BACKGROUND: Proximal tibial fractures with high energy trauma are extremely complex injuries with significant associated soft-tissue damage. This Retrospective study was used to evaluate advantage of two stage reconstructions as a protocol in management of high-energy proximal tibia fractures regards of soft-tissue management, development of complications, and functional outcomes. **MATERIALS AND METHODS**: This retrospective study includes 40 patients who had sustained high energy Proximal tibial fractures were managed at our institute. In all patients, we placed immediate temporary distraction external fixation with management of soft-tissue injuries with delayed definitive fixation. The mean clinical and radiographic follow-up was 1year (range 12-18 months), and functional outcome was assessed by knee society score. **RESULTS**: The mean follow-up is minimum 1 year (range 12-18 months) with KSS was good in 26 cases (65%), fair in 10 cases (25.0%) and poor in 4(10.0%) at latest follow-up. The complications includes 6 patients (15%) suffered superficial infection, deep infection was observed in 4 patients (10%), 2 patients had developed compartment syndrome. 2 patients had deep venous thrombosis. Malalignment included 4 (10%) cases of residual varus and 3 (7.5%) cases of residual valgus deformity. **CONCLUSION**: The use of this protocol, with the initial application of a temporary distraction external fixator followed by delayed internal fixation, is suggested for treatment of high energy tibial plateau fractures. The benefit of this protocol includes access to soft tissues, and prevention of further articular damage and relatively low rates of complications in patients who sustain high-energy proximal tibia fractures. In addition, this protocol allows use of MIPO technique which is based on combination of the principles of stability, restoration of anatomy and early motion, while eliminating the need for excessive soft tissue dissection.

KEYWORDS: Proximal tibial fractures, two stage reconstruction, Knee society score

INTRODUCTION: Intra articular Fractures of the proximal tibia encompass a wide range of severity, from stable undisplaced fractures with minimal soft tissue injury to highly comminuted unstable fractures, and severe soft tissue involvement. [1,2,3] Careful and thorough assessment of injury is critical in achieving optimal outcomes and avoiding complications.[4]
Various treatment modalities have been used over the years, with mixed results. These includes circular frames[5], percutaneous screw fixation, open reduction/internal fixation (ORIF) [2,4] have also been advocated.

Staged management of tibial plateau fractures refers to the use of temporizing methods of care in the form of spanning external fixator and delaying definitive fracture surgery till the skin and soft tissue status is satisfactory and initial soft tissue inflammation and edema subsides. Staged management also refers to employing fracture stabilization techniques that are more "friendly" to injured soft tissues. [5]

This Retrospective study evaluated the use of a two stage management protocol involving temporary distraction external fixation and delayed definitive fixation in the management of high-energy intraarticular proximal tibia fractures in terms of soft-tissue management, development of complications, and functional outcomes.

MATERIALS AND METHODS: The present retrospective study conducted between August 2008 and September 2011, 52 patients who had sustained high-energy intraarticular proximal tibial fractures (high-energy fractures, Schatzker , type IV-VI; OTA types 41A, B, C) were treated at P.D.U. Medical College, Rajkot. According to protocol made, we used to place immediate temporary distraction external fixator and take care of soft tissue for all high-energy intraarticular proximal tibia fractures. 12 patients were lost to follow-up were excluded from the final analysis. Thus, 40 patients (27 males, 13 females, mean age 52 [range 18–94 years]) form the basis of this report.

All these patients were subjected to detailed history to ascertain age, sex, mechanism of injury, related injuries and pre-existing local and systemic diseases that may affect recovery. Laboratory investigations were done as per requirement.

Initial Evaluation

The high-energy trauma patient is usually hypotensive, coagulopathic, and may have multi-system injuries. They were initially managed using accepted ATLS protocols for trauma victims. Iintravenous antibiotics are administrated and tetanus prophylaxis is given.

RADIOGRAPHY: Every patient with tibial plateau fractures was subjected anteroposterior (AP) and lateral plain radiographs of the knee. In situations where the fracture line propagates to the tibial shaft, full-length AP and lateral radiographs of the tibia should also be obtained. Because of the prevalence, ease, and superior-quality images obtained from computed tomography (CT), this modality has replaced additional plain radiographs during the initial work up.

CLASSIFICATION: Tibial plateau fractures are commonly classified using the Schatzker classification, which subdivides these injuries into six types; study includes high-energy fractures, Schatzker[6], type IV-VI. The OTA/AO classification can be used to classify these injuries, both intra- and extra-articular ones. The Gustillo-Anderson[7] classification was used for open injuries.

The use of this protocol is unnecessary in low-energy tibial plateau injuries such as Schatzker type I-III fractures, which are amenable to early definitive fixation or stabilization using an external splint.

TECHNIQUE: The patient after initial resuscitation may be taken to the operating room for further care. Open injuries need to be treated with appropriate debridement. Compartments syndromes are identified with clinical examination and pressure monitoring, and a four-compartment fasciotomy performed if needed. An attempt is made to apply the external fixator

within 6 hours of the injury for open fractures and within 24 hours for closed injuries with no compartment syndrome.

Using standard precautions, the patient is preferably positioned supine on a radiolucent table. This allows approach to the head, chest, abdomen, or the pelvis. Further radiographs or fluoroscopy may be obtained as needed.

The position of the half pins in the tibia should be considered carefully so as to avoid all future definitive fixation hardware. Haphazard pin placement may result in compromising future incisions and also increase the risk of pin tract infections. The pins are generally placed percutaneously using soft tissue protectors. The bone should be pre-drilled and the pins not placed too close to each other for fear of creating a stress riser after pin removal. One should try and avoid injured soft tissue including areas of blistering, avulsion, or open wounds. The two femoral pins are inserted anteriorly, or anterolaterally, approximately 10 centimeters proximal to the superior pole of the patella. The two tibial pins are inserted on the anteromedial border. The pins are connected using bars and clamps at the level of the knee joint after traction is applied to regain length and alignment. The reduction relies on ligamentotaxis and care must be taken to avoid over-distraction. The knee is kept in 20° of flexion for comfort. The frame is either anterior or anterolateral depending on soft tissue status and the need for further debridements or other wound care and surgeon preference. A posterior splint may be added for comfort.

POSTOPERATIVE CARE AND FURTHER PLAN: Once the fixator has been applied additional enhanced imaging can be obtained in order to identify the fracture lines in both coronal and sagittal planes and delineate the size of various fracture fragments. It also prepares the surgeon for the degree of comminution and any joint depression that exists but may be unclear on plain radiographs. Chan [8] showed the importance of a CT scan as it changed the classification and operative plans in a significant number of patients with tibial plateau fractures.

The pins and the clamps are usually cleaned and used in the final procedure as a reduction tool allowing traction for distraction and joint visualization. The fixator can be used as supplemental fixation, if minimal internal fixation is used for the articular fracture fixation, or converted to a non-joint spanning frame for fracture stabilization. Postoperatively, it may be retained as a splint for comfort and wound care. By the time of definitive fixation, all open wounds and/or fracture blisters will be clean, closed, or covered.

TIMING OF DEFINITIVE SURGERY: While definitive fixation can frequently be carried out within a week of injury, it is not uncommon to wait as long as 3 weeks, when the soft tissues are deemed “settled” – classical signs being healing and re-epithelialization of blisters and absence of pitting edema and the “wrinkle sign.” As long as limb length and general limb alignment have been maintained in the external fixator, waiting 3 weeks to perform definitive surgery is acceptable.[9]

GOALS OF DEFINITIVE TREATMENT: It has long been taught that anatomic reduction of the articular surface was the most important factor affecting outcome following tibial plateau fractures. Careful review of the literature, however, seems to indicate that the health of the articular cartilage, mediolateral stability of the knee, presence of the menisci, and overall alignment of the tibia are of equal or greater importance.[10,11,12] On the basis of these principles, the choice of definitive fixation may include plates may be dual platting [13], LISS (Less invasive

stabilizing system)[14] or single lateral platting with or without non-bridging external fixation with minimal invasive lateral platting.[15]

As such, perioperative antibiotic usage for delayed fracture surgery should be the same as for clean, elective orthopedic procedures. A first-generation cephalosporin begun within an hour prior to surgical incision and continuing for 24 hours is the regimen routinely employed. Sutures are removed after approximately 2 weeks.

FOLLOW-UP ASSESSMENT: Union was defined as evidence of bone healing by direct or indirect means in at least two radiographic planes and a full painless weight bearing joint. Functional assessment was performed using the Orthopedic assessment done at latest follow-up included a clinical and radiographic examination and functional outcome measurement with the Knee Society score (KSS).[16]

RESULTS: There were 10 type IV, 22 type V and 8 type VI fractures. 93% of the fractures was closed type and 7% were open. 6 patients were treated with closed reduction and percutaneous cannulated screw fixation, 2 patients with medial MIPO platting, 10 patients with single lateral platting (MIPO), 8 with lateral platting (MIPO) with medial fixators, 4 patients with dual platting (MIPO), 8 patients with open reduction and internal fixation and 2 patients with Illizarov's circular frame. The average time to union was 13 weeks (range 8–36). Out of all the included cases, excellent joint reduction and alignment were achieved initially in 34 cases (85%), but was reduced to 30 (75%) at final follow-up. Malalignment included 4 (10%) cases of residual varus and 3 (7.5%) cases of residual valgus deformity (up to 10 degrees). Five patients developed leg-length discrepancy (two with 2.0 cm, two with 1.5 cm and one with 1.0 cm).

Type of fractures	Modality of fixation	No. of patients
Type IV (*n*=10)	CR + Percutaneous Cannulated Screw Fixation CR+ MIPO OR+ IF	6 2 2
Type V(*n*=22)	CR+MIPO(single lateral platting) CR+MIPO(lateral platting with medial fixator) OR+IF (Dual platting)	10 8 4
Type VI(*n*=8)	CR+MIPO (Dual platting) OR+IF (Dual platting) Illizarov's circular frame	4 2 2

The complications includes 6 patients (15%) suffered superficial infection; All were successfully treated with local wound care and antibiotics. Deep infection was observed in 4 patients (10%). One of them had been treated with external fixation and developed deep sepsis within two weeks of the operation and was managed with surgical debridement. Two patients were diagnosed with deep sepsis at six months following surgery and required metalwork removal. One patient developed deep infection one year after surgery and required sinus excision and metalwork removal.

Two patients had developed compartment syndrome. This was treated by fasciotomies and subsequent split skin grafting. Two patients had deep venous thrombosis (DVT) required anticoagulation therapy. Postoperatively, one patient developed a foot drop but fully recovered 12 months later without permanent damage.

The Knee society score (KSS) was good in 26 cases (65%), fair in 10 cases (25.0%) and poor in 4 cases (10.0%) at latest follow-up. One case with poor results was seen in patient who developed deep infections. The other 3 had multiple injuries that significantly affected outcome.

Type of fractures	Results (Knee Society Score)	No. of patients
Type IV (*n*=10)	Good	8
	Poor	2
	Fair	0
Type V (*n*=22)	Good	14
	Poor	6
	Fair	2
Type VI (*n*=8)	Good	4
	Poor	2
	Fair	2

DISCUSSION: The management of high energy proximal tibia fractures is a challenging task for the surgeon, as they are often associated with a number of complications.[2,4,6,17] Problems of classification have previously been addressed in the literature[6]; however, evaluation criteria and optimal management remain controversial.

The most devastating complication associated with the management of high energy proximal tibia fractures is infection. Its incidence can be decreased by careful surgical timing and soft tissue handling. Indirect reduction techniques and minimally invasive surgery also decrease the likelihood of further devascularisation. In our series the incidence of superficial and deep infection was 15.2% and 9.6%, respectively. Four patients (3.9%) with bicondylar fractures, developed septic arthritis and were treated by metalwork removal and wash out. Infection rates range between 0 and 87.5% in the literature.[12,13,19]

There was 10% incidence of residual varus deformity. Gaudinez et al[18]reported 19% of varus deformity in his series of 18 complex (types V and VI) fractures. We had 4 patients with 10 degrees or less of varus, which did not require any further intervention; 3 of our patients developed <10º of valgus and did not require further surgery.

Complex tibial plateau fractures are associated with nonunion and malunion, as a result of comminution, unstable fixation, failure to bone graft, infection or combination of these factors. The rate of nonunion in this series was 1.6% which is comparable to other studies[18].

We achieved excellent joint reduction in 34 cases at the time of initial surgery but this number was reduced to 30 at the final visit. Loss of reduction was directly proportional to Schatzker type, which was worse in the bicondylar group (Schatzker type V & VI).

Compartment syndrome is often associated with high energy trauma. Two (5%) patients developed this complication. A recent series of 41 bicondylar fractures reported 9.76% incidence of compartment syndrome.[12,13]

Patients with tibial plateau fractures are at high risk of thromboembolic complications. Despite anticoagulation measures and early mobilisation, 2 (5%) patients developed DVT and one (2.5%) PE; however, all recovered satisfactorily. Other studies have reported rates ranging between 1.7% and 20%.[12,13]

Different scoring systems have been used to evaluate functional outcome of tibial plateau fractures. We used the KSS[16] which is graded between 0 and 100. A score of <60 was graded as poor, 60–70 as fair, 70–85 as good and 85–100 as excellent. The outcome was good in 28 cases (70%), fair in 10 (25%) and poor in 2 (5%). Others have reported good/excellent scores in 65–89% of subjects [20]. Poorer outcomes can be expected following complex fractures; however, Ali reported 80% of satisfactory functional and radiological outcomes using fine wire fixators for these injuries. Thus, Our results are comparable with other published studies.[12,13,18,19]

The introduction of minimally invasive techniques and limited dissection where appropriate, can provide stable fracture configurations and promote reliable bone healing, while preserving the soft tissue envelope.[14] 30 patients in our study treated with MIPO technique either single medial platting, single lateral platting or dual platting. All of those patients are associated with either good results (20) or fair results (10).

This study illustrates that proximal fractures continue to remain an important cause of morbidity. Treatment goals should include a congruent articular reduction, adequate knee stability, anatomical limb alignment and avoidance of complications. Functional outcome is directly dependent on achieving these targets.

Finally, we are aware that this study has a number of limitations including a follow-up period of less than ten years, use of different methods of fracture fixation, it is not a single surgeon's series and it is of retrospective nature. Despite these limitations, we believe that it provides useful information with regard to the intermediate functional outcome following these injuries. Although pain severity was associated with the degree of OA, this association was not linear. This can be valuable in informing patients about the outcome that can be expected.

CONCLUSION: The use of this protocol, with the initial application of a temporary distraction external fixator followed by delayed internal fixation, is suggested for treatment of high energy tibial plateau fractures. The use of staged protocol for pilon fractures has been successful in reducing the historically high rates of wound complications associated with these high-energy injuries. The benefits of this protocol includes, access to soft tissues, and prevention of further articular damage and relatively low rates of complications in patients who sustain high-energy proximal tibia fractures as well as, access this technique affords in open fractures and those with compartment syndrome. This study supports the practice of delayed internal fixation until the soft-tissue envelope allows for definitive fixation; as well this protocol allows use of MIPO technique which is based on combination of the principles of stability, restoration of anatomy and early motion, while eliminating the need for excessive soft tissue dissection.

REFERENCE:

1. Shepherd L, Abdollahi K, Lee J, Vangsness CT Jr: The prevalence of soft tissue injuries in nonoperative tibial plateau fractures as determined by magnetic resonance imaging. J Orthop Trauma 16(9): 628-631, 2002.
2. Lansinger O, Bergman B, Korner L, Andersson GB. Tibial condylar fractures. A twenty-year follow-up. J Bone Joint Surg Am. 1986;68:13-19.
3. Pape HC, Giannoudis P, Krettek C: The timing of fracture treatment in polytrauma patients: Relevance of damage control orthopedic surgery. Am J Surg 183(6): 622-629, 2002.
4. Young MJ, Barrack RL. Complications of internal fixation of tibial plateau fractures. Orthop Rev 1994;23:149-54.

5. Mikulak SA, Gold SM, Zinar DM. Small wire external fixation of high energy tibial plateau fractures. Clin Orthop Relat Res 1998;356:230-8
6. SchatzkerJ,McBroom R, Bruce D:The tibial plateau fracture: The Toronto experience 1968-1975. Clin Orthop (138):94- 104, 1979.
7. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: Retrospective and prospective analyses. J Bone Joint Surg Am. 1976;58:453–8.
8. Chan PS, Klimkiewicz JJ, Luchetti WT, et al: Impact of CT scan on treatment plan and fracture classification of tibial plateau fractures. J Orthop Trauma 11(7): 484-489, 1997.
9. Egol KA, Tejwani NC, Capla EL, Wolinsky PL, Koval KJ. Staged management of high-energy proximal tibia fractures (OTA types 41). The results of a prospective, standardized protocol. J Orthop Trauma. 2005;19:448-456.
10. Lachiewicz PF, Funcik T. Factors influencing the results of open reduction and internal fixation of tibial plateau fractures. Clin Orthop Relat Res. 1990;(259):210-215.
11. Delamarter RB, Hohl M, Hopp E Jr. Ligament injuries associated with tibial plateau fractures. Clin Orthop Relat Res. 1990;(250):226-233
12. Barei DP, Nork SE, Mills WJ, Coles CP, Henley MB, Benirschke SK. Functional outcomes of severe bicondylar tibial plateau fractures treated with dual incisions and medial and lateral plates. J Bone Joint Surg Am. 2006;88:1713-1721.
13. Barei DP, Nork SE, Mills WJ, Henley MB, Benirschke SK. Complications associated with internal fixation of high-energy bicondylar tibial plateau fractures utilizing a two-incision technique. J Orthop Trauma. 2004;18:649-657.
14. Ricci WM, Rudzki JR, Borrelli J,Jr. Treatment of complex proximal tibia fractures with the less invasive skeletal stabilization system. J Orthop Trauma. 2004;18:521-527.
15. Gosling T, Schandelmaier P, Muller M, Hankemeier S, Wagner M, Krettek C. Single lateral locked screw plating of bicondylar tibial plateau fractures. Clin Orthop Relat Res. 2005;439:207-214.
16. Insall JN, Dorr LD, Scott RD et al (1989) Rationale of the Knee Society clinical rating system. Clin Orthop Relat Res 13–14.
17. Papagelopoulos PJ, Partsinevelos AA, Themistocleous GS, et al. Complications after tibia plateau fracture surgery. Injury. 2006;37:475–484. doi: 10.1016/j.injury.2005.06.035.
18. Gaudinez RF, Mallik AR, Szporn M (1996) Hybrid external fixation of comminuted tibial plateau fractures. Clin Orthop Relat Res 203–210.
19. The Canadian Orthopaedic Trauma Society Open reduction and internal fixation compared with circular fixator application for bicondylar tibial plateau fractures. Results of a multicenter, prospective, randomized clinical trial. J Bone Joint Surg Am. 2006;88:2613–2623. doi: 10.2106/JBJS.E.01416.
20. Koval KJ, Sanders R, Borrelli J, et al. Indirect reduction and percutaneous screw fixation of displaced tibial plateau fractures. J Orthop Trauma. 1992;6:340–346. doi: 10.1097/00005131-199209000-00012.

PARAGANGLIOMA OF URINARY BLADDER - A RARE CASE

Dr. Srujana Shyamala, Dr. S. S. S Quadri. Dr. N Sreemani Kumari, Dr. P Jijiya Bai, Dr. Jahagirdar.R

1. Assistant Professor, Department of Pathology, Gandhi Medical College, Secunderabad.
2. Assistant Professor, Department of Pathology, Gandhi Medical College, Secunderabad.
3. Associate Professor, Department of Pathology, Gandhi Medical College, Secunderabad.
4. Professor and Head, Department of Pathology, Gandhi Medical College, Secunderabad.
5. Associate Professor, Department of Urology, Gandhi Medical College, Secunderabad.

CORRESPONDING AUTHOR
Dr. Srujana Shyamala,
H.no – 11-3-378/2,
Srinivasnagar, Padmarao Nagar,
Secunderabad, Andra Pradesh,
E-mail: pvsrujana@gmail.com,
Ph: 91-9490312560.

ABSTRACT: A 27 year old male presented with hematuria & dysuria since 9 months. Hematuria was transient at times, pain less & terminal. History of passing clots occasionally. Ultrasound revealed echogenic isolated mass measuring 5x4.6 cm arising from anterolateral wall of bladder with evidence of small foci of calcification at the periphery.

Partial cystectomy was done-but marked fluctuation in blood pressure and tachycardia was observed during the procedure. The histopathological findings were diagnostic of paraganglioma of the urinary bladder. The diagnosis was confirmed by means of immuno histochemical studies.

KEY WORDS: Paraganglioma, extraadrenal pheochromocytoma, bladder neoplasms.

INTRODUCTION: Paragangliomas originating in urinary bladder are extremely rare, arising from chromaffin tissue of the sympathetic nervous system. It is usually benign. The tumor usually develops in young adult women. Common symptoms and signs are dysuria, hematuria, and hypertension. We report a single case of non-functional paraganglioma of the urinary bladder clinically diagnosed as transitional cell carcinoma.

CASE REPORT: A 27 years old male presented to outpatient clinic of urology department with a 9 month history of hematuria and dysuria. He had no significant past medical history. His family history was non contributory. Physical examination was unremarkable, no organomegaly was observed. At admission, his blood pressure was 120/80mm of Hg, pulse rate was normal. Both ultrasound and CECT demonstrated isolated, enhancing sessile mass measuring 5x4.6 cm arising from right anterolateral wall of urinary bladder with specks of calcification. Perivesical fat planes were preserved. Wall thickness appeared normal.

No calculi or evidence of any metastatic disease was observed.

Routine blood tests like CBP, LFT RFT were within normal limits. Urine examination showed pus cells 50-55/ hpf, Epithelial cells-10-12/ hpf, RBC 12-15/ hpf, Albumin 3+ (strip / manual method).

On the basis of the clinical diagnosis of primary bladder tumor, patient was admitted for partial cystectomy. During surgery, marked fluctuation in blood pressure with rise of blood pressure upto 230/80 mm of Hg and pulse rate of 200/minute was observed. Patient developed intraoperative cardiac arrest and was revived after DC shocks and was kept on ionotropic

infusion and mechanical ventilator. He attained conscious state on 2nd day of surgery. On follow up, his blood pressure and pulse rate are within normal limits.

MACROSCOPIC AND MICROSCOPIC FINDINGS: Grossly specimen received consisted of two soft tissue masses showing brown to yellow color. Largest mass measured 5x2.5x1 cm and smaller mass 3x2x1 cm. On cut section, the both masses displayed uniform/homogenous yellowish brown appearance with no evidence of hemorrhage or necrosis.

Histopathological examination revealed a neoplasm composed of tumor cells arranged in nesting pattern and zell ballen pattern with delicate fibrovascular stroma. Individual cells are large round to polygonal with distinct cell border, abundant eosinophilic granular cytoplasm and vesicular nucleus. Mitoses are absent. A provisional histopathological diagnosis of paraganglioma of urinary bladder was made.

On immunohistochemistry, this bladder tumor was negative for epithelial marker cytokeratin and positive for the neuroendocrine markers – chromogranin, synaptophysin. Sustentacular cells showed positivity for S-100, thereby confirming the diagnosis of Paraganglioma of urinary bladder.

DISCUSSION: Paragangliomas are extra adrenal neoplasms arising from chromaffin tissue of the sympathetic nervous system. According to the latest edition Campbell Walsh Urology [1], trigone and posterior wall of urinary bladder have been stated as the most common sites for paraganglioma, while in our case,the tumor arised from the antero lateral wall. The first case of paraganglioma of the urinary bladder was reported by Zimmerman[2] in 1953. Paraganglioma of the urinary bladder accounts for <1% of all bladder tumors and 0.6% of extra adrenal pheohromocytomas [3]. In most cases, paragangliomas of the urinary bladder often causes micturitional attacks, headache, palpitation, fainting and visual disturbances. No such symptomatology was seen in our case. During surgery, marked fluctuation in blood pressure with tachycardia was observed [4].

The tumor usually show females preponderance in (F: M = 3:1), occurring in 20-40 years of age, while this case was 27 years old male.

In most series patients may present with headache, palpitation, paroxymal HTN due to catecholamine excess especially during micturition. As the patient in our study had none of these symptoms, hence no endocrine tests were performed [5]. As many as 50% of the paragangliomas are hereditary and may be associated with familial paragangliomas, neurofibromatosis type1,Von Hippel Lindau disease and the Carney triad [6].

Macroscopically, these tumors are red-brown lobulated solid, submucosal or intramural masses covered by intact epithelium. They are as large as 10 cm but most are only a few centimeters in greatest dimensions. They usually appear as fungiform rounded or pedunculated masses that bulge into the lumen with variable ulceration.

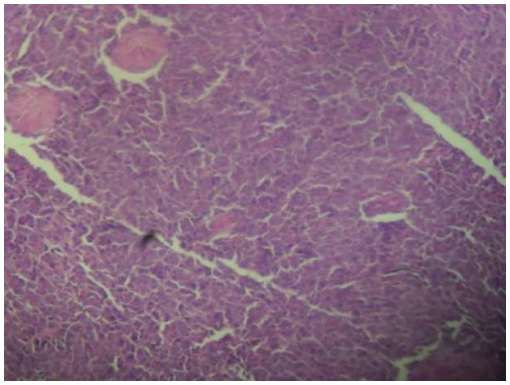
On microscopic examination: tumor shows nests of cells arranged in zellballen pattern or trabecular architecture or a mixture of the two, diffuse or solid architecture can also be seen[7]. Individually, tumor cells are large with abundant granular and basophilic to amphophilic cytoplasm with cellular and nuclear pleomorphism. Sometimes prominent nuclear pseudoinclusions are present in some cases. Spindle cells are present in about 2% of cases.

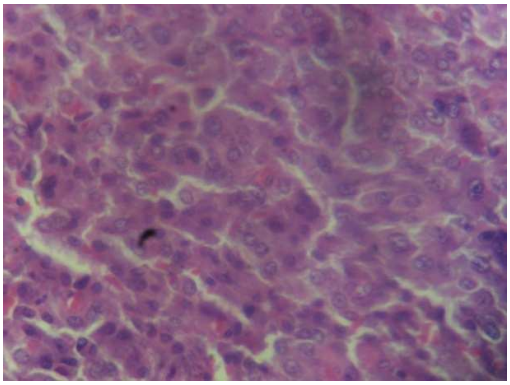
A small cell variant is also described. Intracytoplasmic hyaline globules occur commonly. These are PAS positive and diastase resistant. Hemorrhage and hemosiderin deposits are common.Oncocytic variants have also been reported.

IHC paragagliomas are positive for chromogranin-A, synaptophysis and NSE. S-100 protein demonstrates sustentaular cells.

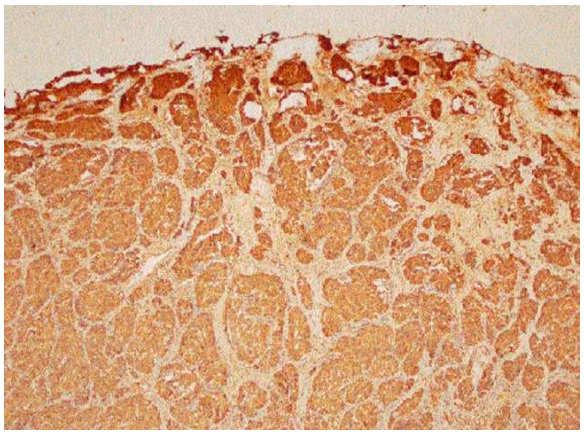
CONCLUSION: Bladder paragangliomas may be misdiagnosed when characteristic symptoms are absent. Although it shows female preponderance, our case is a 27 year male.
It should be considered as a differential diagnosis in neoplasm of urinary bladder. Laparoscopic partial cystectomy may be the first choice in treating paraganglioma of the urinary bladder. Recurrence and metastasis though infrequent, have been reported in the literature; therefore, long term follow up is required.

GROSS: Both masses displayed uniform/homogenous yellowish brown appearance with no hemorrhage or necrosis. Largest measuring 5x2.5x1 cm and smaller3x2x1 cm.





HPE: LP (10x) view: neoplasm composed of tumor cells arranged in nesting and zell ballen pattern with delicate fibrovascular stroma. HP view (40x): Individual cells are large round to polygonal with distinct cell border, abundant eosinophilic granular cytoplasm and vesicular nucleus. Mitoses are absent



I H C: Tumor showing positivity for neuroendocrine markers – Chromogranin A

REFERENCES:

1. Messing EM.Urothelial tumors of bladder. In : Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA,editors Campbell Walsh Urology,9th edPhiladelphia:Saunders;2007.p.2407
2. Zimmerman IJ, BironRE, Macmohan HE: Phaeochromocytoma of the urinary bladder.
1. N Engl J Med 1953, 249(1):25 -26.
2. 3.Yadav R,Das AK,Kumar R.Malignant non functional paraganglioma of bladder presenting with azotemia.Int Urol Nrphrol,2007;39: 449- 51.
3. Ikeda M, EndoF,Shiga Y, Oguchi T, Yashi M, Hattori K, Maraishi O:Cystoscopic assisted partial cystectomy for paraganglioma of the urinary bladder.Hinoyokika Kiyo 2008,54(9):611-614.
4. 5.Thrasher JB, Rajan RR,Perez LM, Humphrey PA, Anderson EE:Pheochromocytoma of urinary bladder:contemporary methods of diagnosis and treatment options.Urology 1993,41(5):435-439.
5. Young WF Jr.Paragangliomas:clinical overviewAnn N Y Acad Sci.2006;1073:21-9.
6. Zhou M,Epstein JI,Young RH: Paraganglioma of urinary bladder: a lesion that may be misdiagnosed as urothelial carcinoma in transurethral resection specimens. Am J Surg Pathol 2004, 28(1):94- 100.

COMPARISON OF DIRECT IMMUNOFLUORESCENCE, IODINE-SALINE WET MOUNT AND MODIFIED ACID FAST STAINING METHODS FOR DETECTION OF CRYPTOSPORIDIUM AND GIARDIA SPP. IN HUMAN FECAL SPECIMENS

Prakriti Vohra, Pooja Singla, Madhu Sharma, Aparna Yadav, Uma Chaudhary

1. Senior Resident, Department of Microbiology, Pt. B. D. Sharma, PGIMS, Rohtak.
2. Senior Resident, Department of Microbiology, Pt. B. D. Sharma, PGIMS, Rohtak.
3. Professor, Department of Microbiology, Pt. B. D. Sharma, PGIMS, Rohtak.
4. Professor, Department of Microbiology, Pt. B. D. Sharma, PGIMS, Rohtak.
5. Senior Professor & Head, Department of Microbiology, Pt. B. D. Sharma, PGIMS, Rohtak.

CORRESPONDING AUTHOR
Prakriti Vohra,
Senior Demonstrator,
Department of Microbiology,
Pt. B. D. Sharma, PGIMS, Rohtak.
E-mail: prakriti@yahoo.com,
Ph: 91-9992289955.

ABSTRACT: BACKGROUND: Cryptosporidiosis and Giardiasis are leading causes of parasitic intestinal diseases. There are various methods to detect them including iodine-saline wet mount for Giardia, Modified Acid fast (MAF) staining for Cryptosporidia and Direct Fluorescent Antigen detection (DFA) method. **OBJECTIVES:** This study was conducted to compare three methods for the detection of Cryptosporidium oocysts and Giardia cysts in fecal samples. **METHOD:** 44 stool specimens from patients with history of diarrhea were examined for Giardia lamblia and Cryptosporidium parvum by using a direct immunofluorescent-monoclonal antibody stain (for unspun specimens), Iodine- saline wet mount method for Giardia cysts & trophozoites and Modified acid-fast for Cryptosporidium oocysts. **RESULTS:** From the total of 44 specimens; 29 (65.9 %) Cryptosporidia oocysts were isolated and 6 (13.6%) Giardia cysts by all the Iodine-Saline wet mount, MAF, DFA methods. The direct immunofluorescent-monoclonal antibody method resulted in a significantly increased detection of both Giardia cysts (5 versus 1 specimens, p value =0.01) and Cryptosporidium oocysts (19 versus 10 specimens, p value=0.01). **CONCLUSIONS:** DFA is a sensitive method for detection of Cryptosporidium oocysts and Giardia cysts in fecal samples whereas MAF is a reliable screening method.
KEY WORDS: Cryptosporidium, Giardia, DFA, Modified Acid-fast Staining.

INTRODUCTION: Cryptosporidiosis and Giardiasis are two of the most commonly seen protozoal causes of diarrhea. Outbreaks of diarrhea have been frequently attributed to these organisms.[1] These often affect children and the immunosuppressed[2,3] Many new diagnostic techniques have been reported to detect these pathogens in stool samples, including various concentration and staining methods.[4,5,6] The diagnostic sensitivity of Iodine-Saline mount for Giardia and that of modified acid-fast staining for Cryptosporidia are often limited by the shedding of organisms intermittently or in low numbers. Sensitivity of these conventional staining methods also depends on the skill of the microscopist, and it is often highest when full-time parasitology technologists are examining specimens.[7]

The majority of patients with cryptosporidiosis suffer with various degrees of diarrhea. In these symptomatic patients, the presence of large number of oocysts ensures that the diagnosis can be made by a number of techniques. However, there may be some situations e.g. some patients may be asymptomatic or exhibit fewer symptoms or when the number of oocysts present is very few. In these situations, the use of routine methods such as concentrations and modified acid-fast stains may be insufficient to demonstrate the presence of the parasite.

Giardia spp. cysts are shed sporadically and may vary in numbers**.** Examination of several sequential fecal specimens may not reveal infection with this parasite, particularly if the numbers are low. Co infection with both organisms have also been documented.[8,9]

Monoclonal antibody reagents with increased sensitivity offer an alternative method to the routine concentrations and stains, both of which may not reveal infections when few parasites are present. The purpose of the present study was to evaluate various methods for detection of Cryptosporidia and Giardia in fecal speimens.

To the best of our knowledge, this study is the first of its kind from North India in which we did comparison of different techniques for the identification of parasitic protozoa.

MATERIALS AND METHODS: Forty four human fecal specimens received from patients with diarrhea in the department of microbiology, Pt. B.D. Sharma PGIMS, Rohtak over a period of two months (3rd July to 3rd September 2012) were analyzed retrospectively and processed in following manner:

1. **IODINE- SALINE WET MOUNT METHOD[10]**
   Stool samples were collected in wide-mouthed disposable containers. With the help of applicator stick the stool sample was emulsified in a drop of saline on a clean dry slide and in a drop of Lugol's iodine on different slides. These were covered with coverslips and observed under the microscope at 400X magnification for the detection of ova and cysts.

2. **MODIFIED ACID FAST STAINING METHOD[11] (MAF)**
   Smears approximately 20 by 20 mm were prepared from samples. These were first stained with Carbol fuchsin (5 minutes) followed by decolorization with 1% Sulphuric acid (2 minutes), followed by counter staining with Methylene blue (1-2 minutes). All these smears were then examined with a 100X oil immersion objective, and the presence or absence of Cryptosporidial cysts was recorded.

3. **DIRECT FLUORESCENT MONOCLONAL ANTIBODY STAINING (DFA)**
   The smears were prepared from the stool samples preserved in 10% formalin and stained with the DFA Merifluor Cryptosporidia/Giardia kit (Meridian Diagnostics Inc., Cincinnati, Ohio) according to the manufacturer's instructions. This kit contained 50 treated slides along with detection reagent, Counter stain, 20X wash buffer, Positive control, Negative control, Mounting medium and transfer loops. A loopful of fecal material was spread over entire well. Positive and negative controls were put up in respective wells. Slides were air-dried for 30 minutes at room temperature. A drop of detection reagent followed by Counter stain was placed in each well. After incubating in humidified chamber for thirty minutes, slides were rinsed with a wash buffer. After removing excess buffer by tapping long edge of slides, a drop of mounting medium was

added to each well and coverslip was applied. Each well was scanned thoroughly under fluorescent microscope (with a filter system for fluorescein isothiocyanate with following parameters: Excitation wavelength – 490-500; Barrier filter- 510-530) using 100-200X magnification. Cryptosporidium oocysts were round to slightly oval shaped, 2-6 µm in diameter and Giardia cysts were oval-shaped, 8-12 µm long. Both of these gave bright apple green fluorescence with DFA staining.

Statistical analysis was done by using Chi square test. SPSS version 19 was used.

RESULTS:

Among 44 patients, 30 were males and 14 were females. Out of these, 28 were children and 16 were adults. 71.43% females and 63.3% males were positive for C.parvum in this study. Neonates (75%) were the most affected and least affected were adults (52.94%).

In the case of Cryptosporidium oocysts, 29 specimens were positive by at least one method. Of these, 8 were positive by both the DFA and the modified acid-fast stains, 11 were positive by DFA alone, and 10 were positive only by the modified acid-fast stain [Table 1]. Overall, DFA staining significantly increased the detection of cryptosporidia (p value=0.01). Of the two false-negative DFA results in the cryptosporidium group, these specimens showed no fluorescence at all. The lack of staining in these specimens could be due to sampling error.

28.57% females and 6.7% males were positive for cysts of Giardia spp. in this study. Adults (23.5%) were the most affected and least affected were neonates (5%). Of the 44 specimens examined, 6 were positive for Giardia cysts by at least one method. Of these, 1 was positive by both DFA and routine methods, 4 were positive only by DFA, and 1 was positive only by the routine method [Table 2]. Thus the overall detection of Giardia cysts was increased significantly (p value=0.01). The single false-negative DFA result was obtained in the Giardia group. It may be due to the sampling error.

During screening of stool samples, cysts of Entamoeba histolytica were detected in 3 samples and ova of Ascaris lumbricoides were detected in 1 sample. All these samples were negative for cysts of Cryptosporidium and Giardia.

Table 1. Comparison of DFA and MAF for Cryptosporidium oocysts

Method	No. of specimen		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative				
DFA	19	25	80%	100%	100%	94.4%
MAF	10	34	40%	91.7%	80%	64.7 %

Table2. Comparison of DFA and Iodine-Saline wet mount method for Giardia cysts

Method	No. of specimen (n=44) Positive Negative	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
DFA	5 39	100%	100%	100%	100%
Iodine-Saline wet mount	1 43	20%	100%	100%	90.7%

DISCUSSION: Our results show that the DFA method, in addition to having excellent specificity, exhibits vastly improved sensitivity over those of the routine methods used. These findings support and supplement the initial experiences of others with this technique.[12,13] Results of DFA in this study matches with that of Alles et al[14] who reported sensitivity of 93%, specificity-100%, Positive predictive value-100% and negative predictive value- 99.9% for Cryptosporidium parvum & sensitivity-99.2%, specificity-100%, Positive predictive value-100% and negative predictive value- 99.96% for Giardia spp. Zimmerman et al[15] also reported 100% sensitivity and 99.8% specificity of DFA. 96.4% sensitivity of DFA was reported by Kehl et al[16] who observed that DFA test detected 53 out of 55 positive fecal samples for these parasitic pathogens. Immunofluorescence assay for antigen detection of Cryptosporidium parvum offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories. The sensitivities and specificities of various commercially available kits for antigen detection range from 66.3% to 100% and 93% to 100% respectively.[17]

For the diagnosis of cryptosporidiosis Kashyap B. et al[18] found that Kinyoun staining gave a sensitivity of 80% and a specificity of 100%. The positive predictive value (PPV) and the negative predictive value (NPV) of Kinyoun staining were 100 and 96.5%, respectively. While in our study, the sensitivity, specificity, Positive predictive value & Negative predictive value for MAF staining was 40%, 91.7%, 80%, 64.7% respectively.

Barua P. et al[19] found Modified Z-N technique to be efficacious method for routine screening of fecal specimens for Cryptosporidium spp in comparison with ELISA.

The 9 fecal specimens which came to be positive by DFA test, but not detected by the conventional acid fast stain, can be considered in fact true positive specimens with low intensity, based on reports by Kehl et al[16] who found that carbol fuchsin failed to stain some of the oocysts in fecal specimens and that DFA is more specific than the acid fast staining.[12,20]

The laboratory diagnosis of both Cryptosporidiosis and Giardiasis from fecal specimens can be improved upon by increasing use of DFA technique. Its advantages over wet mount and staining method include faster detection time and need for comparatively less skilled staff. Moreover, C. parvum and G. lamblia can be detected in the same sample by a single test. However, the DFA method (Both Kit & Fluorescent microscope) is considerably more expensive than other two methods, and this probably is limiting factor in its use in the routine stool examination.

BIBLIOGRAPHY

1. G H Tee, A H Moody, A Hunt Cooke, P L Chiodini. Comparison of techniques for detecting antigens of Giardia lamblia and Cryptosporidium parvum in faeces. Jr Clin Pathol 1993;46:555-558.
2. Current, W.L., L. S. Garcia. Cryptosporidiosis. Clin Microbiol Rev.1991;4:325–358.
3. Farthing M.J. Diarrhoeal disease: current concepts and future challenges. Pathogenesis of giardiasis. Trans R Soc Trop Med Hyg.1993;87 Suppl. 3:17–21.
4. Baxby D., Blundell N. Sensitive, rapid, simple methods for detecting Cryptosporidium in faeces. Lancet.1983; ii:1149.
5. Garcia, L. S., D. A. Bruckner, T. C. Brewer, R. Y. Shimizu. Techniques for the recovery and identification of Cryptosporidium oocysts from stool specimens. J Clin Microbiol.1983;18:185-190.
6. Arora DR, Arora B. AIDS-Associated Parasitic Diarrhoea. Ind J Med Microbiol.2009;27:185-90
7. Mohr, E., Mohr,I. Statistical analysis of the incidence of positives in the examination of parasitological specimens. J Clin Microbiol.1992;30:1572–4.
8. Jokipii L, Pohjola S, Jokipii AM. Cryptosporidiosis associated with traveling and giardiasis. Gastroenterology.1985;89:838-842.
9. Skeels R.M., Sokolow R., Hubbard V.C., Foster R.L. Screening for Coinfection with Cryptosporidium and Giardia in Oregon Public Health Clinic Patients. American Journal of Public Health,1986;76:270-273.
10. Arora DR, Arora B. Medical Parasitology: 2nd ed. CBS Publishers and Distributors; New Delhi, Bangalore, India: 2004. p. 235.
11. Koneman E.W., Allen S.D., Janda W.M., Schreckenberger P.C., Winn Jr. W.C. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott-Raven Publishers, 6th edition. Philadelphia:2006. p.1075-1076.
12. Garcia S.L., Shum A.C., Bruckner A.D. Evaluation of a New Monoclonal Antibody Combination Reagent for Direct Fluorescence Detection of Giardia Cysts and Cryptosporidium Oocysts in Human Fecal Specimens. J Clin Microbiol.1992;30:3255-3257.
13. Xiao L., Herd. R.P. Quantitation of Giardia cysts and Cryptosporidium oocysts in fecal samples by direct immunofluorescence assay. J Clin Microbiol.1993;31:2944–2946.
14. Alles A.J., Waldron M.A.,Sierra L., Mattia A.R. Prospective Comparison of Direct Immunofluorescence and Conventional Staining Methods for Detection of Giardia and Cryptosporidium spp. in Human Fecal Specimens. J.Clin Microbiol.1995;33:1632-1634.
15. Zimmerman S.K. , Needham C.A. Comparison of Conventional Stool Concentration and Preserved- Smear Methods with Merifluor Cryptosporidium/Giardia Direct Immunofluorescence Assay and ProSpecT Giardia EZ Microplate Assay for Detection of Giardia lamblia. J Clin Microbiol..1995;33:1942–1943.
16. Kehl K.S., Cicirello H, Havens PL. Comparison of four different methods for detection of Cryptosporidium species. J Clin Microbiol.1995;33:416–418.
17. Mehta P. Laboratory diagnosis of Cryptosoridiosis.Ind J Med Microbiol.2002;48:217.
18. Kashyap B., Sinha S., Das S., Rustagi N., Jhamb R. Efficiency of diagnostic methods for correlation between prevalence of enteric protozoan parasites and HIV/AIDS status––an experience of a tertiary care hospital in East Delhi. J Parasit Dis.2010 ;34:63–67.

19. Barua P., Hazarika NK, Barua N, Rasul E.,Laskar N. Microscopy for Cryptosporidiosis Screening in Remote Areas. Indian J Med Microbiol.2008;26:203-204.
20. Weber R, Bryan RT, Bishop HS, Wahlquist S.P., Sullivan J.J., Juranek D.D. et al. Threshold of detection of Cryptosporidium oocysts in human stool specimens for low sensitivity of current diagnostic methods. J Clin Microbiol.1991;29:1323-7.

CLINICO – EPIDEMIOLOGICAL TREND OF SPUTUM POSITIVE PULMONARY TUBERCULOSIS IN PATIENT ATTENDING TERTIARY CARE HOSPITAL AT GARHWAL – UTTARAKHAND

Dr. Deependra Kumar Rai, Dr. Amit Kumar Singh.

1. Assistant Professor, Department of TB & Chest,VCSGGMSRI-Srinagar, Garhwal (Uttarakhand)
2. Associate Professor, Department of Community Medicine, -Srinagar, Garhwal (Uttarakhand)

CORRESPONDING AUTHOR
Dr. Amit Kumar Singh,
Associate Professor, Community Medicine,
VCSGGMSRI – Srinagar
Garhwal (Uttarakhand)
E-mail: Singh120377@yahoo.co.in,
Ph: 91 9410392588.

ABSTRACT:BACKGROUND & OBJECTIVE: The present study was undertaken to know the epidemiological trend of sputum positive Pulmonary Tuberculosis in Garhwal region of Uttarakhand which may be useful for programme planners to plan the resource requirement and for monitoring the RNTCP. **MATERIAL & METHOD:** Socio demographic profiles of all patients coming to medical OPD between Jan 2008 to Dec. 2011 with cough more than three weeks and from Oct 2010 Patients with cough for more than 2 weeks were recorded. Data from DOTS centre and medical OPD were collected and trend as well as proportion of sputum positive PTB (pulmonary Tuberculosis) in patients coming from different districts of Garhwal region coming to Tertiary care hospital was studied. **RESULT:** The proportion of sputum positive pulmonary tuberculosis in tertiary care hospital was six cases per thousand in adult patients in 2008, in next three year it was 4 cases per thousand. The sputum positivity in pulmonary suspect varies from 11.91% (2010) to 14.06% (2008). If we compare district wise, sputum positivity was maximum in patients coming from Tehri district (14.37%) and least in Pauri (12.50%) district. The number of PTB suspect required for detection of each sputum positive pulmonary tuberculosis patients was 7.10 in 2008, 8.18 in 2009, 8.36 in 2010 and 7.36 in 2012. **CONCLUSION:** The proportion of sputum positive PTB was high in patients coming from Garhwal region of Uttarakhand .The sputum positivity rate and number of Pulmonary Tuberculosis suspect required for detection of one sputum positive Pulmonary tuberculosis in patients coming from different district of Garhwali varies in different district in different year.
KEYWORDS: TB, Tuberculosis, PTB. Pulmonary Tuberculosis, DOTS. Directly observed treatment short course, **RNTCP**, Revised National Tuberculosis Control Programme

INTRODUCTION: Tuberculosis, an infection caused by Mycobacterium Tuberculosis is a worldwide pandemic and the Centre for Disease Control and Prevention (CDC) state that one-third of the world's population is infected with the bacteria. India, with its population of over 1000 million is estimated to account for nearly 30 per cent of the global tuberculosis burden.[1] Tuberculosis (TB) continues to be a major health problem in India because of its high mortality and morbidity.[2] Data on the burden of tuberculosis are vital for programme planners in order to calculate the resource requirements and monitor the nation-wide TB control programme. The National Tuberculosis Control Programme (NTP) was implemented in 1962. However when

reviewed in 1992, after three decades of implementation the NTP was shown to have made no epidemiological impact, mainly due to poor case finding and low treatment completion rates[3]. As a result, the Government of India (GOI) in 1993 developed the Revised National Tuberculosis Control Programme (RNTCP) based on the internationally recommended Directly Observed Treatment – Short course (DOTS) strategy. Since 1998, the RNTCP has undergone a rapid expansion, and by November 2004 covered a population of over 920 million.[4] Based on the findings of the National Sample Survey (NSS) conducted by Indian Council of Medical Research (ICMR) during 1955-58, [5] an estimate of the burden of TB in India of 3.5 million bacillary cases and 14 million persons with abnormal chest X-rays suggestive of tuberculosis with negative sputum (hereafter, referred to as X-ray abnormal) was made at the time of introduction of RNTCP in 1997. [6] The nationwide survey by Indian Council of Medical Research in the late 50's (1955-58) had shown an average prevalence of sputum positive pulmonary tuberculosis of about 400 per 100,000 populations amounting to an absolute number of 1.5 million infectious cases at that time with similar prevalence rates in rural and urban areas. [7] Unfortunately, we do not have the latest nation-wide data after this report nearly 50 years ago although scattered confined regional data is available off and on. Disease surveys conducted in different parts of the country since the 1950s have reported prevalence of smear-positive pulmonary TB (PTB) of 0.6-7.6 per 1000 population, culture-positive TB of 1.7-9.8 and culture and/or smear-positive TB of 1.8 - 12.7. The incidence of smear-positive PTB has been observed in the range of 1.0-1.6/1000 and that of culture-positive PTB 1.0-2.5/1000 in the limited number of studies carried out.

Indian population is composed of people of diverse cultural, linguistic, biological, ethnic and genetic backgrounds, living in different socio-cultural and socio-economic settings.

The present study was conducted to estimate prevalence and socio - demographic trend of sputum positive Pulmonary Tuberculosis in patients coming to HNB base hospital in last four years.

MATERIALS & METHODS: Study Area The study was conducted in Chest & TB department of VCSG Government medical college, Srinagar - Garhwal, Uttarakhand situated in mid Himalayan region of Uttarakhand. This medical college drains patients mainly from districts of Garhwal like Pauri, Rudraprayag, Chamoli and Tehri.

Study design & Data collection
Type of Study: Hospital record based study.
Study Period: The study was conducted by analysing the data from Jan 2008 to Dec. 2011.

METHOD: Standard RNTCP diagnostic algorithm includes all patients coming in medical OPD between Jan 2008 to Dec. 2011 with cough with more than three weeks and from Oct 2010 Patients with cough for more than Two weeks. Information on patients' socio-demographic characteristics, cough duration in days or weeks and sputum results were studied. The proportion, Sputum positivity in Pulmonary TB suspect and socio demographic trend of Patients coming from four district of Garhwal was analysed.

OPERATIONAL DEFINITIONS: A **PTB suspect:** All patient attending medical OPD with history of cough for three weeks (from Oct 2010, two weeks) or more.

SPUTUM POSITIVE PTB: Patients with at-least two sputum specimens positive for acid –fast bacilli (from Oct 2010 at least one sputum specimen positive for acid-fast bacilli by microscopy)

ETHICAL CONSIDERATIONS: Permission was sought from Principal/Dean of the institute to carry out the present study. Data analysis has been done using SPSS version 15.0 and Microsoft Office Excel 2007.

RESULT:

Table 1 Total suspect case in four districts in different years

Year	Pauri		Rudraprayag		Chamoli		Tehri		Total
	Suspects	%	Suspects	%	Suspects	%	Suspects	%	
2008	285	18.86	355	23.65	368	22.77	300	23.06	**1308**
2009	433	28.66	381	25.38	429	26.55	365	28.06	**1608**
2010	379	25.08	372	24.78	468	28.96	346	26.59	**1565**
2011	414	27.4	393	26.18	351	21.72	290	22.29	**1448**
Total	**1511**		**1501**		**1616**		**1301**		**5929**

Table 2 Total sputum positive pulmonary TB cases

Year	Total Suspects	Total[+ve]	Total[-ve]
2008	1308	184	1124
2009	1608	204	1404
2010	1565	187	1378
2011	1448	197	1251
Total	5929	772	5157

Chi-square: 3.447, The P value is 0.3276.
Chi-squared for trend = 0.2318 (1 degree of freedom). The P value is 0.6302.
There is no significant linear trend of Tuberculosis cases in the population.

Table 3.Sputum positivity in different years

Year	Total PTB suspects	Total sputum positive cases	Sputum positivity (%)
2008	1308	184	14.07
2009	1608	204	12.69
2010	1565	187	11.95
2011	1448	197	13.60
Total	**5929**	**772**	**13.02**

Sputum positivity in pulmonary TB suspect varies from11.91% to 14.06%

Table 4 Sputum positivity in different district

District	Total suspect	Total sputum positive cases	Sputum positivity rate	Number of suspect required for each sputum positive cases
Pauri	1511	189	12.5	7.99
Rudraprayag	1501	200	13.32	7.5
Chamoli	1616	196	12.12	8.24
Tehri	1301	187	14.37	6.95
Total	**5929**	**772**		

Table 5.Proportion of sputum positive pulmonary tuberculosis in different quarter

Year	1st Q	2nd Q	3rd Q	4th Q	Total
2008	49	56	44	35	**184**
2009	49	75	43	37	**204**
2010	47	50	40	50	**187**
2011	42	67	55	33	**197**
Total	**187**	**248**	**182**	**155**	**772**

Table 6. Age and sex wise distribution of sputum positive Pulmonary TB cases

Year	0 - 19		20 - 39		40 - 59		>60		Total	
	M	F	M	F	M	F	M	F	M	F
2008	5	6	51	26	47	11	34	4	**137**	**47**
2009	5	10	61	26	48	8	37	9	**151**	**53**
2010	9	8	58	21	49	10	26	6	**142**	**45**
2011	12	8	61	23	49	10	31	3	**153**	**44**
Total	**31**	**32**	**231**	**96**	**193**	**39**	**128**	**22**	**583**	**189**

Chi-square: 42.997, The P value is < 0.0001. The age and sex are significantly associated.

Table 7. Proportion of sputum positive pulmonary TB cases in Tertiary care hospital of Garhwal in different yrs. (2008 – 2011)

Year	Total OPD patients	Total Pulmonary suspect	Total sputum positive PTB	Proportion of sputum positive pulmonary Tuberculosis in base hospital
2008	30428	1308 (4.29%)	184	6 cases /1000
2009	40037	1608 (4.02%)	204	4 cases /1000
2010	44372	1565 (3.52%)	187	4 cases /1000
2011	42402	1448 (3.41%)	197	4 cases /1000
Total	**157239**	**5929 (3.77%)**	**772**	

Table 8. Sputum positive pulmonary Tuberculosis cases and suspects (2008 -2011)

Year	Total sputum positive PTB	Number of suspect for each sputum positive cases
2008	184	9.16
2009	204	10.15
2010	187	9.30
2011	197	9.80
Total	**772**	**13.02**

A total number of 5929 patient which is equivalent to 3.75% of new adult OPD patient between 2008 to 2011 were referred for sputum microscopy in DOTS centre, 762 pts were sputum positive giving sputum positivity rate of 12.89% The proportion of sputum positive pulmonary tuberculosis in tertiary care hospital was six cases per thousand adult OPD patient in 2008, in next three years it was 4 cases per thousand adult OPD per year. The sputum positivity in pulmonary suspect varies from 11.91% (2010) to 14.06% (2008). If we compare district wise, sputum positivity was maximum in patient coming from Tehri district(14.37%) and least in Pauri (12.50%). The maximum number of sputum positive pulmonary Tuberculosis was found in 2nd Quarter in comparison to other quarter. The proportion of sputum positive pulmonary Tuberculosis and sputum positivity was highest in 20 to 40 years age group. The number of sputum positive Pulmonary Tuberculosis was three times more common in male compares to female. The number of pulmonary tuberculosis suspect required for detection of each sputum positive Pulmonary Tuberculosis patients was 7.10 in 2008, 8.18 in 2009, 8.36 in 2010 and 7.36 in 2012. If we compare the number of suspect required for detection of one sputum positive pulmonary Tuberculosis case in patients coming from different district were 7.99 for pauri district and 7.50, 8.24, 6.95 for Rudraprayag, Chamoli and Tehri respectively.

DISCUSSION: The proportion of Sputum positive Pulmonary Tuberculosis in patients coming to chest and medical OPD was varies from 4-6 cases per 1000 OPD patients which was similar to

prevalence of Sputum positive pulmonary tuberculosis in India. [8] There is no precise information on case detection rate from Garhwal region of Uttarakhand. Our finding suggests that sputum positive pulmonary tuberculosis was more in male than female patients except in patient between 0-19 years where ratio between male and female were almost equal this finding was similar to previous studies. [9] The proportion of sputum positive PTB were almost equal between male and female patients in 0-19 age group this was a different finding in compare to other study. The reason for this is not known, but one study interprets that women have inability to produce good and quality sputum.[10] Nevertheless this indicates that expanding TB case detection activities to maternal and child health clinics, antenatal and family planning clinics may lead to detection of more females suspects. In this study highest burden of sputum positive pulmonary Tuberculosis and maximum sputum positivity rate was found in productive age group which is similar to earlier studies.[11] In our study number of TB suspect from elderly group were as high as in productive age group but sputum positivity rate were very low and this may be due to inadequate, poor quality sputum production and number of other causes of sputum production like chronic bronchitis etc.[12] The number of TB suspect examined for each sputum positive case in patients coming from Garhwal region were high in 2009 and 2010 but again in 2011 less number of suspect were examined for detection of each sputum positive cases and this may suggest underperformances of the national Programme . There was an average of 7 to 8 suspect required for each sputum positive cases. The number of suspect require for detection of one sputum positive PTB was also influenced by change in Pulmonary Tuberculosis suspect definition, change in case definition and number of sputum positive Pulmonary tuberculosis .There was also an observation that number of suspect require for detection of one sputum positive case was maximum in patients coming from Chamoli district in compare to other district like Rudraprayag, Tehri and Pauri.

CONCLUSION: The highest burden of sputum positive pulmonary Tuberculosis and maximum sputum positivity rate was found in productive age group. The proportion of sputum positive PTB was more in male than female patients. All age group except in patient between 0-19 year's ratio between male and female were almost equal. The number of suspect required for detection of one sputum positive PTB was also influenced by change in PTB suspect definition and number of sputum positive PTB. In this study number of suspect required for detection of one sputum positive pulmonary tuberculosis was maximum for Chamoli district in compare to other districts of Garhwal .

REFERENCES:

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 1999; 282 : 677-86.
2. Murray CJL, Lopez AD, editors. The global burden of disease. Cambridge: The Harvard School of Public Health; 1996 p. 660. 3. World Health Organization. Tuberculosis Programme Review - India. 1992. Geneva: WHO; 1992.
3. TB India 2005. RNTCP Status Report.Central TB Division, Directorate of General of Health Services, Ministry of Health and family Welfare, New Delhi. http:// www.tbcindia.org.
4. Tuberculosis in India (special report series No. 34). A Sample Survey 1955-58; Indian Council of Medical Research, New Delhi.

5. Kant L. On estimation of burden of tuberculosis in India (Editorial).Indian J Tuberc2000; 47 : 127-8.
6. Tuberculosis in India (special report series No.34). A sample Survey 1955-58; Indian Council of Medical Research, New Delhi
7. 8.ChadhaVK.Epidemiological situation of tuberculosis in India. Journal of the Indian Medical Association2003; 101: 144–147.
8. 9.World Health Organization: Global Tuberculosis Control: Sur-veillance, Planning, financing. WHO report. Geneva2007
9. 10.13Murthy KRJ, Yazdani A, Lakshmi KA: Use of oral salbutamol in improving quality sputum microscopy in the DOTS strategy. Int J Tuberc Lung Dis2000, 4(12):1191-1192
10. 11.Godo y P, Dominguez A, Alcaide J, Camps N, Jansa J, Minguell SM et al. Patients belief regarding tuberculosis & its outcome. European Journa l of public health.2001; 14(10): 71-75.
11. 12.Nagami P, Yoshikawa TT. Tuberculosis in the geriatric patient. J Am
12. Geriatr Soc1983; 31:356–63.

GIANT EXTRAPLACENTAL CHORANGIOMA: A CASE REPORT AND REVIEW.

Dr. Adil S. A. K, Dr. Shilpa K, Dr Bharthi M, Dr Anubha A.

1. Assistant Professor, Department of Pathology, Mysore Medical College and Research Institute, Mysore.
2. Associate Professor, Department of Pathology, Mysore Medical College and Research Institute, Mysore.
3. Professor, Department of Pathology, Mysore Medical College and Research Institute, Mysore.
4. Post Graduate Student, Department of Pathology, Mysore Medical College and Research Institute, Mysore.

CORRESPONDING AUTHOR
Dr. S. A. K. Adil, Assistant Professor,
#259, 5th Main, Bannimantap, 'C' Layout,
Mysore –570015.
E-mail: saka291@yahoo.co.in,
Ph: 91 9019691259.

ABSTRACT: Chorangioma or placental hemangioma is a primary benign neoplasm of the placenta. It consists of vascular mass arising from chorionic tissue. Chorangiomas provide useful insights into pathophysiology and pathogenesis of fetal and maternal disorders .We herewith, present a case of giant extraplacental chorangioma associated with maternal preeclampsia and low birth weight baby.
KEYWORDS: Placenta, pathophysiology, chorangioma.

INTRODUCTION: Chorangioma is a non-trophoblastic tumor of the placenta, characterised by excessive vascular proliferation within chorionic villi. It is also referred to as a hamartoma or a hyperplastic capillary lesion. Though a benign lesion, large chorangiomas are usually associated with maternal or foetal complications. Small intraplacental chorangiomas are common but large extraplacental chorangiomas are quite rare. Only, few similar cases are reported in literature.

CASE HISTORY: A 26 year old female,$G_2P_1l_1$ at 36 weeks of gestation was admitted for pre-eclampsia and emergency cesarean section was done. A live female baby of 2 kg was extracted. Postpartum course for both baby and mother was uneventful.

PATHOLOGICAL FINDINGS: Gross examination, showed a lobulated firm mass attached to the placenta by a short pedicle, measuring 13x8x4 cms. (Fig 1) The pedicle was cut and the whole soft tissue mass weighed 300 gms .Placenta, membranes and umbilical cord were unremarkable. Cut section from the mass showed grey-white to grey-tan areas. Microscopic examination revealed a lesion composed of capillary-type blood vessels within a cellular stroma, showing occasional mitosis (Fig 2, Fig 3,Fig 4). Few cavernous spaces were also interspersed. The histological features were consistent with chorangioma.

DISCUSSION: The incidence of large chorangiomas range from 1:3500 to 1:9000.[1]The occurrence increases in placentas at high altitudes.[2] They are often found in primipara and twin pregnancies. Risk factors associated with the occurrence of chorangiomas include women over 30 years, diabetes and hypertension. The recurrence risk is not yet known. Examination of the placenta is essential, as the presence of chorangioma helps in elucidation of specific etiologies of

abnormal pregnancies. The histological diagnosis of a chorangioma is not a problem. Variants include capillary, cavernous, endotheliomatous, fibrosing and fibromatous **chorangiomas**.

In this study, the lesion was surrounded by a layer of trophoblast and composed of small size capillary vessels lined by single layer of flattened endothelial cells. There was no evidence of atypia. Few cellular areas in the lesion showed 3 mitosis per 10 high-power fields .If the mitotic count is more than 7 per 10 high-power fields and associated with cytologic atypia and necrosis it is termed as atypical chorangioma.[3]Chorangiocarcinoma, though a misnomer, is considered if there is trophoblast proliferation.[4]

As the lesion involved the stem villi and was expansile, it was distinguished from chorangiosis and chorangiomatosis . In **chorangiosis,** terminal villi are involved and a minimum of 10 villi are required, each with 10 or more vascular channels[5] and in chorangiomatosis lesion permeates the normal villi instead of an expansile lesion.[6] The diagnosis of chorangiomas is problematic, if it undergoes infarction or shows degenerative changes such as hyalinization, myxoid stromal changes or calcification.

Chorangioma is a potentially hazardous vascular lesion. It is clinically significant if the size is greater than 5 cms, which is then referred to as a giant chorangioma.[7] Extensive literature review, reveals a distinct relationship between chorangiomas and feto-maternal complications, which include, fetal cardiomegaly, fetal anemia, thrombocytopenia, polyhydramnios, non-immune hydrops, fetal heart failure, fetal growth restriction, preterm delivery.[1,2,6.7,8,9,]

Few authors have reported its association with maternal preeclampsia, which was apparent in this case.[1, 2] Fetal growth restriction is a cause of low birth weight, its relation to chorangioma has been documented in earlier studies.[1, 2, 7, 9.]Chorangioma, acts as a physiological dead space, resulting in chronic hypoxia leading on to fetal growth restriction.[7] This explains, the low birth weight in this infant. Though, in our study it was a giant chorangioma many of the complications were not observed. It is speculated, that placental tissue compensates for the foetal demands, thus decreasing the incidence of complications. Earlier studies have described the pathophysiology and pathogenesis of various complications associated with chorangioma.[1, 2,7] Pathophysiologically, fetal heart failure can arise from increased blood flow through the low resistance vascular channels, fetal anemia and thrombocytopenia may occur due to sequestration of blood in the vascular mass[7] and hydrops fetalis may result from microangiopathic haemolytic anaemia.[8, 9.]Other complications include, Polyhydramnios which may be related to fetal heart failure[7] and Ante partum haemorrhage, resulting from rupture of vascular pedicle.[10]Therefore, chorangiomas cannot be overlooked as they have a significant impact and can result in serious problems for the developing fetus and the mother. Literature search reveals that MRI, Doppler and ultrasound imaging helps in prenatal diagnosis of chorangiomas[11] and the different modalities of treatment are intrauterine transfusions through cordocentesis, amniodrainage for polyhydramniosis, endoscopic devascularization, alcoholic ablation and interstitial laser coagulation.[12]

CONCLUSION: Examination of placenta is crucial in complicated pregnancies for identifying chorangiomas. The pathophysiology and pathogenesis of various feto-maternal complications can be accounted by this biologically indolent entity.

Antenatal diagnosis and treatment of placental chorangioma can reduce fetal morbidity and mortality.

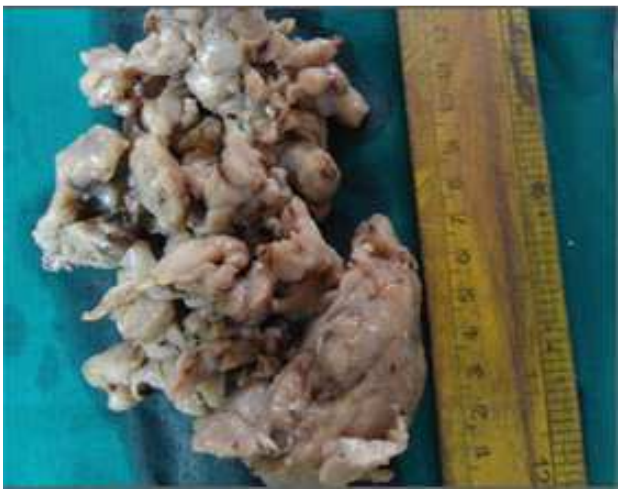


Fig 1-Gross, large lobular, soft tissue mass.

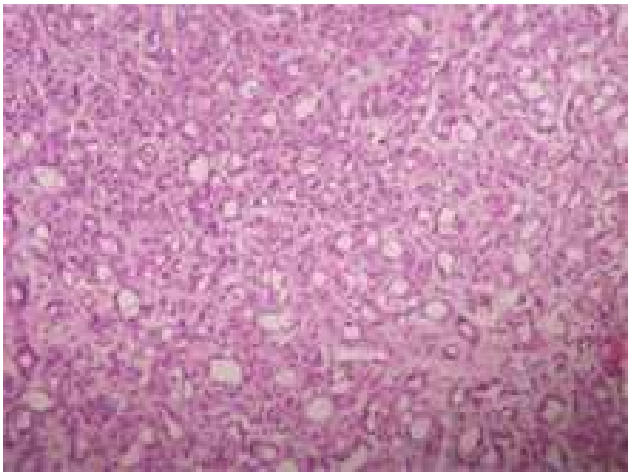


Fig 2-Showing predominantly capillary size vessels within the stroma (H& E, X 200)

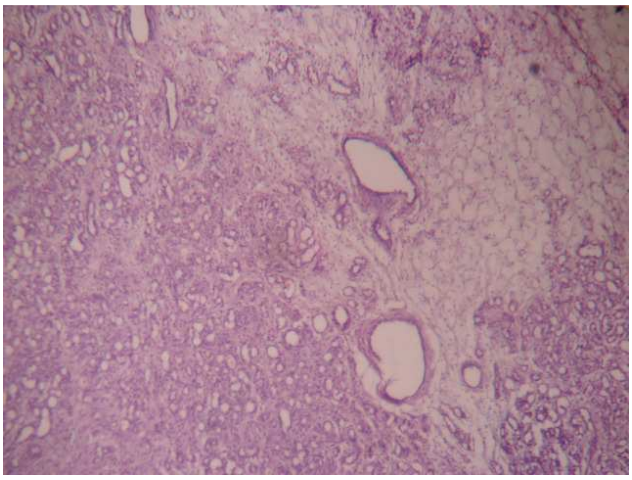


Fig -3 Microphotograph showing varying size vessels within the stroma (H& E, X 100)

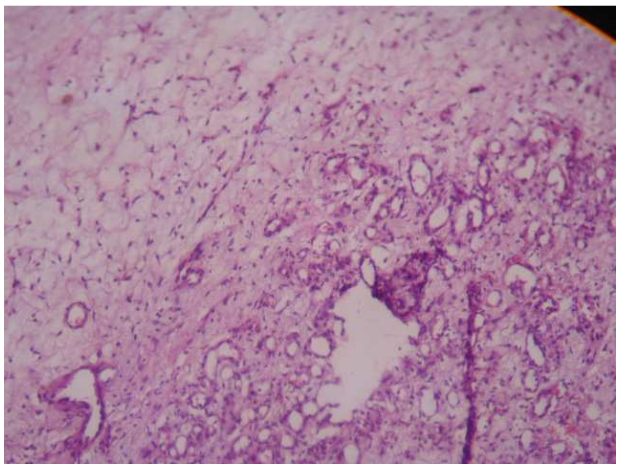


Fig-4 AnotherView showing blood vessels lined by flat endothelial cells (H& E, X 200)

REFERENCES:

1. Fox H, Sebire N.J.Non-trophoblastic tumors of the placenta. In: Fox H.Pathology of the placenta.2nd ed.Philadelphia: Saunders Elsevier; 2007. 401-430.
2. Kay H, Nelson DM, Wang Y. The Placenta: From Development to Disease.UK;Wiley- Blackwell;2011:283-284
3. Mesia AF,Mo P,Ylagan LR.Atypical cellular chorangioma.Arch Pathol Lab Med 1999;123(6):536-538
4. Baergan.R.N.Manual of Pathology of the Human placenta. 2ndedition,New York; Springer, 2011.p420-42
5. Altshuler G. Chorangiosis: an important placental sign of neonatal morbidity and mortality. Arch Pathol Lab Med. 1984;108:71-74
6. Ogino S., Redline R. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and chorangiosis. Hum Pathol. 2000;31(8).945-954
7. Stryjak KG, Lesiak MR, Grzegorz H, Reborowicz H.Nontrophoblastic placental tumors. Archives of Perinatal Medicine 2011; 17(2):113-117.
8. Sabhikhi AK,Chaudhury MC, Singh D,Raja LN.Chorangioma of the Placenta with Hydrops Fetalis .J Ind Ped .1996;33:520
9. Zanardini C., Papageorghiou A. Giant placental chorioangioma: natural history and pregnancy outcome. Ultrasound Obstet. Gynecol.2010; 35: 332-336.
10. Tan SA, Yeo SH.Placental Chorangioma: A case report and review. Singapore Med J.1992; 33:83-85.
11. Shafgat G, Iqbal F, Rizvi F.Chorangioma of the Placenta with hydrops Foetalis . JPMA 2009;6:23
12. Lez C,Fures R,Hrgovic Z,Belina S,Fajdic J,Munstedt K.Chorangioma placentae.Rare Tumors. 2010; 2(4): doi: 10.4081/rt.2010.e67

A STUDY ON NASAL CARRIAGE OF MRSA AND ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN HEALTHY INDIVIDUALS AND HOSPITALISED PATIENTS.

Dr. M. Bharathi, Dr. I . Jyothi Padmaja.

1. Assistant Professor, Department of Microbiology, Andhra Medical College, Vishakapatnam.
2. Professor and HOD, Department of Microbiology, Andhra Medical College, Vishakapatnam.

CORRESPONDING AUTHOR
Dr. M. Bharathi, Assistant professor
Dept. of Microbiology,
Andhra Medical College,
Visakhapatnam, Andhra Pradesh, India.
E-mail: bharathikanthi65@gmail.com,
Ph: 9440615206.

ABSTRACT: BACKGROUND: Staphylococcus aureus is an important pathogen in hospital associated infections as well as in community acquired infections. About 20-40% of healthy persons carry Staphylococci in the nose. Most dreadly strains of S.aureus are Methicillin resistant S.aureus (MRSA) strains. Till recent times these MRSA strains were restricted to hospitals only as Health care associated MRSA (HA-MRSA). But now these strains have also emerged in community, called as Community associated MRSA (CA-MRSA).
AIM: 1. To study the nasal carriage rate of, Staphylococcus aureus in community and in hospital setting in adult males and it's Antimicrobial susceptibility pattern.
2. To compare the nasal carriage rate of HA-MRSA with that of CA-MRSA and their Antimicrobial susceptibility patterns. **MATERIAL AND METHODS:** Nasal swabs were collected from anterior nares of control group (100 healthy adult males with no risk factors for acquisition of HA-MRSA) and study group (100 patients) from June 2011- August 2011. Samples were inoculated on Mannitol Salt Agar and incubated at 37^0c for 24-48hrs. Yellow colonies were considered as Staphylococcus aureus and after confirmation, they were subjected to antimicrobial susceptibility test by Modified Kirby-Bauer disc diffusion test. **RESULTS:** Nasal carriage rate of Staphylococcus aureus and MRSA was 14% & 4% in healthy individuals and 27% & 11% in patients, with significant p value. Community associated S.aureus and CA-MRSA were more susceptible to antimicrobials than Hospital associated S.aureus and HA-MRSA. All hospital strains and 85.78% community strains were resistant to penicillin. All strains were more susceptible to Amikacin and ciprofloxacin and least susceptible to co-trimoxazole. **CONCLUSIONS**: Nasal carriage rate of MRSA was higher in hospitalized patients than in general population. Resistance pattern was more in hospital strains.
KEY WORDS: Nasal carriage, CA-MRSA, HA-MRSA, Antimicrobial susceptibility pattern.

INTRODUCTION: Staphylococcus aureus is an important pathogen in hospital associated infections as well as in community acquired infections. About 20-40% of healthy persons carry Staphylococci in the nose. Most dreadly strains of S.aureus are Methicillin resistant S.aureus (MRSA) strains. Till recent times these MRSA strains were restricted to hospitals only as Health care associated MRSA (HA-MRSA). But now these strains have also emerged in community, called as Community associated MRSA (CA-MRSA). The known risk factors for CA-MRSA are

antibiotic use in the past 3 months, hospitalization during past 12months, diagnosis of skin and soft tissue infections (SSTIs) at admission [1], chronic illness, IV drug use, and close contact with health care personnel[2].

Although several studies were conducted on the nasal carriage of S.aureus in school going children or in students and most of the Indian studies were on the prevalence of CA-MRSA but not on HA-MRSA. Hence we made an attempt to study nasal carriage rate of community associated and health care associated S.aureus and MRSA in adult males.

AIM:

1. To study the nasal carriage rate of Staphylococcus aureus, in community and in hospital setting in adult males and its Antimicrobial susceptibility pattern.
2. To compare the nasal carriage rate of HA-MRSA with that of CA-MRSA and their Antimicrobial susceptibility patterns.

MATERIAL AND METHODS: Nasal swabs were collected from anterior nares from both control group and study group from June 2011- August 2011 after taking informed written consent. Institutional ethics committee approval was taken. Statistical analysis was done using Chi-Square method.

CONTROL GROUP: Comprised 100 healthy individuals in the age group of 20-40 yrs, who were not on antibiotics in the past 3 months , not hospitalized in the past 1yr and not having respiratory infections or skin and soft tissue infections at the time of study.

STUDY GROUP: Comprised post operative patients who were admitted to hospital for more than 1wk duration, without any ENT problem and were negative for nasal carriage of S.aureus at the time of admission.

A single sterile moistened swab was inserted into anterior nares and was rotated gently to collect nasal flora.

PROCESSING OF SAMPLE: Samples were inoculated on Mannitol salt agar (MSA), incubated at 37^0c for 24-48 hrs. Processing was done by using standard methods [3]. Yellow colored colonies were considered as Staphylococcus and S.aureus was identified by positive, tube coagulase test and Voges Proskauer test and then subjected to antimicrobial susceptibility test by Modified Kirby-Bauer disc diffusion test using the following antibiotic discs – oxacillin (1µg), Penicillin (10u), Cotrimoxazole (Trimethoprim-Sulfamethoxazole: 1.25/23.75µg), Vancomycin (30µg), Ciprofloxacin (5µg), Gentamycin (30µg), Doxycyclin (30µg), Roxythromycin (30µg), Amoxyclav (Amoxycillin + Clavulinic acid: 20/10µg) and Cefotaxime (30µg) discs on Mueller Hinton agar. Next day susceptibility was determined by measuring diameter of zone of inhibition according to CLSI guide lines. Quality control was ensured by comparing the results with that of Staphylococcus aureus ATCC 25923 strain.

RESULTS: 14 persons (14%) in control group and 27 patients (27%) in study group were positive for nasal carriage of S.aureus. 4 of the 14 strains of S.aureus from control group (28.57%) and 11 of the 27 strains from patients were MRSA (40.74%) as shown in Table 1 and the values were statistically significant.

All strains isolated from patients showed resistance to penicillin. 100% of community associated S.aureus (CA-SA) isolates and 96.29% of health care associated S.aureus (HA-SA) isolates were susceptible to Vancomycin. Least susceptibility was found to Cotrimoxazole. Antimicrobial susceptibility pattern of S.aureus and MRSA from both groups was shown in Table 2 &3.

DISCUSSION: Many bacteria are transmitted from one person to another through hands. A person with S.aureus carriage in the anterior nares may rub his nose, pick up Staphylococci on the hands and spread the bacteria to other parts of the body or to other person, where infection results. The normal resident microbial flora is harmless, but if introduced into foreign locations, they may produce disease [4]. Nasal carriage of S.aureus acts as endogenous reservoir for clinical infections in the colonized persons, but also act as a source of cross colonization for others [5].

In the present study nasal carriage of S.aureus was 14% in control group and among them 28.57% were MRSA. In the test group it was 27% and among them 40.74% were MRSA. MRSA nasal carriage was 4% and 11% in control group & test group respectively. In an unpublished study (2002) from the same author nasal carriage rate of S.aureus was 11.5% in burns patients at the time of admission and all strains were methicillin sensitive. It showed increase in the nasal carriage rate and development of resistance to methicillin over the years. Most of the Indian studies were on the prevalence of CA-MRSA but not on HA-MRSA. To the best of our Knowledge, our study was first of this kind in our area.

Nasal carriage of S.aureus in healthy adults was 14% in our study, 22.5% in the study of Goud R et al [6] and 46.1% in Dev Jyothi Majumdar et al study [7] and 6.3% in Pathak's study [5]. Rajendra Goud N et al [8] found it was 48.43% in the age group of 13-16 yrs. The above studies were from India. Nasal carriage was 29% in 2wks-21 yrs age group in a study by Nakamura [9], 29% in students by Bischoff et al [10], 31.5% in 20-64yrs age group in AG Mainous III et al study [11], 40.8% in 18-27 yrs age group in Karina A Prates study [12] and 18% in healthy volunteers by Nicola Best [13]. The above findings suggested that there was a wide variation (14-52.3%) in the nasal carriage of S.aureus among different age groups and in different geographical regions.

MRSA carriage rate in healthy adults also had wide range – 26.6% (Dev Jyothi Majumdar), 9.9% (Goud R), 0.84% (AG Mainous III) , 0.3%(Munckhof) [14], 2.4% (Karina A Prates), 0.2% (Nicola Best) and 4% (Present study) suggested higher rates in India than in other countries. CA-MRSA nasal carriage rate in the present study is lower than that from other areas in India.

Nasal carriage rate of HA-SA was 27% and HA-MRSA was 11% in the present study. No Indian studies were available in this regard. HA-MRSA carriage rate was 7.3% in Al Hidron et al study [1] and 0.7% in Munckhof study [14] again shows the higher rate in India than in other countries.

In the present study 100% strains of CASA & CA-MRSA, as in some studies [2,8,12], 96.29% strains of HASA and 90.9% strains of HA-MRSA were susceptible to vancomycin, where as it was 56% in one study [15]. In an unpublished study (2002) from the same institute all MRSA strains were vancomycin sensitive, suggested development of vancomycin resistance in the sensitive strains, stresses the importance of adhering to hospital antibiotic policy. Indiscriminate use of antibiotics leads to development of resistance by selective pressure. Extensive use of penicillin for any infection especially in rural India results in the development

of almost total resistance to penicillin. Similarly indiscriminate use of vancomycin may result in the development of vancomycin resistance in future. Hence the need of the hour is to take containment measures by strict implementation of antibiotic policy.

Least susceptibility was seen to co-trimoxazole, as in some studies [5,15]. Susceptibility of CA-S.aureus to amikacin, roxithromycin, ciprofloxacin, cefotaxime, doxycycline and amoxyclav was 78.57%, 57.14%, 64.28%, 78.57%, 64.28% and 57.14% respectively. Where as for HA-S.aureus it was 62.96%, 44.44%, 44.44%, 62.96%, 55.55% and 48.14% to the above drugs.

The antimicrobial susceptibility pattern of CA-MRSA varied widely among different studies. In the present study CA-MRSA strains were more resistant than CA-SA strains. HA-MRSA strains showed still more resistance. The susceptibility rate of HA-MRSA to amikacin, roxithromycin, ciprofloxacin, cefotaxime, doxycycline and amoxyclav was 36.36%, 36.36%, 27.27%, 54.54%, 45.45% and 36.36% respectively. From the above findings it was clearly evident that hospital strains were more resistant to antimicrobials than community strains. Unless we take measurements, microbes will develop 100% resistance to these antibiotics also as in case of penicillin.

CONCLUSIONS: 1. Nasal carriage rate of S.aureus and MRSA was higher in hospitalized patients than in healthy individuals.

2. Health care associated S.aureus and MRSA strains showed more resistance to antimicrobials than CA-SA and CA-MRSA.

Table 1 showing S.aureus and MRSA isolates from anterior nares of healthy individuals and patients

Group	Total No. of cases	S.aureus*	%	MRSA**	%
Control	100	14	14%	4	4%
Test	100	27	27%	11	11%

*p value significant (< 0.05)

** p value significant (< 0.05)

Table 2 showing antimicrobial sensitivity pattern of S.aureus isolated from both groups in percentage

Group	P	V	OX	CO	Ak	Ro	Cf	CTX	Do	AMC
Control	14.21	100	71.42	21.42	78.57	57.14	64.28	78.57	64.28	57.14
Study	0	96.29	59.25	18.51	62.96	44.44	44.44	62.96	55.55	48.14

Table 3 showing antimicrobial sensitivity pattern of CA-MRSA and HA-MRSA in percentage

Group	V	CO	AK	RO	Cf	CTX	DO	AMC
CA-MRSA	100	25	75	75	50	75	75	75
HA-MRSA	90.9	18.18	36.36	36.36	27.27	54.54	45.45	36.36

AKNOWLEDGEMENTS: We are thankful to **Dr. Krishnaveni,** Professor, Dept. of Community Medicine for her guidance in statistical analysis.

REFERENCES:

1. Alicia I. Hidron, Ekaterina V Kourbatova, Sue Halvosa, Bianca J. Terrell, Linda K. Mc Dougal, Fred C. Tenover, Henry M. Blumberg and Mark D. King " Risk factors for Colonization with Methicillin -Resistant Staphylococcus aureus (MRSA) in patients admitted to an Urban hospital: Emergence of Community- Associated MRSA Nasal carriage" Clin Infect Dis, 2005; 41 (2): 156-166.
2. Mandelia C, Shenoy S "Community- Associated - Methicillin Resistant Staphylococcus aureus in Skin and Soft tissue Infections" Journal of Clinical and diagnostic Research, 2010 August; (4):2673-7.
3. Mackie &McCartney Practical Medical Microbiology.14thed, 2008: Publisher Churchill Livingstone. Chapter 11 :pp 247 &253.
4. Jawetz,Melnick & Adelbag's Medical Microbiology, 25th Edition, publisher Mc Graw Hill company, 2010; Chapter 9 &10 : pp 147 &160.
5. Ashish Pathak A, Yogyata Marothi, Rama V Iyer, Binita Singh, Megha Sharma, Bo Eriksson, Ragini Macaden, Cecilia S Lundborg " Nasal carriage and Antimicrobial Susceptibility of Staphylococcus aureus in healthy preschool children in Ujjain, India" BMC Pediatrics 2010, 10: 100 doi: 10. 1186/1471-2431-10-100
6. Goud R, Gupta S, Neoqi U, Agarwal D. Naidu K, Chalannavar R, Subhaschandra "Community Prevalence of Methicillin and Vancomycin resistant Staphylococcus aureus in and around Bangalore, South India" Rev. Soc. Bras. Med. Trop. 2011, May-June; 44 (3): 309-12.
7. Dev Jyothi Majumdar, Ankur Barua, Barnali Paul. "Nasal carriage of methicillin resistant staphylococci in healthy population of east Sikkim." Indian Journal of Community Medicine 2009; 34(4): pp 364-365.
8. Rajendra Goud N, Deepali Agarval, Prasanth H Nadagoudar and Gaddad S M " Antibiotic sensitivity pattern of Community- Associated Methicillin resistant Staphylococcus aureus (CA-MRSA) in High schools, Bangalore city, Karnataka, South India" International Medical Journal of Student's Research, October 2011; 1(1): 27-35.
9. Mari M Nakamura, Kasey L. Rohling, Michael Shashaty, Hongzhou LU, Yi-Wei Tang, and Kathryn M. Edwards "Prevalence of Methicillin- resistant Staphylococcus aureus Nasal carriage in the Community Paediatric Population" Pediatr Infect Dis J, 2002; 21 (10):917-21
10. Bischoff WE, Wallis ML, Tucker KB, Reboussin BA, Sheretz RJ "Staphylococcus aureus nasal carriage in a student community: Prevalence, clonal relationships and risk factors" Infect Control Hosp Epidemiol 2004 June; 25 (6): 485-91.
11. Arch G Mainous III, William J.Hueston, Charles J Everett and Vanessa A. Diaz "Nasal carriage of Staphylococcus aureus and Methicillin- resistant Staphylococcus aureus in the United States, 2001-2002" Annals of Family Medicine March 1, 2006; 4 (2): 132-7.
12. Karina Aparecida Prates, Ana Maria Torres, Lourdes Botelho Garcia, Sueli Fumia Yamada Ogatta, Celso Luiz Cardoso, Maria Cristina Bronharo Tognim "Nasal carriage of Methicillin- resistant Staphylococcus aureus in University Students" Brazilian Journal of Infectious Diseases, 2010; 14 (3) http:// dx. Doi. Org/10..1590/S1413-86702010000 300021
13. Nicola Best, John D Fraser, Paul B Rainey, Sally A Roberts, Mark G Thomas, Stephen R Ritchie "Nasal carriage of Staphylococcus aureus in healthy

Aucklanders" Journal of The NewZealand Medical Association, 2011; vol 124, No 1332

14. Munckhof WJ, Nimmo GR, Schooneveldt JM, Schlebusch S, Stephens AJ, Williams G, Huygens F, Giffard P "Nasal carriage of Staphylococcus aureus including Community- Associated Methicillin- resistant strains in Queensland adults" Clin Microbiol Infect 2009; 15 (3): 296.
15. D H Tambekar, D V Dhanorkar, S R Gulhane, and M N Dudhane " Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant Staphylococcus aureus from Healthcare and community Associated sources" African J Infect. Dis 1 (1): 52-56.

INTESTINAL PARASITIC INFECTIONS AMONG PATIENTS ATTENDING A TERITIARY CARE HOSPITAL IN SOUTH INDIA

Dr. Champa H. Mrs. Sreeshma P.

1. Assistant Professor, Department of Microbiology, K.V.G. Medical College and Hospital, Sullia, Dakshina Kannada.
2. Assistant Professor, Department of Microbiology, K.V.G. Medical College and Hospital, Sullia, Dakshina Kannada.

CORRESPONDING AUTHOR

Mrs. Sreeshma. P,
Assistant Professor, Department of Microbiology,
K. V. G. Medical College and Hospital,
Kurunjibag, Sullia. Dakshina Kannada,
E-mail: sremolu@yahoo.co.in,
Ph: 09844190161.

ABSTRACT: BACKGROUND: Intestinal parasitic infections are among the most common infections in the world and are responsible for considerable morbidity and mortality. "There is increased prevalence of these infections in rural areas of developing countries." **AIM**: The present study was done to determine the intestinal parasitic infection among patients attending to the hospital, who are residents of Sullia taluk only. **MATERIALS AND METHODS**: Faecal samples from 500 randomly selected patients were collected and screened using conventional saline and iodine wet mount and examined by direct microscopy. Samples were further concentrated by formal-ether sedimentation technique. **RESULTS**: In the present study the occurrence of intestinal parasitic infection was found to be 14.60%. The common parasite detected was *A. lumbricoides* (47.94%) followed by hookworm (30.13%). However *E. histolytica* and *G. lamblia* were only 1.36%, 5.74% respectively. Multiple infection was detected only in 0.80% of infected cases. **CONCLUSION**: Soil transmitted helminthic infections are more common than protozoal infections. Hence there is a need for health programmes to be held regularly that will involve health education regarding personal hygiene, hand washing, importance of sanitary procedures and periodic deworming.

KEY WORDS: Intestinal parasites, Occurrence, Ascaris.

INTRODUCTION: Human intestinal parasitic infections have a worldwide distribution, with the greatest incidence and intensity occurring in developing countries[1]. The high prevalence in rural area of developing countries is probably due to poverty, poor sanitation, inadequate personal hygiene and lack of clean water supply. Helminths and protozoa are the two groups of pathogens causing intestinal illness with both direct and indirect method of transmission among people[2]. Helminthic infections are the main cause of loss of appetite, iron deficiency anemia, gastrointestinal symptoms, perianal pruritis, gastrointestinal or biliary obstruction[3]. They also cause malnutrition in children[4] which leads to impairments in physical, intellectual and cognitive functions[4,5,6].

In India overall prevalence rate of intestinal parasitic infection ranges from 12.5% to 66% with varying prevalence rate for individual parasite. About 50% of the urban population and 68% of the rural population in India is affected[7]. Intestinal parasitic infections are found in

every age group and in both sex[8]. The purpose of this study was to determine the intestinal parasitic infection in the patients who are residents of Sullia attending to the hospital.

MATERIALS AND METHODS: The present study was carried out in the Department of Microbiology, in K.V.G. Medical College & Hospital, Sullia. Five hundred patients of both sexes aged between 1 to 50 years with or without symptoms suggestive of parasitic infections were included in the study. Inpatient and outpatients residing only in Sullia taluk were included in the study group. The study was conducted between November 2010 and September 2011. Informed oral consent was obtained from the patients. Questionnaire was administrated on each subject so as to collect sociodemographic data including age, sex, type of toilet used, family size, source of drinking water and method of water treatment. Each patient was provided with a wide mouthed clean, dry, screw- capped container for collection of stool sample. The stool samples were examined within one hour of collection. Macroscopic examination was done to look for consistency, presence or absence of blood and mucus, adult intestinal worms and proglottids of *Taenia* species. The microscopic examination was done by normal saline and iodine mount directly from the stool. The samples found negative by microscopy were further concentrated by formal- ether sedimentation technique[9]. The mounts were screened under the microscope for the presence of helminthic ova, larva, trophozoites and cysts of protozoa. The modified Ziehl- Neelsen stain was used for the identification of coccidian parasites[10].

RESULTS: The overall occurrence of intestinal parasitic infections was 14.60% (73/500). Out of which 54 cases were detected by direct microscopy, 19 more cases were found positive after formal-ether concentration technique. Single infection was detected in 13.80% and dual infection was detected only in 0.80% of infected cases. *Ascaris lumbricoides* (47.94%) was the most common intestinal parasite, followed by hookworm (30.13%), *Trichuris trichiura* (9.58%), *Giardia lamblia* (5.47%), *Enterobius vermicularis* (4.10%), while *Entamoeba histolytica* and *Strongyloides stercoralis* (1.36%) has the same frequency [Table 1]. *A. lumbricoides* was found in single infection as well as in association with other helminthes in mixed infection [Table 2]. No coccidian parasites were found.

Infection rate was highest (32.87%) in the age group 11-15 years, followed by 6-10 years (15.06%). The distribution of intestinal parasitic infections in various age groups is shown in Table 3. Among the 73 positive cases, 59 were outpatients and remaining 14 were inpatients. Out of 500 patients, 318 were males and 182 were females. Infected case distribution among males and females was 15.40% and 13.18% respectively [Table 4]. The sociodemographic data of the study group is given in Table 5. Among the positive cases, 66 (90.41%) used well water of which 59 (80.82%) boiled water before drinking. 61 positive cases did not use footwear regularly, 46 of them also gave history of defecation in open fields.

DISCUSSION: Intestinal parasitic infections are endemic worldwide and remain a major public health concern in many tropical and subtropical countries. The prevalence rates of intestinal parasitic infections and type of parasite exhibit wide variation from country to country, between geographical areas, communities and even seasonal variations also occurs[11]. Study on the occurrence of infection by various intestinal parasites is important to formulate appropriate intervention measures.

The present study showed 14.60% overall occurrence of infection, which is nearly comparable to the study of Baragundi *et al* (12%)[8]. However, our results are considerably lower

than that of earlier studies[1,4,12]. In our study, *A. lumbricoides* (47.94%) was found to be the dominant intestinal pathogen, followed by hookworm (30.13%) and *T. trichiura* (9.58%). The possible reason for this, is the infective stage of *A. lumbricoides*, the embryonated egg have enormous capacity for withstanding the environmental extremes. Furthermore, Ascaris eggs are coated with a mucopolysaccharide that renders them adhesive to a wide variety of environmental surfaces[13]. They readily adhere to raw fruits and vegetables when fertilised with contaminated night soil or washed with contaminated water. They have also been found adherent to eating and cooking utensils and under fingernails. The results showed occurrence rate of helminthic infection are higher than those reported in other studies[14].

In contrast to helminthic infection, the occurrence of protozoan infection in this study was significantly low (6.84%), but higher in other studies[12,15]. This may be due to people having access to clean drinking water, well water being the main source of water. Water is prime source of spread of infection along with contamination of food in protozoal infection[16]. Larvae of S. *stercoralis* (1.36%) were present in patients negative for HIV testing. *Taenia* and H. nana infections were not seen in our study, probably due to restricted consumption of pork and beef and *H. nana* infection significantly occurs by the presence of rodents and beetle in house. Contrary to our study some studies have shown high prevalence of *H. nana*[8]. All other parasites showed low occurrence when compared to other studies. This could be due to variation in geographical distribution of different parasite[11,17].

We found that in our study dual infection was 0.80%, this coincides with the findings of most of the previous studies[7]. This is a clear indication of large number of various species of parasites in the locality. It was noted that occurrence of intestinal parasitic infection was highest among the age group 11-15 years (32.87%), followed by 6-10 years (15.06%). This finding is in agreement with previous studies[12]. 26.67% of the children were malnourished. Even though, gender is not a significant risk factor for intestinal parasitic infection[18], in this study males were more infected than females. This finding is endorsed by earlier studies[19]. *A. lumbricoides* was the most common helminthic parasite in both male (50.12%) and female (41.66%), followed by hookworm (26.53% in male; 37.5% in female). This may be due to the contamination of soil by human faeces, use of raw sewage for agricultural purposes; use of waste water irrigated vegetables and contaminated imported vegetables[20]. 83.56% of the study population do not use footwear, and still 63.01% defecate in open fields which increase the susceptibility to helminthic infections. Mostly children are affected as they come in contact with contaminated water, food, faeces and other source of infection through play and other unhygienic behaviour[20]. No immunocomprised patients found in our study which may be the reason we found no coccidian parasites.

CONCLUSION: Human parasitic infection is a global problem of enormous proportion, responsible for mild but chronic morbidity. This region of study has high rainfall, high humidity, dense shade, poor sanitation and human host, which helps to complete the life cycle of the helminths. Hence there is a need to improve sanitation facilities. Regular health education should be conducted regarding personal hygiene, hand washing using soap, wearing shoes, periodic deworming and food hygiene. These multiple interventions will result in lowering infection rate but would require further investigation to verify the effect of these interventions.

ACKNOWLEDGMENT: We are deeply thankful to Dr. K.V. Chidananda, Director, K.V.G. Medical College & Hospital, Sullia, for being supportive towards all research activities.

Table 1. Distribution of intestinal parasites (single infection).

Parasites	Total number of cases (percentage)
Ascaris lumbricoides	35 (47.94%)
Hookworm	22 (30.13%)
Trichuris trichiura	7 (9.58%)
Giardia lamblia	4 (5.47%)
Enterobius vermicularis	3 (4.10%)
Entamoeba histolytica	1 (1.36%)
Strongyloides stercoralis	1 (1.36%)

Table 2. Occurrence of dual infection.

Parasites	Total number of cases (percentage)
A.lumbricoides + Hookworm	2 (2.73%)
A.lumbricoides + T.trichiura	1 (1.36%)
Hookworm + S.stercoralis	1 (1.36%)

Table 3. Distribution of parasites in different age groups.

Parasites	Age in years									Total
	<5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	>40	
A.lumbricoides	3	4	19	5	1	0	1	1	1	**35**
Hookworm	2	4	4	2	2	0	0	1	7	**22**
T. trichiura	1	2	1	1	0	0	1	0	1	**7**
E. vermicularis	2	1	0	0	0	0	0	0	0	**3**
G. lamblia	0	0	2	1	1	0	0	0	0	**4**
E .histolytica	0	0	0	0	0	1	0	0	0	**1**
S. stercoralis	0	0	0	0	0	0	1	0	0	**1**
Total	**8**	**11**	**26**	**9**	**4**	**1**	**3**	**2**	**9**	**73**

Table 4. Sex wise distribution of parasitic infection.

Parasites	Males	Females
A. lumbricoides	25 (51.02%)	10 (41.66 %)
Hookworm	13 (26.53%)	9 (37.5 %)
T. trichiura	5 (10.20%)	2 (8.33 %)
G. lamblia	3 (6.12%)	1 (4.16 %)
E. vermicularis	2 (4.08%)	1 (4.16 %)
E. histolytica	1 (2.04%)	0
S. stercoralis	1 (2.04%)	0
Total	**49 (15.40%)**	**24 (13.18%)**

Table 5. Sociodemographic data of the study group

Parameter	Positive cases- 73 (%)	Negative cases- 427 (%)
Source of drinking water		
Well water	66 (90.41%)	346 (81.03%)
River water	6 (8.22%)	-
Bore water	1 (1.37%)	81 (18.97%)
Method of water treatment		
Boiling	59 (80.82%)	358 (83.84%)
Chlorination	7 (9.59%)	43 (10.07%)
No treatment	7 (9.59%)	26 (6.09%)
Usage of toilet	27 (36.99%)	324 (75.88%)
Open defecation	46 (63.01%)	103 (24.12%)
Usage of footwear	12 (16.44%)	333 (77.99%)
No footwear used	61 (83.56%)	94 (22.01%)
Deworming done	10 (13.70%)	64 (14.99%)
Deworming not done	63 (86.30%)	363 (85.01%)

REFERENCES:

1. Naish S, McCarthy J, William GM. Prevalence, intensity and risk factors of soil transmitted helminth infections in South Indian fishing village. Acta Tropica 2004; 91:177-87.

2. Cleaveland S, Laurenson MK, Taylor LH. Diseases of human and their domestic mammals: pathogen characteristic host range and the risk of emergence. Phil Trans R Soc Land B 2001; 356:991-9.
3. Samuel L, Stanley Jr. Amebiasis and infection with free living amebas. In: Longo DL, Fauci AS, Kasper DL, editors. Harrisons's principles of internal medicine. 18th ed. USA: McGraw Hill Medical 2012; 1683-8.
4. Srihari N, Kumudini TS, Mariraj J, Krishna S. The prevalence of intestinal parasitic infections in a teritiary care hospital- a retrospective study. J Pharm Biomed Sci 2011; 12(08):e1-4.
5. Jardim- Botelho A, Raff S, Rodrigues, et al. Hook worm, A. lumbricoides infection and polyparasitism associated with poor cognitive performance in brazilian school children. Trop Med Int Health 2008; 13(8):994-4.
6. Bethony J, Brooker S, Albonico M,et al. Soil transmitted helminth infections: Ascariasis, trichuriasis, hookworm. Lancet 2006; 367(9521):152-3.
7. Ragunathan L, Kalivaradhan SK, Ramadas S, Nagaraj M, Ramesh K. Helminthic infection in school children in Puducherry, South India. J Microbiol Immunol Infect 2010; 43(3):228-32.
8. Baragundi MC, Sonth SB, Solahannwar S, Patil CS. The prevalence of parasitic infections in patients attending teritiary care hospital. Nat J Bas Med Sci 2011; 2(1):31-4.
9. Chatterjee KD, Parasitology: 12th ed. India: Chatterjee medical publisher, Culcutta 1995:211.
10. Ortolani EL. Standardisation of the modified Ziehl –neelsen's technique to stain oocysts of Cryptosporidium species. Braz J Vet Parasitol 2000; 9(1):29-31.
11. Tappe KH, Mohammadzadeh H, Khashaveh S, Rezapour B, Barazesh A. Prevalence of intestinal parasitic infection among primary school attending students in Barandooz-chay region of urmia, Weat Azerbaijan province, Iran in 2008. Afr J Microbiol Res 2011; 5(7):788-91.
12. Mbuh JV, Ntonifor HN, Ojong JT. The incidence, intensity and host morbidity of human parasitic protozoan infections in gastrointestinal disorder outpatients in Bura subdivision Cameroon. J infect Dev Ctries 2010; 4(1):38-43.
13. Cromptein DWT. Biology of Ascaris lumbricoides. In: Cromptein DWT, Neshem MC, Pawlowski ZN editors. Ascaris and its prevention and control. London: Taylor& Francis 1989; 9-44.
14. Choubsia SL, Choubsia L. Intestinal helminthic infections in tribal population of southern Rajasthan, India. J Parasit Dis 2006; 30(2):163-7.
15. Bisht D, Verma AK, Bharadwaj HD. Intestinal parasitic infestation among children in a semi- urban Indian population. Trop Parasitol 2011; 104-07.
16. Ravdin JI, Stauffer WM. Entamoeba histolytica (Amebiasis). In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of infectious diseases. 6th ed. Pennsylvania: Elsevier Churchill Livingstone 2005; 3097-107.
17. WHO Guidelines for evaluation of soil transmitted helminthiasis and schistosomiasis at community level. Schistosomiasis and intestinal parasite unit, WHO, Geneva WHO/CTD/SIP/98.1, 1998.
18. Wani SA, Ahamad F, Zargar SA, Fomda BA, Ahmad Z, Ahmad P. Helminthic infestastion in children of Kupwara district: A prospective study. Indian J Med Microbiol 2007; 25(4):398-400.

19. Wani SA, Ahamad F, Zargar SA, Amin A, Ahmad Z, Ahmad P. Intestinal helminthiasis in children of Gurez valleyof Jammu& Kashmir state,India. J Glob Infect Dis 2010;2(2):91-4.
20. Brooker S, Bundy DAP. Soil transmitted helminths (Geohelminths). In: Cook GC, Zumla AI, editors. Manson's Tropical diseases. 22nd ed. China: Saunders Elsevier 2009; 1515-44.

PREVALENCE OF URINARY TRACT INFECTION IN PREGNANT WOMEN

Dr. Nawaz Umar, Dr. Siddesh Basavaraj. Sirwar

1. Assistant Professor, Department of Microbiology, Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga Karnataka.
2. Professor, Department of Microbiology, Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga Karnataka.

CORRESPONDING AUTHOR
Dr. Nawaz Umar,
C/O Mohd Umar "Ayesha Mansion",
Near Baogi Hospital, Aiwan Shahi colony,
Gulbarga, Karnataka 585102,
E-mail: drnawazumar@gmail.com,
Ph: 0091 9945270149.

ABSTRACT: Urinary tract infection is one of the most frequently seen medical complications in pregnancy. UTI in pregnancy is an important concern, as it possesses risk of complications such as acute and chronic pyelonephritis, toxaemia, anaemia, hypertension, intrauterine growth retardation and increased perinatal mortality. The detection of bacteriuria allows an approach to be made for the prevention of chronic urinary disease in the community and to avoid complications in pregnancy at an early stage. **OBJECTIVES:** (1) To study the prevalence of bacterial pathogens causing urinary tract infection among pregnant women; (2) To evaluate the sensitivity of the screening test with culture. **MATERIALS AND METHODS**: A total of 500 samples were investigated from pregnant women aged between 18 to 35 years, with varying gravida and from all three trimesters were included in a period of one year i.e., from January to December 2011. The samples were collected aseptically from women attending the antenatal clinic at the Khaja Banda Nawaz Hospital, Gulbarga. Urine was collected in the sterile urine container. Both macroscopic and microscopic examination was done. Screening tests like Griess Nitrite test and TTC tests were done. Culture was done by standard loop technique.
RESULTS: The prevalence rate of UTI in pregnancy was 10.40% i.e., with significant bacteriuria (1,00,000 or more bacterial count/ ml of urine) by Kass concept. The incidence of bacteriuria increased along with age and rising parity. Incidence was similar during all three trimesters. Gram's staining, TTC and Griess nitrite gave 88.46%, 73.07% and 57.69% respectively correlate with culture positive bacteriurics. **CONCLUSIONS**: UTI, the most commonly seen complications in pregnancy was 10.4%. Early treatment of bacteriuria not only averts the occurrence of other complications, but also diminishes the risk of premature and perinatal mortality.
KEYWORDS: Bacteriuria; Urinary tract infection; Pregnancy.

1. INTRODUCTION: Urinary tract infection in women is more prevalent and is one of the most frequently seen medical complications in pregnancy[1]. Ideal pH, temperature and constituents like glucose present in urine predispose to bacterial growth[2]. During pregnancy, urethral compression at the pelvic brim by the enlarging uterus leads to stasis of urine, incomplete emptying and residual urine, which is the single most important factor that can initiate the proliferation of microorganisms[3]. Patients with UTI present with classical symptomatology of frequency, urgency, urinary tenesmus, and fever or it may be completely without any symptoms (asymptomatic bacteriuria)[4].

The detection of bacteriuria allows an approach to be made for the prevention of chronic urinary disease in the community and to avoid complications in pregnancy at an early stage.

2. MATERIALS AND METHODS: The objective of the study was to know the prevalence of UTI in pregnant women.

A total of 500 urine samples were collected from pregnant women attending KBN Hospital and subjects comprised of varying ages from 18 to 35 years during January to December 2011. Subjects from varying gravida and from all three trimesters were included.

1. **COLLECTION OF URINE**: Subjects were instructed to collect midstream urine sample with all aseptic precautions in sterile urine container.
2. **EXAMINATION OF URINE**:

a) Macroscopic examination: Urine was observed by naked eye for altered colour, presence of turbidity, deposit and the findings were recorded.

b) Microscopic examination:

I. A drop of uncentrifuged urine was allowed to air dry. The smear was Gram stained and examined under oil immersion. Twenty fields were examined. Presence of at least one organism per field was considered as significant ($\geq 10^5$/ml organisms)[13].

II. For the microscopic examination of urine 10 ml of urine was transferred into conical centrifuge tube. The urine was then centrifuged at 1500 rpm for 5 minutes. The supernatant was decanted, to have sediment suspended in 1 ml volume of urine. This preparation was examined in low and high power. Several fields were searched to identify and count the number of cells, crystals and casts. More than 10 pus cells per high power field were considered as significant[5].

3. **SCREENING TESTS**:

i) Griess Nitrite Test: 1 ml of urine was taken in a clean sterile test-tube. One ml of 10% potassium nitrate was added and incubated at 37ºC for two hours. To this one ml of Griess nitrite reagent was added i.e., solution-A: Sulphanilic acid and solution-B: α-naphthylamine. Development of pink to dark red colour indicates positive test[14].

ii) Triphenyl tetrazolium chloride test (TTC): 2 ml of urine was taken in a sterile test tube and 0.5 ml of working triphenyl tetrazolium chloride reagent was added. This mixture was incubated at 37ºC for four hours. Formation of red precipitate indicated a positive test[15].

4. **PLATING OF URINE SAMPLES**:

STANDARD LOOP TECHNIQUE: Plating of urine was done by standard loop technique on blood agar, nutrient agar and McConkey's agar.

METHOD:

I. I Flame a calibrated wire inoculating loop and allow it to cool without touching any surface.
II. Mix the urine thoroughly and remove the top of the container.
III. Insert the loop vertically into the urine to allow urine to adhere to the loop.
IV. Spread the loopful of urine without flaming or re-entering urine, loop is drawn across the entire plate, crossing the first inoculum, which is the centre streak numerous times to produce isolated colonies.
V. Incubate plates for at least 24 hours at 37ºC. Colonies are counted on each plate. The number of CFU's is multiplied by 1000 (if a 0.001ml loop is used) to determine the number of microorganisms per millilitre in the original specimen[6].

3. RESULTS: Out of the 500 urine samples collected from pregnant women attending the antenatal clinics, every sample was subjected to routine urine analysis including microscopic examination. Among these 500 samples, the present study revealed that 52 pregnant women had significant bacteriuria (1,00,000 or more bacteria/ ml of urine) giving a prevalence of 10.4%.

Age wise distribution in pregnant women with bacteriuria is shown in table-1. It can be concluded that the incidence of bacteriuria increased with age among the pregnant women. Similarly a positive association is observed between parity of women and bacteriuria. As majority i.e. 24.4%(11out of 45) of grand multi para had bacteriuria followed by 10.52% (8 out of 76) with 3rd gravid, 8.7% of (19 out of 217) primi and 8.64% (14 out of 162)of second gravid had bacteriuria .The incidence was almost thrice when compared to that in the primigravida. Table-2 reveals that significant bacteriuria during pregnancy had a similar incidence during all the three trimesters.

Significant proteinuria was present in 75% (39) of bacteriuric cases. However, 13.46% (7)of the bacteriuric women did not show any proteinuria, 11.54%(6) bacteriuric women revealed a trace of proteinuria .

Table-3 reveals the correlation of the Gram negative isolates and Gram positive bacteria to the screening tests viz., TTC and Griess nitrite. The TTC test gave an overall better correlation as compared to the Griess nitrite test. However, the correlation with Gram positive bacteria was very poor, being 26.92%. With Gram negative bacilli, it was better, being 46.15%. The Griess nitrite test gave a even more poor correlation, being 38.46% positive with Gram negative bacilli, and 19.23% positive with Gram positive bacteria. Table-4 shows the comparison of other screening method with standard loop technique.

4. DISCUSSION AND CONCLUSION: From this study, the prevalence of UTI in pregnancy was 10.4%. Hitherto the studies conducted reveal that the prevalence of bacteriuria during pregnancy ranges from 3-12.8%. This variation is related to factors like socioeconomic status of the group of women studied[11,12].

It was observed that increasing age or greater parity is associated with a higher frequency of significant bacteriuria[7]. In the present study the incidence of bacteriuria in all the 3-trimesters was not much different, being 10.9% in the 1st trimester, and 10.12% and 10.45% in the second and third trimesters respectively.

The TTC test gave a 73.07% correlation with culture positive bacteriuria and Griess nitrite gave a 57.69% positive correlation with culture positive bacteriuria. These two tests could be supportive to culture for bacteriuria, but not conclusive on their own.

The observations made by Simmon and Williams[8] and Kincaid Smith et al[9], for the prevalence of TTC test positive was 94% and 79% respectively. Our findings of TTC test positivity of 73.07% and nitrite test of Griess gave a positivity in 30 cases of bacteriurics (57.69%).

Mere isolation of microorganisms from the urine does not always mean infection, organisms has to be 1,00,000 or more/ ml of urine. When qualitative estimation is carried out indicating growth and multiplication of the organisms in the urinary bladder[3], Gram positive cocci multiply slowly in the urine as compared to Gram negative bacilli. It has been suggested by Pead et al[10] that 10^4 organisms/ ml of uncentrifuged urine in case of Gram positive cocci should be considered consistent with urinary infection instead of 10^5 bacteria/ ml, which is appropriate for Gram negative bacilli[13].

Significant bacteriuria during pregnancy has not yet been accorded the status of a disease despite the isolation of almost similar type of bacterial flora as in the symptomatic cases. However, this study will serve as a reference for researchers interested in this field. Controlled trials and large-scale studies are required to establish pathogenic potential of the isolates. This study has revealed that screening for bacteriuria during pregnancy is an appropriate investigation and that culture was the most effective means of detecting bacteriuria, which may help in the prevention of complications associated with UTI in pregnant women.

Table-1: Age Distribution in Pregnant Women with Bacteriuria

Age (years)	Total No. of samples screened	Cases with Bacteriuria	
		No.	Percentage
18-20	89	7	7.86
21-25	203	20	9.85
26-30	166	16	9.64
31-35	38	6	15.78
35 & above	4	3	75.00
Total	500	52	10.40

Table-2: Relationship of Duration of Pregnancy and Bacteriuria

Trimester	Total No. of samples screened	Cases with Bacteriuria	
		No.	Percentage
First	55	6	10.90
Second	158	16	10.12
Third	287	30	10.45
Total	500	52	10.43

Table-3: Correlation of Screening Tests with Culture Positive Bacteriurics

Bacteriuric cases on culture	TTC test positive		Griess Nitrite Test	
	No.	Percent	No.	Percent
Gram negative bacilli	24	46.15	20	38.46
Gram positive bacteria	14	26.92	10	19.23
Total	38	73.07	30	57.69

Table-4: Technique wise distribution of cases with significant bacteriuria in comparison with Standard Loop Technique

Name of Technique	No. of Cases	Percentage
Standard loop technique	52	100.00
Gram staining	46	88.46
TTC test positive	38	73.07
Griess nitrite test positive	30	57.69

ACKNOWLEDGEMENT: The authors are very much grateful to the Dean, Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga for providing all the necessary facilities.

REFERENCES

1. MaClean AB: Urinary tract infection in pregnancy. International Journal of Antimicrobial Agents, 2001; 17, 4, 279.
2. Asscher, AW. Urine as a medium of bacterial growth. Lancet-II, 1966; 1037.
3. Pritchard JA, MacDonald PC. Maternal adaptation to pregnancy. Williams Obstetrics, 16th Edition, Appleton Century Croff/ New York, 1976: 211.
4. Kass EH, (1956): Quoted by Whalley, p. 1967.
5. McNeely, Michael. Urinalysis. In: Sonnerwith AC, Jarett L (eds) Gradwall's Clinical Laboratory Methods & Diagnosis, 8th Edn., Vol. I, BI Publications Ltd., New Delhi, 478: 1990.
6. Bailey & Scott's Diagnostic Microbiology, Chap-60. Infections of the Urinary Tract, 937: 2002.
7. Whalley PJ, Martin FG, Peters PC. Significance of asymptomatic bacteriuria detected during pregnancy. Journal of American Medical Association, 1965; 193: 879.
8. Simmons and Williams. A simple test for significant bacteriuria. Lancet, 1962; 1: 1377.
9. Kincaid Smith P, Bullen M. Bacteriuria in Pregnancy. 1965; 1: 395.
10. Pead C, Cump J, Maskell R. Staphylococcus as urinary pathogen. Journal of Clinical Pathology, 1977; 30: 427.
11. Turck M, Goffee BS, Petersdorf RG. Bacteriuria of pregnancy – relation to socioeconomic factors. New England Journal of Medicine, 1962; 266 (17): 857.

12. Kaitz AL, Hodder EW. Method for the detection of significant bacteriuria in large groups of patients. Journal of Clinical Pathology, 1964; 17: 498 Quoted by Leigh DA and Williams JD.
13. Mackie and McCartney. Practical Microbiology. Chapter-4, 14th Edn., Elsevier, 2007: p. 84-85.
14. Weltman O. Griess nitrite test. In: Diagnosis of Urinary Tract Infection. Journal of American Medical Association, 1956; 161: 528 quoted by Schaus R (1956).
15. Simmons and Williams. A simple test for significant bacteriuria. Lancet 1962; 1: 1377.

STUDY OF ANTIBIOTIC RESISTANCE PATTERN IN UROPATHOGENS AT A TERTIARY CARE HOSPITAL

Dr. Prasanna Gupta[1].

1. Assistant Professor, Department of Microbiology, Konaseema Institute of Medical Sciences, Amalapuram, Andhra Pradesh, India.

CORRESPONDING AUTHOR
Dr. Prasanna Gupta[1] , Dept. of Microbiology,
Konaseema Institute of Medical Sciences,
Amalapuram- 533201, Andhra Pradesh, India,
E-mail: pramin1414@yahoo.co.in,
Ph: 09630238572.

ABSTRACT: BACKGROUND: Background and objective: Empiric treatment of urinary tract infections (UTI) is determined by the antibiotic sensitivity patterns of uropathogens in a population. There is increased resistance to the first line empirical drugs used in the treatment of urinary tract infection. This study was conducted to determine patterns of resistance amongst uropathogens in Amalapuram (India), to help establish local guidelines on treatment Of UTI. **METHODOLOGY:** This is a retrospective study on 323 urine cultures from July 2011 to June 2012. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and compared. Analysis was done using simple percentage method. **RESULTS:** Out of the 323 samples subjected for culture, 152(47.06%) were positive for growth. Out of the 152 culture isolates, E. coli was the most common (46.7%) followed by Klebsiella spp (18.4%), Candida spp (8.5%), Staphylococcus aureus (7.8%), Pseudomonas spp (6.5%), Citrobacter spp(6.5%), Proteus spp (2.6%), and Acinetobacter spp(2.6%). All bacterial isolates were 100 % sensitive to Imipenem except Pseudomonas and Klebsiella which were 90 % and 92.8 % sensitive respectively. All the isolates were 100 % resistant to Ampicillin except Staphylococci which was 16.6 % sensitive. E. coli and Klebsiella were sensitive in range of 21.1% to 50 % to Ciprofloxacin, Ceftriaxone, and Cefotaxime. Staphylococci were 100 % sensitive to Vancomycin followed by 91.6 % sensitive to Linezolid, Cefotaxime and Amikacin. Acinetobacter and Proteus were having 100% sensitivity to Amikacin and Ciprofloxacin. **CONCLUSION:** The alarming rate of resistance to Ciprofloxacin, Cefotaxime, Ceftriaxone , SXT and Ampicillin for major urinary isolate E. coli and Klebsiella, precludes the use of these commonly used antibiotics for empiric treatment of UTI in India. . Urine culture for screening and diagnosis is recommended.
KEY WORDS: Urinary tract infection, antibiotic susceptibility, India

INTRODUCTION: Urinary tract infection (UTI) is one of the most frequent conditions encountered by general practitioners .[1–3]An acute uncomplicated urinary tract infection (UTI) is one of the most common bacterial infections in women.[1–3] It is estimated that as many as 60% of all women report having had a UTI atleast once in their lifetime.[4,5] Worldwide, about 150 million people [6] are diagnosed with UTI each year, costing in excess of 6 billion dollars [7]. Among both outpatients and inpatients, Escherichia coli is the most common isolate, accounting for 75% to 90% of uncomplicated UTI isolates [8,9]. Staphylococcus saprophyticus, Klebsiella spp., Proteus spp., Enterococcus spp., and Enterobacter spp. are organisms less commonly isolated from outpatients. In the majority of cases, antibiotics are given empirically before the final bacteriology results are available. Therefore, area-specific

monitoring studies to document the microorganisms causing UTI and their antimicrobial susceptibility is mandatory for helping the selection of an effective empirical treatment.[10]UTIs are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity maybe appropriate because of concerns about infection with resistant organisms. Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in area where resistance is likely to be of concern [11, 12]. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens[13-15]. The resistance pattern of community acquired UTI pathogens has not been studied extensively. [13]The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide [16]. The etiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years, both in community and nosocomial infection [17, 18]. However, there is no much information on etiology and resistance pattern of community acquired UTIs in India . This retrospective study was done to compare the frequency and drug resistance pattern in uropathogens isolated from patients with UTIs in Amalapuram, India .

MATERIALS & METHODS: This study was designated as a retrospective survey of 323 urine culture specimens from July 2011 to june 2012. As we had no control over collection of specimens, we excluded those culture isolates, which are likely to be contaminants, except those, which were isolated in 2 consecutive cultures. Cultures which yielded more than one isolate were excluded from the study group. All the culture isolates were identified in the department of Microbiology, KIMS, Amalapuram by standard laboratory techniques. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as per CLSI criteria. ATCC control strains(E.coli ATCC 25922, for Pseudomonas ATCC 27853 and for S.aureus ATCC 25923) were used as per CLSI guidelines. All the analysis was performed using simple percentage method.

RESULTS: In the present study total 323 samples were studied. Out of them 152(47.06%) were culture positive. Out of the 152 culture isolates, E. coli was the major isolate, followed by Klebsiella spp (28), Candida spp(13), Staphylococcus aureus (12), Pseudomonas spp(10), Citrobacter spp(10), Proteus spp(4) and Acinetobacter spp(4). E. coli and Klebsiella spp were sensitive in range of 21.1% to 50 % to Ciprofloxacin, Ceftriaxone, and Cefotaxime. All bacterial isolates were 100 % sensitive to Imipenem except Pseudomonasspp and Klebsiella spp, which were 90 % and 92.8 % sensitive respectively. All the isolates were 100 % resistant to Ampicillin except staphylococci which were 16.6 % sensitive to Ampicillin. Staphylococci were 100 % sensitive to Vancomycin followed by 91.6 % sensitive to Linezolid and Cefotaxime and Amikacin .Acinetobacterspp and Proteus spp were having 100% sensitivity to Amikacin and Ciprofloxacin. Pseudomonasspp, Staphylococcus aureus, Acinetobacter spp and Proteus spp were sensitive to Piperacillin- Tazobactum in a range of 75 to 83.3 %. Staphylococcus aureus, Pseudomonas spp and Citrobacter spp were also sensitive to ciprofloxacin in a range of 7 0 to 75 % . **(Table - 2)**

DISCUSSION: The most commonly isolated organism in our study was E. coli. The proportion of bacterial species isolated was similar to those described in several previous studies [19,20]. This study shows the distribution and antibiotic susceptibility pattern of microbial species isolated from patients with UTIs in KIMS, Amalapuram. These organisms cause a variety of infections including UTIs [21]. Most common age group affected in the present study was 21-30

years (31.57%) followed by 31-40 years (25.66%). Females (76.97%) were more frequently affected than males(23.03%).The age and sex distribution of the study group is shown in the table.3.

It has been extensively reported that adult women have a higher prevalence of UTI than men, principally due to anatomic and physical factors [22,23]. The UTIs are more frequent in women than in men, which corresponds to our findings because 76.97% of our patients were females. [24]

High resistance rates to the oral antibiotics in our study may be due to the uncontrolled consumption of these antibiotics in the community in the past decade in our region [25,26]. On the other hand, resistance to Amikacin, Piperacillin- Tazobactum and Meropenem are low, likely reflecting lower usage of these drugs. Our study demonstrates extremely low susceptibility to the first-line agents (Ampicillin, Ampicillin/ Sulbactum, Ciprofloxacin, Cotrimoxazole) in uropathogens in our population. However, recent studies have demonstrated therapeutic failure in more than 50% of patients infected with Cotrimoxazole resistant urinary pathogens [27,28].

The worldwide trend of empirically treating UTI may not apply for specific geographical regions such as India, where decreased susceptibility rates are documented for common urinary pathogens. In the Indian setting, routine urine cultures may be necessary, since treatment failure with empirical therapy is likely to occur. International guidelines are no longer applicable for treating UTI in India, and development of specific guidelines based on local susceptibility patterns are necessary. Development of regional surveillance programs is necessary to provide information which would then enable the development of Indian UTI guidelines.

CONCLUSION: The alarming rate of resistance to Ciprofloxacin, Cefotaxime, Ceftriaxone, SXT and Ampicillin for major urinary isolates E. coli and Klebsiella spp, precludes the use of these commonly used antibiotics for empiric treatment of UTI in India. . Urine culture for screening and diagnosis purpose is recommended.

Table 1 Organisms isolated from UTI cases (n =152)

Organisms	No.of isolates	% of isolation
Escherichia Coli	71	46.71
Klebsiella spp.	28	18.42
Candida spp.	13	8.55
Staphylococcus aureus	12	7.89
Pseudomonas aeruginosa	10	6.57
Citrobacter spp.	10	6.57
Proteus spp.	4	2.63
Acinetobacter spp.	4	2.63
Total	152	100

Table2: ABST pattern of the isolates (in percentage)

ANTIBIOTIC	E. Coli (n=71)	Klebsiellasp. (n=28)	Staph.aureus (n=12)	Pseudomonas spp. (n=10)	Citrobacter spp. (n=10)	Acinetobacter spp. (n=4)	Proteus spp. (n=4)	Candida spp. (n=13)
G	30.9	42.8	83.3	30	40	50	60	NT
A	0	0	16.6	NT	0	0	0	NT
AS	40.8	25	25	20	30	25	50	NT
AK	56.3	57.1	91.6	70	70	100	100	NT
CA	28.1	46.4	58.3	40	50	75	50	NT
CE	47.8	50	91.6	40	60	50	75	NT
CI	38	53.5	83.3	30	60	50	75	NT
CF	36.6	46.4	75	70	70	100	100	NT
SXT	21.1	39.2	58.3	60	50	25	50	NT
I	100	92.8	100	90	100	100	100	NT
LZ	NT	NT	91.6	NT	NT	NT	NT	NT
NA	49.2	28.5	0	0	40	50	50	NT
NF	50.8	25	75	NT	50	NT	NT	NT
NX	23.9	53.5	58.3	60	60	75	75	NT
P	NT	NT	0	NT	NT	NT	NT	NT
VA	NT	NT	100	NT	NT	NT	NT	NT
PT	46.4	57.1	83.3	80	80	75	75	NT

Table 3.shows age and sex distribution of the study group

Age in years	MALES (%)	FEMALES (%)	Total (%)
1-10 years	2	3	5(3.29%)
11-20 years	3	10	13(8.55%)
21-30 years	6	42	48(31.57%)
31-40 years	5	34	39(25.66%)
41-50 years	6	16	22(14.47%)
51-60 years	8	7	15(9.87%)
61 years and above	5	5	10(6.58%)
Total	35(23.03%)	117(76.97%)	152(100%)

ABBREVIATIONS : G- Gentamycin, A – Ampicillin, AS –Ampicillin- Sulbactum, AK- Amikacin, CE – Cefotaxime, CA- Ceftazidime, CI – Ceftriaxone, CF – Ciprofloxacin, SXT- Trimethoprim-Sulphamethoxazole, I – Imipenem, LZ – Linezolid, NA- Nalidixic Acid , NF – Nitrofurantoin, NX – Norfloxacin, P – Penicillin , VA – Vancomycin, PT – Piperacillin – Tazobactum, NT – Not tested, ABST - Antibiotic sensitivity testing

ACKNOWLEDGEMENTS: Funding: None. Ethical approval: Not required. I would like to express my sincere thanks to Dr. Raghuram V, Associate Professor Dept. of Community Medicine, KIMS for his contribution during preparation of this manuscript.

REFERENCES:

1. Fry J. Medicine in Three Societies. Lancaster: Medical andTechnical Publishing Co. Ltd, 1969.
2. Royal College of General Practitioners Office of Population Consensus & Survey. Department of Health Morbidity Statistics from General Practice, 1991–1992. London: HMSO, 1995.
3. Car J. Urinary tract infections in women: diagnosis and management in primary care. BMJ 2006; 332: 94–7.
4. Foxman B. Epidemiology of urinary tract infections: incidence,morbidity, and economic costs. Am J Med 2002; 113 Suppl 1A: 5–13S.
5. Foxman B, Barlow R, D'Arcy H et al. Urinary tract infection:self-reported incidence and associated costs. Ann Epidemiol 2000; 10:509–15.
6. Stamm WE, Norrby SR (2001) Urinary tract infections: disease panorama and challenges. J Infect Dis 183 Suppl 1:S1-S4.
7. Gonzalez CM, Schaeffer AJ (1999) Treatment of urinary tract infection: what`s old, what`s new, and what works. World J Urol 6:372-382.

8. Gupta K, Hooten TM, Stamm WE (2001) Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med 135:41-50.
9. Nicolle LE (2001) Epidemiology of urinary tract infection. Infect Med 18:153-162.
10. Smith RD , Coast J , 2002 . Antimicrobial resistance: a global response .Bull World Health Organ 80: 126 – 133 .
11. Schaeffer AJ: The expanding role of fluoroquinolones. Am J Med2002, 113(Suppl 1A):45S-54S.
12. Biswas D, Gupta P, Prasad R, Singh V, Arya M, Kumar A: Choice of antibiotic for empirical therapy of acute cystitis in a setting of high antimicrobial resistance. Indian J Med Sci2006,60(2):53-8.
13. Goldstein FW: Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections in France. Multicentre Study Group. Eur J ClinMicrobiolInfect Dis 2000, 19:112-117.
14. Gupta V, Yadav A, Joshi RM: Antibiotic resistance pattern in uropathogen. Indian J Med Microbiol2002, 20:96-98.
15. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U: Evaluation of extended spectrum beta lactamase in urinary isolates. IndianJ Med Res 2004, 120:553-556.
16. Kumar MS, Lakshmi V, Rajagopalan R: Related Articles, Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute.Indian J Med Microbiol2006, 24(3):208-11.
17. Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW: The changing prevalence of drug-resistant Escherichia coli clonal groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiol Infect2006, 134(2):425-31.
18. Kahan NR, Chinitz DP, Waitman DA, Dushnitzky D, Kahan E, Shapiro M:Empiric treatment of uncomplicated urinary tract infection with fluoroquinolones in older women in Israel: another lost treatment option? Ann Pharmacother2006, 40(12):2223-7.
19. Zhanel GG et al. (2005) Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents 26:380-388.
20. Andrade SS, Sader HS, Jones RN, Pereira AS, Pignatari AC, Gales AC (2006) Increased resistance to first-line agents among bacterial pathogens isolated from urinary tract infections in Latin America: time for local guidelines? MemInstOswaldo Cruz 101:741-748.
21. Meharwal SK, Taneja N, Sharma SK, Sharma M: Complicated nosocomialUTI caused by nonfermenters. Indian J Urol2002, 18:123-128.
22. Kumar MS, Lakshmi V, Rajagopalan R: Related Articles, Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute.Indian J Med Microbiol2006, 24(3):208-11.
23. Khan AU, Musharraf A: Plasmid mediated multiple antibiotic resistance in P. mirabilis isolated from the UTI patients. MedicalSci Mon 2004, 10:598-602.
24. Schaeffer AJ ,Rajan N , Cao Q , Anderson BE , Pruden DL , SensibarJ , Duncan JL , 2001 . Host pathogenesis in urinary tract infections Int J AntimicrobAgents 17: 245 – 251 .
25. Mudur G (2000) Drug resistant cholera in India attributed to antibiotic misuse. BMJ 321:1368-1369.
26. Magee JT, Pritchard EL, Fitzgerald KA, Dunstan FDJ, Howard AJ (1999) Antibiotic prescribing and antibiotic resistance in community practice: retrospective study, 1996-8. BMJ 319:1239-1240.

27. McNulty CAM et al. (2006) Clinical relevance of laboratory-reported antibiotic resistance in acute uncomplicated urinary tract infection in primary care. J AntimicrobChemother 58:1000-1008.
28. Gupta K, Stamm WE (2002) Outcomes associated with trimethoprim/sulphamethoxazole (TMP/SMX) therapy in TMP/SMX resistant community-acquired UTI. Int J Antimicrob Agents 19:554-556.

SERUM CREATININE ASSAY: ENZYMATIC VS KINETIC JAFFE'S METHOD

Vijaya Marakala, Avinash S. S, Shivashankara A. R, Malathi M, Arun Kumar

1. Assistant Professor, Department of Biochemistry, Srinivas Institute of Medical Science & Research Centre, Mukka, Surathkal, Mangalore.
2. Assistant Professor, Department of Biochemistry, Father Muller Medical College, Mangalore.
3. Associate Professor, Department of Biochemistry, Father Muller Medical College, Mangalore.
4. Professor, Department of Biochemistry, Father Muller Medical College, Mangalore.
5. Assistant Professor, Department of Biochemistry, Father Muller Medical College, Mangalore.

CORRESPONDING AUTHOR
Dr. Vijaya Marakala, Assistant Professor,
Department of Biochemistry,
Srinivas Institute of Medical Science & Research Centre,
Mukka, Surathkal, Mangalore.
E-mail: vkunder637@gmail.com,
Ph: 0091 9008165850.

ABSTRACT: BACKGROUND: An enzymatic kit method for the determination of serum creatinine was optimized for use with OLYMPUS AU 400 Auto analyzer and its performance characteristics and practicability compared with kinetic Jaffe-based method. Effects of some common interfering substances like glucose and bilirubin on the kinetic Jaffe's and the enzymatic methods were compared. Method comparison between the enzymatic creatinine method (y) and Jaffe's kinetic method (x) gave the following equation for the normal group: y=0.97*x+0.0 and a coefficient correlation R of 0.98.There was very good agreement between both the methods as intra class correlation coefficient (ICC) was between 0.81-1.In the presence of high levels of glucose or bilirubin, the coefficient correlation values were 0.99 and 0.89respectively.
KEYWORDS: Creatinine; Enzymatic assay; Kinetic Jaffe's.

INTRODUCTION: Routine clinical biochemistry laboratories use several methods for the estimation of serum and urinary concentrations of creatinine, most of which are based on the Jaffe's reaction described first by Jaffe in 1886. Over the years, the Jaffe's assay has progressed through many phases. There are major analytical problems associated with the use of the Jaffe's reaction, in particular those relating to positive and negative interference by chromogens. More than 50 chromogenic interfering substances have been documented[1].

Commonly encountered interfering substances of the Jaffe's based methods include glucose, acetoacetate, bilirubin, and cefoxitin[2]. Glucose and bilirubin both inhibit the reaction between creatinine and alkaline picrate. Glucose slowly reduces picric acid to picramate[3], while bilirubin, under alkaline conditions, is oxidized to biliverdin, causing a decrease in absorbance at 520 nm[4]. Acetoacetate and Cefoxitin, conversely, react directly with alkaline picrate and cause positive interference. Acetoacetate, in fact, reacts more rapidly with picrate than creatinine[5].

Enzymatic creatinine assay is widely accepted as one of the most accurate routine methods available at present. Several studies concluded that enzymatic method is suitable as a routine diagnostic laboratory method for the measurement of serum creatinine, particularly for diabetic ketotic patients, neonates, and patients receiving cephalosporins[6]. The enzymatic method exhibits several advantages over Jaffe's based methods-namely, improved specificity,

smaller sample volume and hence a rapid sample throughput. Glucose, acetoacetate, and Cefoxitin do not interfere with the enzymatic method, although bilirubin causes a negative interference which depends on both creatinine and bilirubin concentrations.

The enzymatic creatinine assay deals effectively with most interfering substances but has a greater cost and shorter shelf-life compared with the kinetic Jaffe's method[7].In this study an enzymatic kit method for the determination of serum creatinine was optimized for use with OLYMPUS AU 400Auto analyzer and its performance characteristics and practicability were compared with kinetic Jaffe-based method.

The aim of this study was to compare analytical performance and practicability of the enzymatic method and kinetic method for serum creatinine for routine use and to compare the effects of some common interfering substances like glucose and bilirubin on the enzymatic method and kinetic Jaffe's method.

MATERIALS AND METHODS: The present study was conducted in central diagnostic laboratory, department of Biochemistry, Father Muller Medical College Mangalore. We assessed 318 consecutive serum samples obtained for routine clinical care. Creatinine was analyzed both by kinetic Jaffe's and enzymatic method. The Jaffe's method for serum creatinine determination is based on the principle that picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample. The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which is converted to sarcosine by creatine amidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase producing hydrogen peroxide. In the presence of peroxidase the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye. All measurements were performed using an Olympus AU 400 analyzer. The 2 levels of quality control materials used in this study were supplied from Biorad. We also estimated Serum Total Bilirubin by azobilirubin method and Fasting Plasma Glucose by hexokinase method of the respective subjects and the data were divided into 3 groups. Group I comprising of 167 samples without interfering substances (Samples having Fasting Plasma Glucose < 126 mg/dl and Serum Total Bilirubin ≤ 1 mg/dl).Group II comprising of 33 samples with bilirubin (Samples having Serum Bilirubin >1mg/dl and Fasting Plasma Glucose < 126 mg/dl); Group III comprising of 118 samples with glucose (Fasting Plasma Glucose ≥126mg/dl and Serum Bilirubin ≤ 1 mg/dl).

We determined the mean difference between the two methods and analysed the agreement between them. The relationship between the two methods was also compared by regression analysis. The 2 levels of quality control materials were analysed by enzymatic and kinetic Jaffe's methods for precision.

The Statistical Package for the Social Sciences (version 11.0; SPSS) was used for statistical analyses. Data were analyzed by using independent test to assess the significance of difference between the two methods and regression analysis for comparison of methods. Linear regression model was used to establish correlation coefficients.

RESULTS: Mean differences between enzymatic to kinetic Jaffe's methods were -0.042mg/dl in group I; -0.158mg/dl in group II and -0.116mg/dl in group III. Overall mean difference between the two methods was -0.081 mg/dl. All of the above differences were statistically insignificant ($p>0.05$). Intra class correlation coefficients for the agreement between the two methods for

Group I, Group II, Group III and for all the group together were 0.995, 0.915, 0.997 and 0.995 respectively (Table 1)

Method comparison between the enzymatic creatinine method (y) and Jaffe's kinetic method (x) by linear regression analysis gave the following equation for all groups together (n=318)gave the following equation:y = 0.97x-0.04 with a coefficient correlation R of 0.99 and in the group I (n= 167): y=0.97*x + 0.00 with a coefficient correlation R of 0.98. Regression analysis in group II (n= 33) gave the following equation: y = 0.80*x + 0.11 with a coefficient correlation R of 0.89 and in group III (n= 118) gave the following equation: y = 0.98*x + (-0.08) with a coefficient correlation R of 0.98 (Figure 1).

The Quality control analysis of level 1 for precision by Enzymatic method (n=18) yielded a mean, SD,CV and imprecision of1.45, 0.086, 5.93 and 11.86 respectively. The Quality control analysis of level 1 for precision by Kinetic Jaffe's method (n=79) yielded a mean, SD, CV and imprecision of1.71, 0.082, 4.80 and 9.6 respectively. The Quality control analysis of level 2 for precision by Enzymatic method (n=18) yielded a mean, SD,CV and imprecision of 5.19, 0.365, 7.03 and 14.06 respectively. The Quality control analysis of level 2 for precision by Kinetic Jaffe's method (n=79) yielded a mean, SD, CV and imprecision of 5.36, 0.246, 4.59 and 9.18 respectively (Table 2).

DISCUSSION: The enzymatic method exhibits advantages over Jaffe's based methods namely, smaller sample volume (10µL) and free of interference from substances such as glucose, acetoacetate and bilirubin. The enzymatic technique yields results directly proportional to the kinetic Jaffe's reaction. Access to enzymatic assays can also be useful when interference from substances such as bilirubin and hemolysis is suspected[8]. On the other hand, a very few compounds may interfere with enzymatic procedures. Interference for enzymatic assays has been reported in case of intravenous fluid contamination of plasma samples from dopamine or dobutamine solutions[9].The only drug reported to interfere with currently available enzymatic assays at borderline therapeutic concentrations is calcium dobesilate, used to reduce capillary permeability in diabetic retinopathy[10].

The enzymatic creatinine methods appear to be the only assays giving reliable results when specimens take time to reach the laboratory and blood centrifugation is delayed for 24 h or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine by alkaline picrate assays due to the possible interference effect of some metabolites built up in vitro, such as pyruvate or ketones[11]. A minor disadvantage of the enzymatic method is its relatively high cost.

In our study, estimation of creatinine by enzymatic method showed no statistically significant mean difference (-0.042) with the kinetic Jaffe's method, which is used by several laboratories (including our own center) in samples without glucose and bilirubin interference. In the presence of glucose interference (glucose > 126 mg/dl), the samples showed no statistical significant mean difference (-0.116) between enzymatic and kinetic Jaffe's method. This was higher compared to mean difference between the two methods in samples without glucose and bilirubin interference. When bilirubin was present in the serum samples (bilirubin > 1 mg/dl), the mean difference between enzymatic and kinetic Jaffe's method was statistically not significant (-0.158). The mean difference between the methods was higher amongst the samples containing bilirubin compared to samples with glucose interference and the samples without glucose and bilirubin interference (Table 1).

Method comparison between the enzymatic creatinine method (x) and kinetic Jaffe's method (y) gave the following equation for the whole group of 318 individuals: y = 0.97*x-0.04 and a correlation coefficient of 0.99. The creatinine kinetic Jaffe method gave substantially higher values compared with the enzymatic method. These results are in accordance with several studies that compared an enzymatic method with the kinetic Jaffe method. These results indicate that Jaffe methods, based on an alkaline picrate reaction, overestimate true serum creatinine concentrations due primarily to non-specific protein interference [12–14].

In this study there was no statistically significance mean difference between both methods in all the groups and the difference was also not clinically significant. The Intra class correlation coefficient between the two methods in group I, Group II, Group III and all the groups together, indicates a very good agreement between Kinetic Jaffe's method and enzymatic method (Table 1). Hence in routine clinical care both the methods can be used (Table 1).

Both the methods showed significant correlation with or without the presence of interfering substances (Figure 1). All the above results indicate a very good comparability between the two methods in all the specified settings with or without the presence of interfering substances like glucose and bilirubin and also when all the groups together were analysed. Analysis of quality controls specimens suggested a comparable precision of enzymatic and Jaffe's methods of creatinine analysis (Table 2). However, the imprecision values obtained in our laboratory settings are much higher than the desirable imprecision of 3% as per the guidelines[15].The laboratory is having the mechanism of quality assurance which aims at bringing down the imprecision to the desirable level.

In case of serum creatinine, standardization is of particular importance because of its role in the assessment of renal function and for estimation of glomerular filtration rate[16,17]. Introducing the enzymatic creatinine method to routine laboratory work, instead of the alkaline picrate method, is in accordance with recent recommendations of the Laboratory Working Group of the National Kidney Disease Education Program. This group suggests that the estimated glomerular filtration rate has to be reported using accurate and specific serum creatinine measurements, based on the concept of traceability[18].

Since the sample volume required is lesser, the throughput is higher, the interfering substances are fewer for the enzymatic method and since there is good agreement and good comparability with the kinetic Jaffe's method, the enzymatic method for estimation can be preferred especially in the setting of neonates, diabetic, ketoacidosis, jaundice and hemolytic samples.

In accordance to ours, another study[19] evaluated 29 samples with Bilirubin concentrations between 0.1 and 22.7 mg/dL (1.7-388.2µmol/L) and did not find a significant difference between 2 methods of creatinine measurement (enzymatic [Ortho Vitros 950] and Jaffe's colorimetric on 2 different analyzers [Roche Hitachi 917 and Dade Di-mension RXL]).

In conclusion, enzymatic and kinetic Jaffe's methods of creatinine analysis were comparable with respect to performance in the presence and absence of interfering substances glucose and bilirubin, and imprecision. In this study we employed a small sample size and could test effects of only two interfering substances. Future studies will aim at analyzing the effects of many more interfering substances and validation of two methods by recovery studies, analysis of accuracy, sensitivity and specificity, and evaluation of the methods under allowable imprecision level. Both external and internal quality control programmes will be utilized to increase the accuracy and precision of the creatinine assay methods.

Table1. Comparison (by independent 't' test) and the agreement between two methods (ICC) of the Serum Creatinine values obtained by Enzymatic Methods and Kinetic Jaffe's method

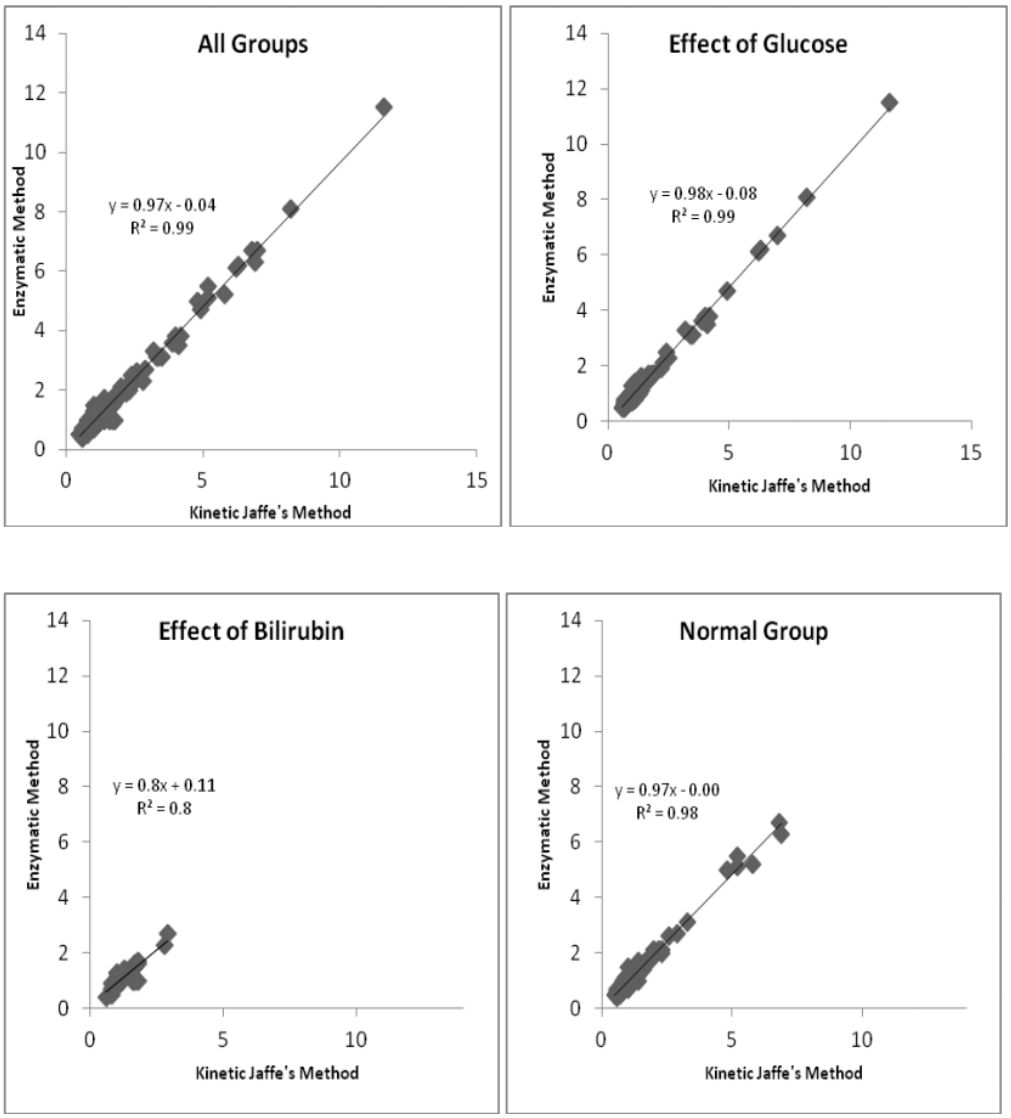
		Mean± SD (mg/dl)	Mean differences± SD (mg/dl)	'p' value	ICC
Group I (Normal)	Enzymatic (n=167)	1.18 ± 0.965	-0.042±0.129	0.565*	0.995
	Kinetic Jaffe's (n=167)	1.23 ± 0.989			
Group II (Bilirubin)	Enzymatic (n=33)	1.20± 0.452	-0.158±0.228	0.186*	0.915
	Kinetic Jaffe's (n=33)	1.35 ± 0.503			
Group III (Glucose)	Enzymatic (n=118)	1.52 ± 1.581	-0.116±0.134	0.577*	0.997
	Kinetic Jaffe's (n=118)	1.63 ± 1.610			
All Groups (Total)	Enzymatic (n=318)	1.31 ± 1.207	-0.081±0.150	0.401*	0.995
	Kinetic Jaffe's (n=318)	1.39 ± 1.239			

* p >0.05 ('p' values for mean differences between two methods by independent 't' test
ICC – Intra class correlation coefficient- used to assess the agreement between two methods.
ICC: <0.2 Poor agreement, 0.21 – 0.4 Fair agreement, 0.41 – 0.6 Moderate agreement, 0.61 – 0.8 Good agreement and 0.81 – 1 Very good agreement.

Table 2. Precision analysis of Kinetic Jaffe's and Enzymatic Methods with Quality Controls Levels 1 and 2

Level 1 Quality Control			**Level 2 Quality Control**	
	Creatinine (mg/dl) Kinetic Jaffe's (n=79)	Creatinine (mg/dl)Enzymatic (n=18)	Creatinine (mg/dl) Kinetic Jaffe's (n=79)	Creatinine (mg/dl)Enzymatic (n=18)
Mean	1.71	1.45	5.36	5.19
SD	0.082	0.086	0.246	0.365
CV(%)	4.80	5.93	4.59	7.03

Figure 1: Method comparison by linear regression between Enzymatic and Kinetic Jaffe's method.



REFERENCES:

1. Cook JGH. Factors influencing the assay of creatinine. Ann Clin Biochem 1975;12:219-32.
2. Spencer K. Analytical reviews in clinical biochemistry: the estimation of creatinine. Ann Clin Biochem 1986;23:1-25.
3. Bowers LD, Wong ET. Kinetic creatinine assays. II. A critical evaluation and review. Clin Chem 1980;26:555-61.
4. Knapp ML, Hadid 0. Investigations into negative interference by jaundiced plasma in kinetic Jaffe methods for plasma creatinine determinations. Ann Clin Biochem 1987;24:85-97.

5. Gerard SK, Khayam-Bashi H. Characterization of creatinine error in ketotic patients. Am J Clin Pathol 1985; 84:659-64.
6. R M Jacobs, J H Lumsden, J A Taylor, and E Grift Effects of interferents on the kinetic Jaffé reaction and an enzymatic colorimetric test for serum creatinine concentration determination in cats, cows, dogs and horses. Can J Vet Res. 1991 April; 55(2): 150–54.
7. H Crocker, M D S Shephard, G H White Evaluation of an enzymatic method for determining creatinine in plasma.J Clin Pathol 1988;41:576-81
8. Peake M, Whiting M. Measurement of serum creatinine -Current status and future goals. Clin Biochem Rev 2006;27:173-84
9. Karon BD, Daly TM, Scott MG. Mechanisms of dopamine and dobutamine interference in biochemical tests that use peroxide and peroxidase to generate chromophore. Clin Chem 1998;44:155-60.
10. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts.Clin Chem 1992;38:1933-53
11. Shepherd J, Warner MH, Kilpatrick ES. Stability of creatinine with delayed separation of whole blood and implications for eGFR. Ann Clin Biochem2007;44:384-87.
12. Panteghini M on behalf of the IFCC Scientific Division. Enzy-matic assays for creatinine: time for action. Clin Chem LabMed. 2008; 46:567–72.
13. Delanghe JR, Cobbaert C, Galteau MM, Harmoinen A, JansenR, Kruse R, et al. Trueness verification of actual creatinineassays in the European market demonstrates a disappointingvariability that needs substantial improvement. Clin Chem LabMed. 2008; 46:1319–25.
14. Chromy V, Rozkosna K, Sedla K P. Determination of serum creatinine by Jaffe method and how to calibrate to eliminatematrix interference problems. Clin Chem Lab Med. 2008; 46:1127–33.
15. Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Mininchela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress" Scand J Clin Lab Invest 1999;59:491-500
16. Fuentes-Arderiu X, A´lvarez-Funes V, Coca-Fa ´bregas L, Cruz-Placer M, Dı´az-Ferna ´ndez J, Herrero-Bernal P, et al. Multi-centre physiological reference values for the concentration of creatininium in plasma and diagnostic specificity of glomerular filtration rate estimated with the MDRD equation. Clin ChemLab Med 2007;45:531–4.
17. Van Biesen W, Vanholder R, Veys N, Verbeke F, Delanghe J, De Bacquer D, et al. The importance of standardization of creatinine in the implementation of guidelines and recommen-dations for CKD: implications for CKD management pro-grammes. Nephrol Dial Transplant 2006;21:77–83
18. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creat-inine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 2006; 52:5–18.
19. Trotter JF, Brimhall B, Arjal R, Phillips C. Specific labo-ratory methodologies achieve higher model for endstageliver disease (MELD) scores for patients listed for liver transplantation. Liver Transpl 2004;10:995-1000

EFFECT OF TRAMADOL ON POST OPERATIVE ANALGESIA WHEN ADDED TO LIGNOCAINE IN INTRA VENOUS REGIONAL ANESTHESIA (BIER'S BLOCK) FOR UPPER ARM ORTHOPEDIC SURGERIES.

Dr. Vivek Chakole, Dr. Ritesh Dixit, Dr. Gayatri Tadwalkar

1. Assistant Professor, Department of Anaesthesiology, R. D. Gardi Medical College, Ujjain (MP).
2. Assistant Professor, Department of Anaesthesiology, R. D. Gardi Medical College, Ujjain (MP).
3. Associate Professor, Department of Anaesthesiology, Government Medical College, Aurangabad (MS).

CORRESPONDING AUTHOR
Dr. Vivek Chakole. Assistant Professor,
Department of Anaesthesiology,
R. D. Gardi Medical College, Ujjain, MP 456006,
E-mail: drvivekchakole@rediffmail.com,
Ph: 0091 9630024978, 0091 7368261280

ABSTRACT: BACKGROUND: Intravenous regional anesthesia (IVRA) is known for its simplicity, effectiveness, safety, reliability and being economical for day care and emergency surgery circumventing the problems of full stomach. **OBJECTIVE:** Comparison of effect of lignocaine alone and lignocaine with Tramadol in surgery of upper extremity under intravenous regional anaesthesia. **MATERIAL AND METHOD:** In our randomized prospective study, total 60 adult ASA class I and II patients undergoing upper limb surgeries were given IVRA and studied for addition of Tramadol on intra operative pain and post operative analgesia. Patients received 0.5% lignocaine 40 ml in one group and we added Tramadol 100 mg in the other group. All the patients were monitored for onset of effect, quality of anesthesia, post op analgesia after deflation of tourniquet, time of first analgesic drug and number of analgesic drug required in first 24 hrs. **RESULT:** Onset of effect was same in both groups. Intraoperative conditions and post operative analgesia were significantly better in Tramadol group. Consequently, number of doses of analgesic required in first 24 hours was less in Tramadol group. **CONCLUSION:** Addition of Tramadol has improved the quality of intravenous anaesthesia.
KEYWORDS: IVRA (Intra Venous Regional Anesthesia), Tramadol.

INTRODUCTION: Intravenous regional anesthesia (IVRA), a type of regional anesthesia was introduced by German surgeon August K.G. Bier (1908); thus the name, "Bier's Block". The main advantages of this technique are its simplicity, effectiveness, safety, reliability and being economical for day care and emergency surgery circumventing the problems of full stomach.
The ideal IVRA solution should have Rapid onset of sensory and motor block with prolonged analgesia after deflation of tourniquet. To achieve this, we evaluated effect of addition of an opioid, Tramadol[08] to lignocaine in IVRA. Tramadol is a synthetic opioid analgesic with a unique dual mechanism of action. It exerts agonist properties at opiate receptors and also interferes with neurotransmitter reuptake[02]. Hence in this prospective, randomized controlled study we compared Tramadol (100mg) for its efficacy as an intra-operative, post-operative and pre-emptive analgesic when added to lignocaine 40 ml solution in IVRA for upper arm orthopedic surgeries.

MATERIALS AND METHODS: A study of IVRA with Lignocaine compared with Lignocaine and Tramadol was carried out in 60 patients undergoing upper arm orthopedic surgery.

Appropriate approval was taken from Hospital Ethics Committee. All patients selected were planned for forearm surgeries, of ASA Grade I or II, aged 15-65 years, Weighing 40-70 Kg. Patients were excluded from study when there was refusal for the procedure from patient's side, patients had hypersensitivity to local anesthetic agents, Opioids, NSAID drugs or when Patients were having past or present history of sickle cell disease, peripheral vascular disease, any neurological disease, hemolytic disease or any major systemic disease.

After detailed examination and informed consent, patients were randomly assigned in two groups of thirty patients each. Preservative free Lignocaine was used.

A. Lignocaine 0.5% 40ml. (now onwards group L)
B. Lignocaine 0.5% 40ml + Tramadol 100mg. (now onwards group LT)

A detailed history and thorough general and systemic examination and required investigations were done. Exanguination was done by using Esmarch Bandage after keeping limb elevated for 3- 5 minutes. IVRA was given using double tourniquet technique and using any of the one drug combination. First the upper tourniquet was inflated to 100 mm of Hg above the systolic blood pressure and drug was injected through angiocath on operative limb. After 10 mins, the lower cuff was inflated and the upper cuff was deflated. All patients were premedicated with inj. Midazolam 0.05 mg/kg. IV. 10 min. prior to inflation of tourniquet for anxiolysis. After completion of surgery, the tourniquet was deflated by intermittent deflation and reinflation method over a period of 2-3min. In no circumstances, tourniquet was released before 20 minutes after injection of drug. Hemodynamic monitoring was done continuously throughout procedure and stay in Post Anesthesia Care Unit. Thereafter, patients were followed every 4 hourly in ward. Any patient who had inadequate analgesia or lengthened procedure were converted into general anesthesia and excluded from study. Postoperatively for V.A.S.≥ 5, Diclofenac Sodium 3 cc. (75 mg.) IV was given as rescue analgesic. Postoperative nausea vomiting was treated with intravenous Ondansetron 4mg intravenously.

The following parameters were studied and observations noted.

1. Onset of sensory analgesia - The time taken for onset of loss of pain sensation by pin prick after injection of drug.
2. Onset of motor paralysis - Time from injection of drug to onset of motor paralysis ≤ grade II.
3. Intra operative condition –
   a) Excellent –Indicated complete loss of touch, position sense, and pain sensation with no sensitivity to pin prick or deep pressure with marked or total paralysis of muscle.
   b) Good – Loss of pain, touch and position sense but retained a sensory response to maximum pressure applied to the fingernail.
   c) Moderate- anesthesia which was complete in most areas but incomplete in small region (patchy). Mild pain or discomfort during the reduction or during the operative procedure.
   d) Poor/Failure- Failure to obtain anesthesia, moderate to marked pain when carrying out the manipulative or surgical procedure or both.
4. Total surgical time
5. Total Tourniquet Time,

6. Time to first analgesic- Time between deflation of tourniquet and consumption of first analgesic dose (V.A.S.≥5).

7) Number of analgesics required- Total number of analgesic doses required in first 24 hours after deflation of tourniquet.

The results were statistically evaluated using SPSS and EPI Info software.

RESULTS:

Group	**Group L**	**Group LT**
Age in years	37.5	37.8
Sex Male	88%	76%
Female	12%	24%
Weight in Kg.	60.26	57.76
Duration of Surgery	82.4 ± 18.35	79± 6.99
Duration of Tourniquet	97. 9 ± 18.53	94.33 ± 7.5
Onset of Analgesia(Sec)	209.17±129	62±18
Onset of motor paralysis	7.65±3.37	6.86±2.56
Analgesia after deflation of tourniquet	10± 4.6	363± 74.88
Tourniquet discomfort	06	01
Rash	00	05
PONV	00	03
Analgesics in 24 hrs	3.7±0.59	1.3±0.46

Demographically both the groups were comparable. There was no significant difference between duration of surgery and tourniquet time. Onset of sensory analgesia was significantly earlier in LT group (62±18 secs compared to 209.17±129) while onset of motor paralysis was similar in both groups. Incidence of tourniquet pain was significantly higher in group L (6) then group LT (1).

The post operative analgesia was significantly longer in group LT (10± 4.6 mins) then group L (363± 74.88mins). Accordingly the number of analgesics consumed in post operative first 24 hours was significantly less in group LT (1.3±0.46) then group L (3.7±0.59). The incidence of rash (5) and PONV (3) was higher in group LT.

DISCUSSION: IVRA introduced by Sir August Bier (1908) and hence named BIER'S BLOCK, was made popular by Sir Holmes[08]. But it has fallen out of routine use due to the limitations of poor muscle relaxation, immediate post operative pain and risk of toxicity of local anesthetic agents. To improve post operative pain relief after IVRA, many adjuncts are tried. As the first family of analgesics, Opioids are a natural choice to try for the same. In this study, we have tried to study the effect of addition of an opioid, Tramadol to local anesthetic solution of IVRA on the parameters of onset of analgesia, quality of intraoperative analgesia, muscle relaxation, analgesia after deflation of tourniquet and number of analgesics required in first 24 hours.

The study was done in 60 of ASA Grade I/ II patients planned for forearm surgeries. They were randomly assigned in two groups of thirty patients each.

A) Lignocaine 0.5% 40ml. (group L) and B). Lignocaine 0.5% 40ml + Tramadol 100mg. (group LT)

Both the groups were comparable in age, sex & weight.

This technique is less suitable for the lower limb due to the difficulty in venepuncture, especially in the affected limb, difficulty in retaining the tourniquet and higher dose of anesthetic agent required (Dawkins 1964, Mittal 1972)[03,09]. We selected all orthopedic patients undergoing surgeries in upper limb.

According to Holmes (1963), any suitable vein distal to the tourniquet can be used while Dawkins et al (1964) opines that site of injection should be as near the region of operation as possible[03]. Prithvi Raj (1972) proved by nerve conduction, radiopaque studies and radio isotope studies that distribution of contrast was similar whether cannula was inserted in to the median cubital vein at the elbow or at the dorsum of the hand[10]. We used veins on dorsum of hand and 22 G angiocath in both groups. Anesthesia appeared from fingertip upward.

The choice of lignocaine as the local anesthetic agent and its dose has been suggested by many studies done before to be 3 mg/kg or 30-40 ml of Lignocaine 0.5% solution [07,08] .In 1983, the American Food and Drug Administration (FDA) specifically contraindicated use of bupivacaine for IVRA following unfortunate incidents of cardiac toxicity followed by use of bupivacaine in IVRA.

We used 40 ml of 0.5% lignocaine solution for both the groups. We used Esmarch bandage for all patients of both groups for exsanguinations.

Tourniquet pain is nociceptive, generated by activation of peripheral nociceptors and direct axonal stimulation of nervous trunks. We used double balloon tourniquet, applied on upper 2/3 [rd] arm. The lower tourniquet was inflated after obtaining the analgesia below the upper tourniquet and then upper tourniquet deflated. The technique of using double balloon tourniquet is very effective in reducing pain due to tourniquet.

Onset of sensory analgesia was tested by loss of sensation to pin prick at least three separate areas innervated by 3 main nerves of upper extremity.

We observed mean time for onset of sensory analgesia for LT group to be 62 ± 18 seconds, While in group L it is significantly more at 209.17 ± 129 second($P<0.001$)[1].

The mean time for onset of muscle relaxation in group L was 7.65 ± 3.37 minutes while in Group LT 6.86 ± 2.56minutes. There is no significant difference in onset of muscle relaxation in between two groups as is echoed by other studies [12,11,05].

One of the major limiting factors has been the minimal or no residual post operative analgesia following IVRA [08,03,09]. Many adjuvants have been tried to counter this limitation. Kulkarni et al (1993) used Lignocaine+ Ketamine+ Gallamine[04], Sunita Goel et al (2002) Lignocaine + Tramadol or Ketorolac[06], Acalovschi et al Lignocaine + tramadol[1] and Reuben et al (1995) Lignocaine with ketorolac[01]. An opioid was considered to be a more acceptable choice because of its good analgesic efficiency. Tramadol is a synthetic opioid analgesic with a unique dual mechanism of action. It exerts agonist properties at opiate receptors and also interferes with neurotransmitter reuptake. We used Tramadol in dose of 100 mg as adjuvant to local anesthetic solution.

POST OPERATIVE ANALGESIA: No significant post operative analgesia was found after deflation of tourniquet when only local anesthetic solution was used[08,09,03]. Various findings have been noted when different adjuvant were added to IVRA solution. Acolovschi et al (2001) demonstrated that the speed of recovery of sensory block was similar (pin prick and cold sensation) but for touch speed of recovery was delayed in Tramadol Lignocaine group[1]. He also

showed that cold sensation recovered slower than the pin prick sensation. Sunita Goel et al (2002) also demonstrated that Tramadol assured significantly longer pain free interval of 16.8 ± 9.07 hours ($p<0.05$) while Ketorolac had 12.9± 8.48 hours[06]. We found analgesia after deflation of tourniquet significantly longer in Tramadol group (363 min.) than Lignocaine group (10 min.) ($P<0.00001$). Thus, it can be stated that addition of Tramadol to Lignocaine solution for IVRA definitely prolongs duration of analgesia after deflation of tourniquet. Another way to evaluate the post operative analgesia is total number of analgesics required in first 24 hours after the procedure. In our study the consumption of analgesic was significantly high in group L (3.7 ± 0.59) than group LT (1.3 ± 0.46) in first 24 hours. ($P<0.005$). For group L the requirement of first and second analgesics was earlier (10 min.) than group LT (363 min.), showing tramadol has postoperative analgesic effect and also has a property of preemptive analgesia. Reuben et al (1995), Sunita Goel et al have also observed similar results.

ADVERSE EFFECTS: We did not find any adverse effect like central nervous system toxicity, restlessness, bradycardia, muscle fasciculation, pain or burning sensation at the site of injection. But we found, in 5 patients out of thirty in LT group, rash below level of tourniquet after injection of drug on the operating forearm. The rash disappeared within ten minutes after its appearance and required no active treatment. The incidence of rash over forearm in LT group was significantly high than L group which had no incidence. Similarly, 3 out of 30 suffered from PONV in LT group, but none required any treatment. The difference was not significant from L group where no one suffered from PONV. ($P<0.05$).

CONCLUSION: Addition of Tramadol 100 mg. with 0.5% Lignocaine for IVRA substantially augments the speed of sensory block, improves intraoperative analgesia and prolongs the duration of analgesia after deflation of tourniquet without any significant side effects.

REFERENCES:

1. Acalovschi I, Cristea T. Intravenous regional anesthesia with meperidine.
2. Anesth Analg 1995; 81: 540–3.
3. C Rhoda Lee, Donna McTavish and Eugene M. Sorkin. Tramadol a preliminary review of its pharmacodynamics and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states. Drugs 46 (2): 313-340, 1993.
4. Dawkins OS, Russel ES, Adams AK, Hooper RL, Okicikosa DA, Flemming. Intravenous regional anesthesia. Canadian anaesthetist society Jn; 1964; 11; 243.
5. DK Kulkarni, R Gopinath, Y Rajender, RS Manimala. Intravenous regional anesthesia with different drug combinations: A comparative study. Ind. J. Anaesth. (41), 114, 1993.
6. Elhakim M. Sadek RA. Addition of atracurium to lidocainefor intravenous regional anesthesia. Acta Anaesthesiol Scand 1994; 38:54-4.
7. Goel Sunita N, Daftary Swati R, Pantavaidya Shanti H. Intravenous regional anesthesia using tramadol hydrochloride and ketorolac: a double blind control study. Indian J Anaesth. 2002: 46 (5): 369-72.
8. Harris WH, Slater EM, Bell HM. Regional anesthesia by the intravenous route. JAMA 1965; 194: 1273–6.
9. Holmes CM. Intravenous regional analgesia a useful method of producing analgesia of the limbs. Lancet 1963; 1: 245 7.

10. Mittal NK, Kakkar SN. Intravenous regional anesthesia a clinical study.
    a. Indian Journal of Anesthesia 1972; May; 185-92.
11. Raj PP, Garcia CE, Burleson JW, Jenkins MT. The site of action of intravenous regional anesthesia. Anesth Analg 1972; 51: 776 86.
12. Reuben SS, Steinberg RB, Kreitzer JM, Duprat KM. Intravenous regional anesthesia using lidocaine and ketorolac. Anesth Analg 1995; 81: 110 –3.
13. Sukhani R, Garcia CJ, Munhall RJ, Winnie AP, Rodvold KA. Lidocaine disposition following intravenous regional anesthesia with different tourniquet deflation technique. Anesth Analg. 1989 May; 68(5): 633-7.

PREVALENCE OF HELICOBACTER PYLORI

Vandana Berry, Vidya Sagar

1. Professor and Head, Department of Microbiology, Christian Medical College and Hospital, Ludhiana, Punjab.
2. Tutor, Department of Microbiology, Christian Medical College and Hospital, Ludhiana, Punjab.

CORRESPONDING AUTHOR
Dr. Vandana Berry, Prof & Head,
Department of Microbiology, Christian Medical College,
Ludhiana, Punjab-141008,
E-mail: vandana_berry@yahoo.com,
Ph: 91 094637 52244.

ABSTRACT: This study was done at Christian Medical College and Hospital, Ludhiana for the period of 5 years from January 2006 to December 2010 in the Department of Microbiology. A total of 306 endoscopic gastric biopsy specimens were collected from the suspected cases of the peptic ulcer and were subjected to rapid urease test for Helicobacter pylori. This test has high sensitivity and cent percent specificity. The prevalence was determined to be 09.48% and male: female ratio was determined to be 68.97:31.03.
KEYWORDS: Helicobacter pylori Endoscopy, Rapid Urease Test

INTRODUCTION: Helicobacter pylori is a common cause of peptic ulcer. It is gram negative, microaerophilic and motile organism with multiple polar flagella. It associated with 90% of duodenal ulcer and 80% of gastric ulcer[1].H.pylori is also associated with gastric adenocarcinoma. H. pylori infection is mainly acquired through the faeco oral route. Water has been shown to be a source of H. pylori infection by Kleinet al[2].A study from Bombay reported that H. pylori is present in the dental plaques of 98% patients with dyspepsia[3].

This study was conducted in the Department of Microbiology, Christian Medical College and Hospital, Ludhiana, Punjab over a period of 5 years from January 2006 to December 2010.During this period a total of 306 endoscopic gastric specimens, collected from patients suspected to have gastric ulcer, were subjected to rapid urease test. The medium used for this test was urea broth which contains urea, phenol red indicator and distilled water. The broth was sterilized by steaming at 100°C for 20 minutes. The medium was distributed in the quantity of 1.5ml to 2.0 ml in aliquots. Each biopsy specimen was inoculated and incubated at 37 ^{0} C for one and a half hour .The change in the colour of the broth from pale yellow to deep pink was taken as positive test.

PREVALENCE OF HELICOBACTER PYLORI

Year	No. of Samples	Positive Samples	Percentage	No. of Males	No. of Females
2006	113	14	12.39	10	04
2007	86	06	6.98	04	02
2008	76	01	1.31	00	01
2009	20	00	00	00	00
2010	71	08	11.26	06	02
Total	306	29	09.48	20(68.97%)	09(31.03%)

Rapid urease test is one of the invasive tests based on the principle that abundant urease enzyme is being produced by H. Pylori that hydrolyses urea to ammonia ; phenol red indicator shows the alkalinity produced thereby and the medium turns deep pink in colour.An earlier study[4] done in the same institution reported a prevalence of 10.93%.The present prevalence is 9.48% which is encouraging and signifies the awareness of public regarding various measures initiated to curtail this troublesome disease.

REFERENCES:

1. Aroori S.Gasteroenterology Today 2001:5:131-33.
2. Klein PD,GrahamDY,GailourA,etel.Water sources as risk factor for Helicobacter Pylori infection in Peruvian children.The Lancet 1991;337:1503-06.
3. DesaiHG,GillHH,Shankaran K et al.Dentalplaque:a permanent reservoir of Helicobacter Pylori.Scand J Gastroentrol 1991;26:1205-08.
4. BerryV,SagarV.Rapid urease test to diagnose Helicobacter Pylori Infection.JK Sciences 2006;8:86-68.

STICKLER SYNDROME: ANAESTHETIC CONSIDERATIONS - A CASE REPORT.

Dr. Ibemhal Heisnam, Dr. S .Thoibahenba Singh, Dr. K. Upendra Singh, Dr. P. Pritam Singh, Dr. L. Chandramani Singh

1. Assistant Professor, Department of Anaesthesiology, Jawaharlal Nehru Institute of Medical Sciences. Imphal.
2. Assistant Professor, Department of Anaesthesiology, Jawaharlal Nehru Institute of Medical Sciences. Imphal.
3. Assistant Professor, Department of Anaesthesiology, Jawaharlal Nehru Institute of Medical Sciences. Imphal.
4. Senior Resident, Department of Anaesthesiology, Jawaharlal Nehru Institute of Medical Sciences. Imphal.
5. Senior Resident, Department of Anaesthesiology, Jawaharlal Nehru Institute of Medical Sciences. Imphal.

CORRESPONDING AUTHOR
Dr. Ibemhal Heisnam
Thangmeiband Khoyathong Polem Leikai,
Imphal – 795001.
E-mail: dribemhal@gmail.com,
Ph: 0091 9856153557.

ABSTRACT: BACKGROUND: Stickler Syndrome is a multi systemic disorder caused by genetic malfunction in the tissue that connects bones, heart, eyes and ears. Symptoms include myopia, cataract and retinal detachment, hearing loss, midfacial underdevelopment and cleft palate and mild spondyloepiphyseal dysplasia and or arthritis. This case reports discusses the systemic problems and anaesthetic management of a patient with stickler syndrome.
KEYWORDS: Stickler Syndrome, Hereditary arthro- opthalmopathy, difficult airway, inhalational induction.

INTRODUCTION: Stickler syndrome is a genetically linked multisystem connective tissue disorder. Its prevalence is estimated to be about 1 in 10000 population. It is inherited in an autosomal dominant manner and involves the tissue that connects bones, heart, eyes, and ears. Because of its inherent problems including airway management and multisystem involvement, safe anaesthetic management of such a case need careful planning and conduct of the same.

CASE REPORT: We present a case of Stickler syndrome in a 13 year old child presented with giant retinal tear at our hospital. Pars Plana Vitrectomy with sub retinal fluid drainage with silicon oil injection was done under general anaesthesia. Her complaint was sudden onset painless loss of vision of the right eye for the last 15 days. She was diagnosed to have retinal detachment of both the eyes, right more than the left and was also myopic with a power of -7 D in the right eye and – 2 D in the left eye. She weighs 40kgs, her pulse rate was 80/min, B.P. was 110/70mmHg andSpO_2 was 98% in room air .On physical examination she had cleft palate (Fig. I), flat nasal bridge with a small nose (Fig. II), and micrognathia (Fig. III). The fingers and toes of both upper and lower limbs showed abnormal length (Fig. IV). She complained of chronic pain in both knee and ankle joint. There was no evidence of kyphoscoliosis. She had hearing defect and audiometry revealed sensory neural deafness of both ears. Clinical evaluation of cardiovascular and respiratory system was unremarkable and her IQ was normal .There was no other similar family history. All the routine investigation was done including an echocardiography, which was normal. X-ray of the spine was normal. On airway assessment,

mouth opening was adequate; thyromental distance was around 5cm and Mallampati grade II with cleft palate. Neck movement and spine was normal.

Patient was premedicated with 5mg oral diazepam on the previous night. On arrival in the operation theatre, pulse, B. P., ECG, and SpO_2were monitored. A trolley for difficult airway was kept ready in the theatre .The patient was premedicated with intravenous ondansetron 3 mg and 0.1mg glycopyrrolate. A gradual gas induction with sevoflurane was done after pre-oxygenating the patient for 3 mins. At a deeper plane laryngoscopic assessment was done which showed a Cormack and Lehane of glottis visualization as class II. Anaesthesia was deepened with propofol 2mg/kg and fentanyl 2 µg/kg IV. After confirming adequate manual ventilation, succinylcholine 1.5mg/kg was given and after a smooth larngoscospy patient was intubated with 6.5 mm I.D. endotracheal tube with the help of a bougie. Correct placement of the tube was confirmed by auscultation and appearance of $ETCO_2$ in the monitor. Anaesthesia was maintained with nitrous oxide in oxygen mixture and sevoflurane and vecuronium (0.08 µg/kg) and fentanyl (0.5 µg/kg). The vitals were stable throughout the surgery which lasted for about two hours. The patient was reversed with IV neostigmine and glycopyrrolate at the end of the surgery and extubation was done once the child was fully awake. Her postoperative recovery was uneventful and she was discharged after three days.

DISCUSSION: Stickler Syndrome also known as 'Hereditary arthro-opthalmopathy' is a connective tissue disorder first described by Dr. Gunnar Stickler in 1960. It is a multi system disorder that can affect the eyes and ears, skeleton and joints and craniofacies. Symptoms may include myopia, cataract and retinal detachment, hearing loss that is both conductive and sensorineural, mid facial underdevelopment and cleft palate, mild spondyloepiphyseal dysplasia and/or precocious arthritis. This case report is an attempt to discuss the characteristic features of Stickler Syndrome and possible problems which can be encountered during anaesthesia.

Stickler Syndrome arises due to mutation of gene involved in collagen synthesis and is inherited in an autosomal dominant manner. But in few cases spontaneous mutation may occur meaning it occurs with no prior family history as in our patient. The diagnosis of Stickler Syndrome is clinically based. The diagnosis can be confirmed by molecular genetic testing, however, it is primarily used to obtain information for genetic counseling. The disorder should be considered in individuals with clinical findings in two or more of the following categories. :

i) Ophthalmologic: Congenital or early onset cataract congenital vitreous anomaly, rhegmatogenous retinal detachment, myopia > -3 Dioptres
ii) Cranio facial: Midface hypoplasia producing a flat facial profile referred as scooped out face, bifid uvula, cleft palate, micrognathia, Robin sequence(micrognathia, cleft palate, glossoptossis)
iii) Audiologic: Sensorineural or conductive hearing loss, hyper mobile middle ear system
iv) Joints: Hypermobility, mild spondyloepiphyseal dysplasia, precocious osteoarthritis and spinal abnormalities
v) Cardiovascular system: mitral valve prolapse has been reported in almost 50% of the individuals with Stickler Syndrome.

Peter S Rose et al in their study described in detail the clinical characteristics of Stickler Syndrome and proposed a diagnostic criteria[1]. Our patient was a classical case with almost all the features in their diagnostic criteria. Anaesthetic consideration includes possibility of difficult airway due to craniofacial abnormality and mitral valve prolapse causing cardiac problem. No cardiac abnormality was detected in our patient. Micrognathia[2] and cleft palate may cause airway difficulties. But the characteristic facial appearance of Stickler Syndrome tends to improve with age, micrognathia tends to become less prominent over time, so anaesthetic problem tend to decrease in this 13 year old patient. The younger the patient, the more is the problem with airway management. [3]

A proper and careful airway assessment[4] helped us to plan our anesthetic technique. Since our patient was a little older, her airway was not much distorted. But still we anticipated a difficult airway due to the presence of micrognathia and cleft palate, so necessary precaution was taken. Although, awake intubation is the gold standard for difficult intubation, we did not try it because of lack of coordination from the child. Retrograde intubation is very uncomfortable and traumatic to awake patients especially in paediatric age group. Laryngeal mask airway (LMA) [5] is another good alternative in patient with difficult airway. Following insertion, it may be used to maintain the airway, or as a route to allow tracheal intubation. Parul Mullick[6] et al have reported a case of Pierre- Robin syndrome in which LMA was used as a conduit for endotracheal intubation. Fiber optic endotracheal intubation through LMA in a child with Treacher Collins Syndrome has also been reported by Lisa Muraka[7] et al.

Deep inhalational induction of anaesthesia[8] is also a widely used technique for patient anticipated to be difficult to intubate. If airway obstruction develops, anaesthesia may be turned off and the patient woken up. During induction, when the patient is deeply anaesthetized, direct laryngoscopy is performed and if the larynx is visible, the patient may be intubated directly or be given a muscle relaxant and then intubated. In our patient, after deep inhalational induction, laryngoscopy showed a Cormack Lehane[9] of glottis visualization as class II and manual ventilation was adequate so we decided to give muscle relaxant and intubate the patient smoothly. Inhalational induction and intubation in deeper plane after directly assessing the airway in a child with Treacher Collins syndrome was reported by Leena Goel[10] et al. Paul J Kuzma et al have also reported successful intubation after deep inhalational induction in a patient with multiple pterygium syndrome. [11]

Other problems of Stickler Syndrome includes early onset of osteoarthritis which may be severe, leading to surgical joint replacement[12] in younger age group. Due to precocious osteoarthritis these patients need great care during positioning.

CONCLUSION: The symptoms and severity of Stickler Syndrome vary from patient to patient even within the family. So anaesthetic technique should be individualized for every patient. Care should be taken when planning anaesthesia because airway management is often difficult in younger age group and emergency surgery may be required.

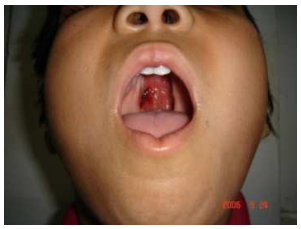


Fig .I: Cleft Palate with Receding Mandible

Fig. II: Front view showing Flat Nasal Bridge

Fig. III: Lateral view showing Micrognathia

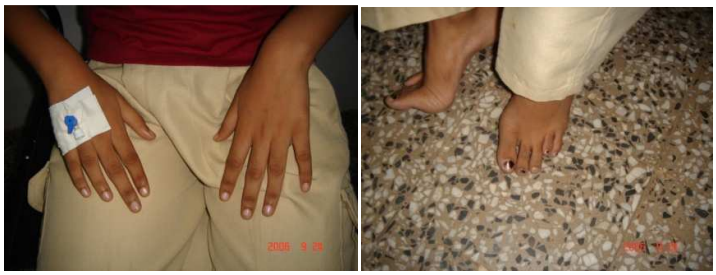


Fig. IV: Skeletal Abnormalities of both Hands and Feet

Fig V: Patient under Anaesthesia

REFERENCES:

1. Peter S. Rose, Howard P. Levy, Ruth M. Liberfarb, Joie Davis, Y.Szymko-Benett, Benjamin I. Rubin et al. Stickler Syndrome: Clinical characteristics and diagnostics criteria. American Journal of Medical Genetics (2005) 138 A: 199- 207
2. Kücükyavuz Z, Ozkaynak O, Tüzüner AM, Kisnisci R. Difficulties in Anesthetic management of patients with micrognathia: Report of a patient with Stickler Syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Dec; 102(6): e33-6
3. Yokota Hiroshi, Takahata Osamu, Sengoku Kazufumi, Okada Hanako, Iwasaki Hiroshi. Anesthetic management of a patient with Stickler Syndrome. Masui (2006) Vol 55; No. 12; Page 1487- 1489
4. Dr Sunanda Gupta, Dr Rajesh Sharma KR, Dr Dimpel Jain. Airway Assessment: Predictors of Difficult Airway. Indian J. Anaesth. 2005; 49(4): 257-262
5. Peery, Michael. Airway Management of the Severely Retrognathic Child: Use of the Laryngeal Mask Airway. Ear Nose and Throat Journal 2002/April/1-p583, ISSN 0145-5613
6. Dr. Parul Mullick, Dr Vandana Talwar, Dr Anoop Raj Gogia. The laryngeal mask- an aid for difficult intubation in a child with Pierre – Robin Syndrome- A case report. Indian J. Anaesth. 2005; 49(1): 51-53
7. Lisa Muraika, Julius S. Heyman, Yuri Shevchenko. Fibreoptic tracheal Intubation through a laryngeal mask airway in a child with Treacher Collins Syndrome. Anesth Analg 2003; 97;1298-9
8. Dr I H Wilson, Dr Andreas Kopf. Prediction and Management of Difficult Tracheal Intubation. Update in Anaesthesia Issue 9 (1998) Article 9
9. Cormack RS, Lehane J. Difficult Tracheal Intubation in Obstetrics. Anaesthesia 1984; 39: 1105-1111
10. Leena Goel, Santosh Kumar Bennur, Shweta Jambhale. Treacher Collins Syndrome – A challenge for Anaesthesiologist. Indian J. Anaesth. 2009; 53(4): 496-500.
11. Paul J Kuzma, Mark D. Calkins, Mark D. Kline, Steven M. Karan, Michael D. Maston. The Anaesthetic Management of Patients with Multiple Pterygium Syndrome. Anaesth Analg 1996; 83: 430-2.
12. GopalKrishna G. Verma, Adel Zarough, KH Suraliwala. Surgical difficulties for total knee replacement in Stickler Syndrome: A case report. Cases Journal 2008, 1:179.

ULTRASONOGRAPHY GUIDANCE FOR CENTRAL VENOUS CATHETER – A PROSPECTIVE STUDY FOR PATIENT'S SAFETY & QUALITY CARE

Dr. Siddharth Kumar. B. Parmar, Dr. Sameera. N. Parikh, Dr. Sharad. S. Vyas.

1. Assistant Professor, Department of Emergency Medicine, B. J. Medical college, Ahmedabad, Gujarat.
2. Professor, Department of Emergency Medicine, B. J. Medical college, Ahmedabad, Gujarat.
3. Professor & Head, Department of Emergency Medicine, B. J. Medical college, Ahmedabad, Gujarat.

CORRESPONDING AUTHOR
Dr. Siddharth Kumar. B. Parmar,
Tenament no. 516, Gayatri society, Sector- 27,
Gandhinagar, Gujarat. Pin 382028,
E-mail: drsid25@gmail.com,
Ph: 0091 9426332701.

ABSTRACT: BACKGROUND: Context: A central venous line access is very importance in management of the critically ill patients even though, it may carry a risk of complications.
AIMS: Objective of this study is to assess and compare success rate, attempts of cannulation and complications like inadvertent arterial puncture, hematoma, and pneumothorax occurred during the Central Venous Catheter (CVC) placement using ultrasound guidance (USG) & anatomical landmark guidance (ALG). **SETTINGS AND DESIGN:** The prospective randomized study was carried out in 64 patients for right sided internal jugular vein CVC placement. Using computer generated randomization chart, all patients were divided randomly into two groups: Group USG and Group ALG. **METHODS AND MATERIAL:** Right sided internal jugular vein (IJV) was cannulated with the guidance of ultrasound and anatomical landmark, respectively in group USG and group ALG. Patients were observed & data were recorded for success rate, no. of attempts, and complications like inadvertent arterial puncture, hematoma, and pneumothorax
STATISTICAL ANALYSIS USED: Database was analysed using graphpad prism 5 software. **RESULTS:** Success rate is 31 out of 32 (96.88%) in group USG while 24 out of 32 (75%) in group ALG (p =0.031). Placement of central venous catheter with 1st attempt is 28 out of 32 (87.50%) in group USG while 18 out of 32 (56.25%) in group ALG (p =0.012). Hematoma and overall complications are 0 versus 6 (18.75%) in group USG & ALG respectively. **CONCLUSIONS:** Ultrasound guided central venous catheter placement is easy, safer & prudent approach than the anatomical landmark guided central venous catheter placement. **KEY MESSAGES:** We believe that Ultrasound guidance should be encouraged for all central venous catheter placements in patients & thereby improving patient's safety and quality care.
KEY-WORDS: central venous line, central venous catheter placement, ultrasound, anatomical landmark guidance.

INTRODUCTION: A central venous line access is very importance in management of the critically ill patients even though, it may carry a risk of complications.

The role of ultrasound has been established in central venous catheter (CVC) placement since a long time in different country.

Objective of this study is to assess and compare success rate, attempts of cannulation and complications like inadvertent arterial puncture, hematoma, and pneumothorax occurred

during the CVC placement using ultrasound guidance (USG) & anatomical landmark guidance (ALG).

MATERIAL & METHODS: A prospective, randomized study was performed in medical & surgical Intensive care unit of a tertiary care hospital between April 2010 and October 2010. Total 64 patients of both sexes with age between 20 – 60 years were enrolled in this study. Inclusion criteria were fresh patients for CVC placement in whom adequate peripheral venous access is unobtainable and who required central venous access as a part of clinical management e.g. invasive hemodynamic monitoring, inotropic supports, dialysis & long term parenteral nutrition. Exclusion criteria were local site infection, coagulopathy.

Using computer generated randomization chart, all patients were divided randomly into two groups:

Group USG: 32 cases Ultrasound guided CVC placement
Group ALG: 32 cases an anatomical landmark guided CVC placement

Right sided internal jugular vein (IJV) was cannulated in all patients in this study. In both groups, the procedure was performed by consultant anaesthetist with more than 3 years experience in ICU & who had undergone workshop on role of ultrasonography in emergency medicine & hands-on training.

Peripheral line was ensured in all patients before the procedure. Standard monitors were applied. The patient was placed in supine position with slightly head down and neck rotation on contra-lateral side. Wedge was placed between shoulder blades. Part preparation & draping was done, taking all aseptic and antiseptic precautions.

For group USG:

A portable ultrasound "Sonosite Micromaxx" machine with 7.5 M Hz Linear array (vascular) probe was used in this group. Lead and transducer probe were cleaned with antiseptic solutions. Sterile jelly was applied on the probe. The probe was covered with sterile sheath. Now, sterile jelly was applied over the area to be scanned. Probe was placed perpendicular to the area at apex of the triangle formed by two heads of sternocleidomastoid (SCM) muscle and clavicle.

Each probe has an orientation marker placed on one side which correlates to a mark on the image screen. In the longitudinal plane, the probe is oriented with the marker towards the patients head, providing a view of the vessels along its long axis. In the transverse plane, the probe is placed with the marker facing towards to the patient's right, which provides an image similar in appearance to a CT scan.

Vessels were visualized in transverse section in 2-D ultrasound. Internal carotid artery (ICA) was seen as a circular pulsatile structure while IJV as an oval non-pulsatile structure. On applying downward pressure with probe, IJV get compressed whereas ICA remained as such. Target vessel patency was checked every time in all patients in this study at time of compressibility test.

Once vein was identified, probe was positioned so that vein was visualized in centre of the screen. Once introducer needle first indents the anterior wall of the IJV, a short stabbing motion of the needle at this point will tend to puncture the anterior wall without opposing it to the posterior wall, thereby avoiding a double-wall puncture. After successful aspiration of blood, a guide wire was inserted through it by visualizing ultrasound image in longitudinal plane. Introducer needle was withdrawn and a dilator was passed once through guide wire to

dilate the tract and withdrawn, CVC was inserted over the guide wire and guide wire was withdrawn. CVC was checked for free flow of blood. CVC was sutured with skin and a dressing was applied.

For group ALG:

The patient was placed in supine position with slightly head down and neck rotation on contra-lateral side. Wedge was placed between shoulder blades. Part preparation & draping was done, taking all aseptic and antiseptic precautions

Apex of the triangle formed by two SCM was palpated for carotid pulsations. With fingers of the left hand, carotid artery was pressed slightly medially and introducer needle inserted lateral to pulsation point, directing toward the ipsilateral nipple at an angle of 20-30 with the skin. After successful aspiration of blood, a guide wire was inserted through it and Introducer needle was withdrawn. Rest of the procedure was similar to that in group USG.

A chest x ray was obtained in all patients of this study after the procedure.

The complications like arterial puncture, hematoma, & pneumothorax were recorded.

Less than 2 attempts for CVC placement by either technique was defined as success in this study while more than 2 attempts (defined as separate skin punctures) for CVC placement by either technique were considered as failure.

Carotid artery puncture was noted by forceful pulsatile expulsion of bright red blood from the needle.

In previous study[1], success rate is 74.2% in first attempt in conventional group (non USG), So, taking this 74.2% (p=0.742) and E=10% of p, in above formula, required sample size[2] is around 32 for each group.

$$(n) = Z^2_{\propto/2}\frac{pq}{E^2}$$

Where n = sample size, $Z^2_{\alpha/2}$ = level of significance i.e. 0.95, p= prevalence, q= 1-p, E= allowable errors (10% of p)

We used graphpad prism 5 version for the statistical analysis. The means of continuous variables compared using the student's t-test and categorical variables were compared using the chisquare test and fisher's exact test. A p- value <0.05 was considered statistically significant.

OBSERVATIONS: The present study was undertaken in 64 indoor patients for comparison between USG guided & anatomical landmark guidance guided CVC placement.

In present study, success rate for CVC placement was 31 (96.88%) for group USG and 24 (75%) for group ALT. Result is statistically significant (*P* =0.031). Successful CVC placement on 1st attempt was 28 (87.5%) for group USG and 18 (56.25%) for group ALT. Result is statistically significant (*P* =0.0123). Successful CVC placement on 2nd attempt was 3 (9.38%) for group USG and 6 (18.75%) for group ALG. Result is statistically not significant (p=0.4721). Failure rate for CVC placement was 1 (3.13%) and 8 (25%) in group USG and group ALT respectively. Result is statistically significant (p=0.031). Average No. of attempts was 1.16 ± 0.45 for group USG and 1.56 ± 0.8 for group ALT. Result is statistically significant (*P* =0.0165).

There was no complications in group USG while there was 6 (18.75%) complications in group ALG. Result is statistically significant (*P* =0.032). Arterial puncture was encountered zero in group USG and 3 (9.38%) in group ALG. Result is statistically not significant (*P* =0.2369). Hematoma encountered zero in group USG and 6 (18.75%) in group ALG. Result is statistically significant (*P* =0.032). There was no any pneumothorax in both groups.

DISCUSSION: CVC line has become an integral part of management in critical as well as in emergency medicine. Traditionally, all CVC insertions have been performed using the anatomical landmark guidance. Even with experienced hands, complications rates of up to 12.3% have been reported for CVC insertions using the anatomical landmark guidance.[3] Considering the increasing use and role of CVC in the management of critical as well as emergency medicine day by day, efforts should be made to minimize & prevent the occurrence of complications and thereby improving patient safety and quality care. So, we studied to assess and compare success rate, attempts of cannulation and complications like inadvertent arterial puncture, hematoma, and pneumothorax occurred during the Central Venous Catheter placement using ultrasound guidance (USG) & anatomical landmark guidance (ALG).

In our study, we could visualise IJV and its relationship with carotid artery, introducer and guide wire entering into IJV very well with ultrasound in group USG. So, success rate was high (96.88%) in group USG because of Workshop and hands-on training which was needed for the successful use of USG guided CVC placement.

In our study, we cannulated IJV in all patients but we needed more than 2 attempts for IJV cannulation in 8 patients which was considered as failure as per definition fixed for it. So, success rate was low (75%) in group ALG.

In our study, there was no complication in group USG. There were 3 (9.38%) arterial puncture and 6 (18.75%) hematoma in group ALG. Arterial puncture was the reason for hematoma in 3 cases. Double wall puncture of IJV might be the reason for hematoma in other 3 cases. So, overall complications remained 6 (18.75%) in group ALG as arterial puncture and hematoma seen in the same patients.

There are many studies done across the world supporting the present study.

E. Paramythiotou et al [1] found in his study that IJV cannulation was successful in 29 cases (82.8%) in the control group and in 30 cases (96.7%) of the ultrasound group ($P = 0.07$). Carotid artery puncture occurred in two cases in the control group and in one case in the ultrasound group ($P = 0.80$). IJV cannulation was successful at the first attempt in 74.2% and 86.6% of cases in the control and ultrasound group correspondingly ($P = 0.25$). So, they concluded that ultrasound guidance improved the success rate and reduced complications as well as the number of attempts clinically but the difference was not significant statistically. The familiarity of the operators with the method is a contributing factor.

Sulek et al [9] found in their study to compare the success rate and incidence of complications of right IJV versus left IJV cannulation using landmark or ultrasound guidance. Cannulation of the left IJV was more time consuming; it required more attempts; and it was associated with a greater number of complications when compared to the right side ($p < 0.05$). The use of ultrasound improved the success rate with less time consuming and ultrasound reduced complications during IJV cannulation.

Hind et al[10] observed in their meta-analysis of 18 trials (1646 participants). Compared with the landmark method, real time 2 D USG guidance for cannulating the IJV in adults was associated with a significantly lower failure rate both overall (relative risk 0.14, 95% confidence interval 0.06 to 0.33) and on the first attempt (0.59, 0.39 to 0.88). Limited evidence favoured 2 D USG guidance for subclavian vein and femoral vein procedures in adults (0.14, 0.04 to 0.57 and 0.29, 0.07 to 1.21, respectively). Three studies in infants confirmed a higher success rate with 2 D USG for internal jugular procedures (0.15, 0.03 to 0.64). Doppler guided cannulation of the IJV in adults was more successful than the landmark method (0.39, 0.17 to 0.92), but the landmark method was more successful for subclavian vein procedures (1.48, 1.03 to 2.14). No

significant difference was found between these techniques for cannulation of IJV in infants. An indirect comparison of relative risks suggested that 2 D USG would be more successful than Doppler guidance for subclavian vein procedures in adults (0.09, 0.02 to 0.38). So, they concluded that evidence supports the use of 2 D USG for central venous cannulation and catheterization under USG guidance is quicker and safer than the landmark method in both adults and children.

P. Temblett[11] studied for USG guided CVC placement in 82 cases, in which 85% CVCs were inserted into IJV and 15% inserted into the femoral veins. USG guided CVCs were inserted majority by senior house officers (88%). Most (82%) were inexperienced with the use of USG (less than 20 previous USG guided CVCs inserted), while most (81%) were experienced in non-USG placement (greater than 20 previous non-US-guided CVCs inserted). There was 4% failure rate. Sixty three per cent of CVCs were inserted on the first attempt, and 21% were inserted on the second attempt. The average duration of insertion was 18 min, with 57% taking less than 15 min. There was a 10% complication rate. They concluded that ultrasound guided CVC insertion by inexperienced junior doctors with minimal training is not only feasible, but also appears to decrease complications and failure rates as well as increasing rates of first pass insertion.

Ankit Agarwal et al[12] studied to compare USG guided CVC and conventional landmark guided CVC in 80 patients, in terms of ease of cannulation, time consumed in procedures and associated complications. They found that mean time to successful insertion was 145 and 176.4 sec in groups USG and control group respectively (p = 0.00). An average of 1.2 attempts per cannulation was required for group USG, while 1.53 for control group (*p= 0.03*); 10% witnessed arterial puncture and 2.5% pneumothorax in control group and none in USG group. So, they concluded that use of ultrasound is a quicker, easier and prudent approach for CVC insertion.

Troianos et al[13] found in his prospective study that cannulation of the right IJV was successful in all 77 patients in the ultrasound group and in 80 of 83 patients (96%) in the control group. Patients with ultrasound guidance required an average ± SD of 1.4 ± 0.7 needle advances, whereas in the control patients an average of 2.8 ± 3.0 advances, were required for cannulation *(*P < 0.05). Fifty-six of 77 (73%) patients in the ultrasound group were cannulated with one attempt as compared with 45 of 83 (54%) in the control patients *(*P < 0.05). There were seven carotid punctures in the control group (8.43%) and one in the ultrasound group (1.39%) *(*P = 0.09, X^2*;* p = 0.04 before Yates' correction). They concluded that use of ultrasound reduces incidence of complications and average no. of attempts for CVC placement.

Ultrasound technology is an emerging science, has brought a revolution in the field of medicine. Modern Ultrasound machine are compact, portable & handy with good resolution, real time guidance & safety to patient and operator. (No radiation)

In emergency or critical setting when patient might not be able to be shifted to other department for imaging and interventions, with availability of USG machine, USG guided interventions can save the time & increase accuracy, efficacy & safety in emergency & critical medicine. Thus USG is a valuable tool to reduce the medical errors and to improve the medical care.

"The Stanford evidence based practice centre" has recommended ultrasound guidance in central venous catheter insertion as one of the 11- point recommendations in "A critical analysis of patient's safety practices" in 2001.[4]

"The agency for healthcare quality and research" (AHRQ), in its 2001 report on reducing medical errors in the United States, placed ultrasound guidance for CVC placement in the list of ways to reduce medical error.[5]

Based on meta-analysis in 2002, "National Institute for clinical excellence" in UK has recommended that the use of 2 D ultrasound guidance should be considered in the most clinical situations where a central venous line is necessary electively or in an emergency. [6, 7]

In some countries, central venous line under ultrasound guidance is likely to be made compulsory in the near future.[8]

LIMITATIONS OF THE STUDY:

- No. of patients in the present study was less.
- Experience of operator in basic Ultrasound physiology & hands-on training was needed for the successful use of CVC placement.
- Familiarity of the operator with methods is probably a contributing factor & must be taken into account.

CONCLUSION:

Based on the present study,

1. Ultrasound guidance increases success rate in CVC placement.
2. Ultrasound guidance also increases successful placement of CVC with 1st attempt and thereby reduces average no. of attempts for CVC placement.
3. Ultrasound guidance decreases overall complications & Hematoma during CVC placement.

Ultrasound guided CVC placement is easy, safe, accurate & prudent approach than the anatomical landmark guided CVC placement. This study evaluated a change in practice for CVC placements.

So, we believe that Ultrasound guidance should be encouraged for all CVC placements in patients & thereby improving patient's safety and quality care.

TABLE 1 Results of the present study

Outcome	USG		ALG		p value *
	count	%	count	%	
Success	31	96.88	24	75	0.0310
1st attempt cannulation	28	87.50	18	56.25	0.0123
2nd attempt cannulation	3	9.38	6	18.75	0.4721
Failure	1	3.13	8	25	0.0310
Overall complications	0	0	6	18.75	0.0320
Arterial puncture	0	0	3	9.38	0.2369
Hematoma	0	0	6	18.75	0.0320
Pneumothorax	0	0	0	0	

Table 2 : Success & Failure Rate In Different Studies.

Study	Success rate (%)		Failure rate (%)	
	USG	ALG	USG	ALG
Present study	96.88	75	-	-
Gopal B. Palepu et al, 2009 India[14]	97.6	91.6	-	-
E. Paramythiotou et al, 2004 Greece[1]	96.7	82.8	-	-
Sulek et al, 2000. USA[9]	-	-	3.3	3.3
Hind et al, 2003. UK[10]	-	-	1.7	22
P. Temblett, 2004. UK[11]	-	-	4	-

Table 3: 1st Attempt Cannulation & Average No. Of Attempts In Different Studies

Study	1st attempt cannulation (%)		Average no. of attempts (%)	
	USG	ALG	USG	ALG
Present study	87.5	56.3	1.16	1.6
Gopal B. Palepu et al, 2009. India	84.4	72.7	1.2	1.5
Ankit Agarwal et al, 2009. India[12]	87.5	67.5	1.2	1.5
E. Paramythiotou et al, 2004. Greece	86.6	74.2	-	-
P. Temblett et al, 2004. UK	63	-	-	-
Troianos et al, 1991. USA[13]	-	-	1.4	2.8
Sulek et al, 2000. USA	-	-	1.5	2.1

Table 4 : Overall Complication In Different Studies

Study	Overall complication (%)	Arterial puncture (%)	
		USG	ALG
Present study	18.75	0	9.38
Gopal B. Palepu et al, 2009. India	7	-	-
Ankit Agarwal et al, 2009. India	-	0	10
Sulek et al, 2000. USA	10	6.7	13.3
P. Temblett et al, 2004. UK	10	-	-
Troianos et al, 1991. USA	-	1.4	8.4
E. Paramythiotou et al, 2004. Greece	-	3.2	5.7

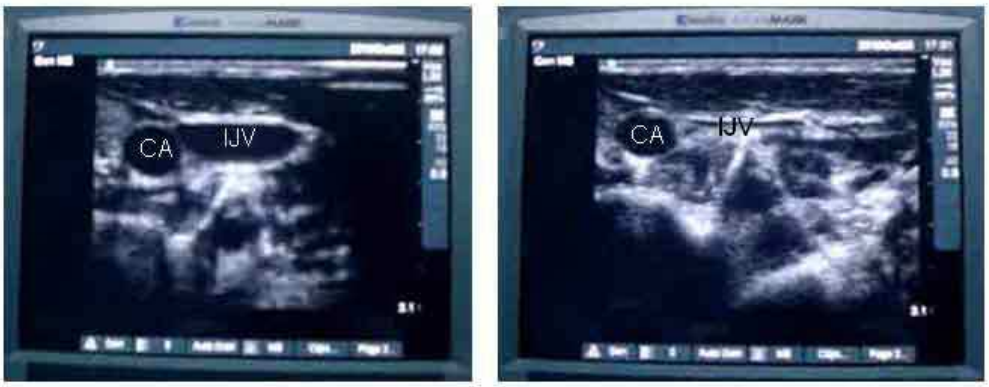


Figure 1: showing compressibility test in transverse plane of 2D USG.

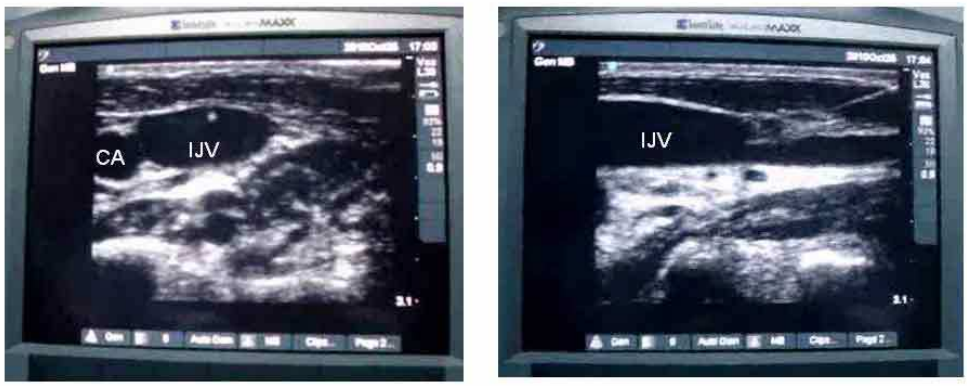


Figure 2: introducer needle entering into IJV in transverse plane of 2D ultrasound (left) and in longitudinal plane of 2 D ultrasound (right).

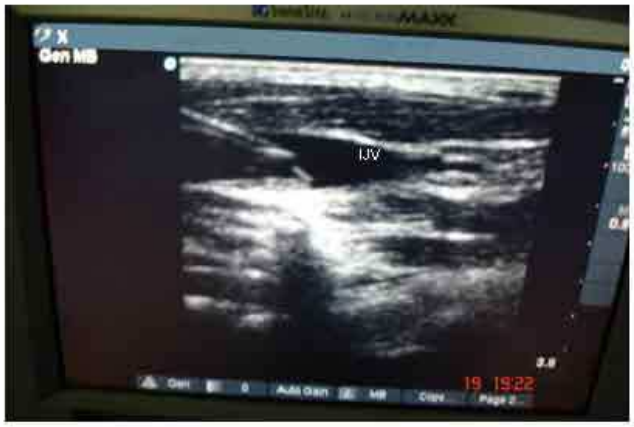


Figure 3: Guide wire from introducer needle entering into IJV in longitudinal plane of 2 D USG

REFERENCES:

1. A 3 year experience of ultrasound guided catheterization of internal jugular vein in an intensive care unit. Critical care 2004
    a. "www.ccforum.com/supplements/8/S1/P71"
2. K Vishweswara Rao -- Biostatistics: A Manual of Statistical Methods for use in Health, Nutrition and Anthropology, 2nd ed. New Delhi: Jaypee Brothers Medical Publisher, 2007: 210-8.
3. Schummer W, Schummer C, Rose N. , Niesen WD --Mechanical complications and malpositions of CVC by experience operators. A prospective study of 1784 catheterization in critically ill patients. Critical care medicine 2007; 33: 1055-9.
4. Shojania KG, Duncan BW, McDonald KM, Wachter RM, Markowitz AZ --Making health care safer: A critical analysis of patient's safety practices. Evid Rep Technol Access (Summ) 2001; 43:1-668.
5. Agency for healthcare Research and Quality (AHRQ). "Making health care safer". A critical analysis of patient safety practices. Evidence Reports/ Technology Assessments, No. 43.
    a. "www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=erta43
6. National Institute for Clinical Excellence. Guidance on the use of ultrasound locating devices for placing central venous catheters. NICE technical report number 49; September 2002.
    a. http://www.nice.org.uk/nicemedia/live/11474/32461/32461.pdf
7. Anthony Espinet, Joel Dunning -- Does ultrasound guided central line insertion reduce complications and time to placement in elective patients undergoing cardiac surgery. Interactive cardio Vascular and Thoracic Surgery 2004; 3: 523-7.
8. Jefferson P, Ogbue MN, Hamilton KE, Ball DR -- A survey of the use of portable ultrasound for central vein cannulation in critical care units in the UK. Anaesthesia 2002; 57: 365-8.
9. Sulek CA, Bias ML, Lobato EB -- A randomized study of left versus right internal jugular vein cannulation in adults. J Clin Anesth 2000; 12: 142-5.
10. Hind D, Calvert N, Mc Williams R, Davidson A, Paisley S, Beverley C. Thomas S -- Ultrasonic locating devices for central venous cannulation: meta-analysis. Br Med J 2003; 327: 361-4.
11. Ultrasound guided central line insertion. Critical care 2004
    a. "www.ccforum.com/supplements/8/S1/P72"
12. Ankit Agrawal, Dinesh K. Singh, Anil P. Singh -- USG A novel approach to central venous cannulation. Indian J Crit Care Med 2009; 13: 213-6.
13. Troianos CA, Jobes DR, Elison N -- Ultrasound guided cannulation of the internal jugular vein. A prospective, randomized study. Anesth Analg 1991; 72: 823-6.
14. Gopal B. Palepu, Juneja Deven, M Subrahmanyam, S. Mohan -- Impact of ultrasonography on central venous catheter insertion in intensive care. Indian J Radiol Imaging 2009; 19: 191-8.

RISK FACTORS FOR SEVERE PNEUMONIA IN CHILDREN: A HOSPITAL BASED CASE – CONTROL STUDY

Dr. Ramesh. K, Varshitha Hemanth, Ashray. V, Safna. C. T, Anup. N.

1. Assistant Professor, Department of Community Medicine, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka.
2. Junior Resident, Department of Community Medicine, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka.
3. Junior Resident, Department of Community Medicine, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka.
4. Junior Resident, Department of Community Medicine, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka.
5. Junior Resident, Department of Community Medicine, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka.

CORRESPONDING AUTHOR

Dr. Ramesh. K MBBS, MD, Assistant Professor,
Dept. of Community Medicine,
Vijayanagara Institute of Medical Sciences (VIMS),
Bellary -583104, Karnataka
E-mail: ramspsm@yahoo.co.in,
Ph: 0091 9481181291.

ABSTRACT: BACKGROUND: Pneumonia is the leading single cause of childhood mortality. The disease accounts for 19 per cent deaths in children less than 5 years. Pneumonia kills more children than any other illness, more than measles, malaria and AIDS combined. Globally 156 million new pneumonia cases are reported every year in the developing world. As many as 8.7 per cent of these cases are severe enough to be life-threatening and require hospitalization. In India, pneumonia is responsible for an estimated 410,000 deaths in children under five. Studies have shown that up to 19% of children hospitalized with pneumonia die in India. Most of the death occurs in severe pneumonia cases. Many studies have revealed the risk factors of pneumonia but very few studies have come out with risk factors of severe pneumonia and no study has been conducted in Karnataka to explore the risk factors of severe pneumonia. So we intended to do a study to find the risk factors of severe pneumonia. **METHODOLOGY:** This was a case-control study conducted between Jan 2010 to Sep 2010 in the Dept. of Pediatrics, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka. . Cases were in-patients with severe pneumonia as ascertained by WHO criteria, while controls were out-patients with non-severe acute respiratory infections. For each case we interviewed two controls. Totally 75 cases and 150 controls were included for the study. After obtaining consent from the parents, data was collected using a semi structured questionnaire and necessary anthropometric measurements were taken. Data was analyzed in SPSS 15.0 **STATISTICAL ANALYSIS**: proportion, Chi square test, odds ratio, logistic regression. **RESULTS:** The study subjects included 75 cases and 150 controls. The significant factors on univariate analysis were paternal and maternal education, joint type of family, delayed initiation of breast feeding, discontinuation of breastfeeding in young infants, PEM, low birth weight, previous history of ARI, immuno-compromised state, similar cases in neighborhood, delivery by LSCS, birth order of 3or more, ANC visits less than 5, delayed initiation of treatment, increased distance of health care facility and use of solid fuel for cooking. Binary logistic regression revealed that PEM, previous history

of ARI, delivery by LSCS and delayed initiation of treatment are strong associates of severe pneumonia. **CONCLUSION:** Many of the factors mentioned are amenable to corrective measures and may help in reducing the alarmingly high global burden of severe pneumonia.
KEYWORDS: Risk factor, Severe Pneumonia, case-control

INTRODUCTION: Pneumonia is the leading single cause of childhood mortality. The disease accounts for 19 per cent deaths in children less than 5 years. Pneumonia kills more children than any other illness, more than measles, malaria and AIDS combined. Globally 156 million new pneumonia cases are reported every year in the developing world. As many as 8.7 per cent of these cases are severe enough to be life-threatening and require hospitalization. India accounts for the maximum 43 million new cases followed by China (21 million cases) and Pakistan (10 million cases).[1]

In India, pneumonia is responsible for an estimated 410,000 deaths[1] in children under five. Studies have shown that up to 19% of children hospitalized with pneumonia die in India.

According to the 2008 estimates of WHO, pneumonia is the second leading cause of mortality in under-5 children (18%) and the cause for highest mortality by a single specific disease among under-5 age group.[2]

As millions of children, specially the poor, remain at high risk of dying from pneumonia, it is a major factor causing delay in achieving the Millennium Development Goal of globally reducing childhood deaths by two-thirds by 2015.[1]

Childhood clinical pneumonia is caused by a combination of exposure to risk factors related to the host, the environment and infection. Possible risk factors[6] include malnutrition ,low birth weight, non-exclusive breastfeeding (during the first 4 months of life), immunization status, indoor air pollution[6][7], overcrowding[10], concomitant diseases, mother's education and birth order etc. [6]

Many studies[4] [5][9] have revealed the risk factors of pneumonia but very few studies have come out with risk factors of severe pneumonia and no study has been conducted in Karnataka to explore the risk factors of severe pneumonia. So we intended to do a study to find the risk factors of severe pneumonia.

Pneumonia constitutes about 17% of admissions to the Pediatric wards of Vijayanagara Institute Of Medical Sciences, Bellary, Karnataka, India. Keeping in view the burden of the disease, this study was conducted with the following objectives:

1) To study the risk factors of severe pneumonia among pediatric age group of 0-5 years
2) To study the difference in socio economic, nutrition, immunization, KAP factors among cases of pneumonia and severe pneumonia

METHODOLOGY;A case control study was conducted among children of the age group 0-5 yrs admitted to the Pediatric Dept. of Vijayanagara Institute Of Medical Sciences, Bellary , from January to September 2010.

Among the total 225 study subjects, cases comprised of in-patients with severe pneumonia as ascertained by WHO criteria, which were 75 in number; while controls were out-patients with non severe acute respiratory infections numbering to 150.

Clinically pneumonia is classified as :[11]

I Very severe disease

II Severe pneumonia
III Pneumonia (not severe)
IV No Pneumonia: cold or cough

The current WHO definition of very severe pneumonia is a clinical diagnosis based on the presence of cough or difficulty breathing plus at least one of the following: central cyanosis; inability to breast feed or drink, or vomiting everything; convulsions, lethargy or unconsciousness; or severe respiratory distress.

Severe respiratory distress is defined as the presence of head nodding in addition to other signs of respiratory distress such as chest indrawing and tachypnoea. This study intends to look at the risk factors for severe pneumonia.

Data was collected using a pre designed semi structured questionnaire which contained information regarding family & socio economic conditions, birth & immunization, nutritional aspects including breastfeeding details, past history, treatment details, KAP and health seeking behaviour.

Age of the child was recorded in completed months. Family type was classified as Nuclear /Joint / Three generation family. The family size and number of members with history of ARI (Acute respiratory infection) in the past one month were recorded.

Education of mother and father was graded according to the following scale:

1) Illiterate
2) Primary schooling
3) Secondary schooling
4) Higher secondary schooling
5) Pre – University.
6) Degree and higher.

The occupation of the mother and father was graded as follows:

1) Unemployed.
2) Unskilled.
3) Semiskilled.
4) Skilled.
5) Professional.
6) Business and others.

The Monthly Family income was recorded.

History of immunization was elicited from parents and verified by checking the written document wherever available. A child was assessed to be completely immunized if he/she had received all vaccinations due for his age according to national immunization schedule. The child was also categorized as being immunocompromised if he had/has HIV/HBV/Measles.

Birth history included birth order [6], antenatal history, perinatal history and postnatal history. Details about the mother during the antenatal check up, tetanus immunization and iron & folic acid supplementation were also collected.

History of breastfeeding and the age of introduction of supplementary feeding was elicited. Caloric intake of the child was calculated by recording the food items given to the child regularly prior to the current illness by 24 hr recall method.

The Indian Association Of Pediatrics classification was used for assessing the grade of malnourishment among the study subjects. BMI was also calculated.

Details of the illness including duration of illness, time of initiation of initial treatment and referral from other places were recorded. History of previous similar attacks,T.B contact, history of measles and similar complaints in the neighbourhood , school or family was elicited.

Housing conditions included type of house, locality (rural/urban & slum/non slum), ventilation, water supply, waste disposal and overcrowding.

Overcrowding was determined by calculating number of family members per room [3]. A child who was not found to be in the following norm was labeled as staying in overcrowded house.

1 Room 2 persons
2 Rooms 3 persons
3 Rooms 5 persons
4 Rooms 7 persons
5 or more rooms 10 persons (additional 2 for each further room).

Usage of solid fuel for cooking[7],[8] was also recorded.
Knowledge , Attitude and Practices of the parents comprising mode of transmission, belief of disease being curable or not, use of handkerchief while sneezing/coughing , sanitary disposal of sputum , isolation of the patient after onset of symptoms (P) and hygiene of the child .

Health seeking behavior such as approaching the nearest health facility at the time of illness was included.

RESULTS: Table.no1 shows the age sex wise distribution of study subjects which includes both cases and controls. 61.3% is contributed by males and the remaining 38.7% by females.107 subjects belonged to the age group of 2 months-12 months. 62.6% males were from the 2mo-12mo age group and among female subjects; majority (ie 41.1%) belonged to the age group of 12mo to 5years.

Table 1- Age sex wise distribution of study subjects

Age group	Male	Female	Total
< 2months	18 (64.3%)	10 (35.7%)	28 (100%)
2 months – 12 months	67 (62.6%)	40 (37.4%)	107 (100%)
> 12 months – 5 years	53 (58.9%)	37 (41.1%)	90 (100%)
Total	138 (61.3%)	87 (38.7%)	225 (100%)

Table 2 shows the Socio-demographic factors considered to study the risk associated with severe pneumonia.

In the study, majority (46.8%) of the children suffering from severe pneumonia were aged between 12mo and 5yrs.Though majority of the cases were males(62.6%), it was not found

to be significant(p=0.88).The joint family type was found to be significantly associated with severe pneumonia (68%)(p=0.02).

The paternal (70.6%)(p=0.01) and maternal education(81.3%)(p=0.01) only upto the primary level was positively associated with incidence of severe pneumonia. Significantly more fathers were found to be either unemployed or unskilled in the severe pneumonia group (84%) (p=0.04), however maternal occupation did not show significant risk associated with severe pneumonia (p=0.29).

Table 2: Socio-demographic factors

Variables	Cases (N= 75)	Controls (N= 150)	Odds ratio (95CI%)	P value
Age				0.35
< 2 months	08 (10.6%)	20 (13.3%)		
2 – 12 months	32 (42.6%)	75 (50.0%)	-------	
> 12 months –5 yrs	35 (46.8%)	55 (36.7%)		
Sex				
Male	47 (62.6%)	91 (60.6%)	1.08	0.88
Female	28 (37.4%)	59 (39.4%)	(0.61 – 1.92)	
Family type				0.02
Joint family	51 (68.0%)	79 (52.6%)	1.91	
Nuclear family	24 (32.0%)	71 (47.4%)	(1.03 – 3.56)	
Family size				
> 5 members	56 (74.6%)	97 (64.6%)	1.61	0.12
< 5 members	19 (25.4%)	53 (35.4%)	(0.83 – 3.14)	
Paternal education				
< Primary	53 (70.6%)	87 (58.0%)	2.13	0.01
> Primary	22 (29.4%)	63 (42.0%)	(1.11 – 4.13)	
Maternal education				
< Primary	61 (81.3%)	99 (66.0%)	2.24	0.01

> Primary	14 (18.7%)	51 (34.0%)	(1.10 – 4.65)	
Paternal occupation Unemployed/unskilled Semi/skilled	 63 (84.0%) 12 (16.0%)	 108 (72.0%) 42 (28.0%)	 2.04 (0.95 – 4.44)	 0.04
Maternal occupation Unemployed/unskilled Semi/skilled	 69 (92.0%) 06 (08.0%)	 131(87.3%) 19 (12.7%)	 1.67 (0.59 – 4.92)	 0.29

Table 3 shows the variables studied for housing conditions. Amongst the 6 housing conditions variables, kuchcha type of housing (73.3%) (p=0.00), residence in slum area(46.6%)(p=0.03),inadequateventilation(76%)(p=0.04),overcrowding(81.3%)(p=0.00) and use of solid cooking fuel (85.3%)(p=0.00) were found to be significant risk factors for severe pneumonia.

Table 3: Housing conditions

Variables	Cases (N= 75)	Controls (N= 150)	Odds ratio (95CI%)	P value
Housing type Kuchcha/semi pucca Pucca	 55 (73.3%) 20 (26.7%)	 70 (46.6%) 80 (53.4%)	 3.14 (1.65 – 6.02)	 0.00
Locality Rural Urban	 48 (64.0%) 27 (36.0%)	 110 (73.3%) 40 (26.7%)	 0.65 (0.34 – 1.22)	 0.14
Area Slum Non slum	 35 (46.6%) 40 (53.4%)	 49 (32.6%) 101 (67.4%)	 1.80 (0.98 – 3.31)	 0.03
Ventilation Inadequate	 57 (76.0%)	 94 (62.6%)	 1.89	 0.04

Adequate	18 (24.0%)	56 (27.4%)	(0.97 – 3.70)	
Overcrowding Present Absent	 61 (81.3%) 14 (18.7%)	 92 (61.3%) 58 (38.7%)	 2.75 (1.35 – 5.67)	 0.00
Cooking fuel Solid fuel Others	 64 (85.3%) 11 (14.7%)	 86 (57.3%) 64 (42.7%)	 4.33 (2.02 – 9.48)	 0.00

Table 4 includes the pregnancy and birth related factors among which less than 3 ante natal check up visits (68%)(p=0.00), Ceasarean section mode of delivery (74.6%)(p=0.00), low birth weight (84%)(p=0.01) and delayed initiation of breast feeding were of significance in children suffering with severe pneumonia.

However, the place of delivery, birth order and exclusive breast feeding were found to have no implications on occurrence of severe pneumonia.

Table 4: Pregnancy and birth related factors

Variables	Cases (N= 75)	Controls (N= 150)	Odds ratio (95CI%)	P value
ANC reg Yes No	 47 (62.6%) 28 (37.4%)	 91 (60.6%) 59 (39.4%)	 1.15 (0.27 – 4.57)	 1.00
ANC visits < 3 visits > 3 visits	 51 (68.0%) 24 (32.0%)	 79 (52.6%) 71 (47.4%)	 2.39 (1.30 – 4.41)	 0.00
Mode of delivery LSCS NVD	 56 (74.6%) 19 (25.4%)	 97 (64.6%) 53 (35.4%)	 2.64 (1.43 – 4.90)	 0.00
Place of				

delivery Home Institution	53 (70.6%) 22 (29.4%)	87 (58.0%) 63 (42.0%)	1.34 (0.70 – 2.57)	0.34
Birth order </= 2 > 2	61 (81.3%) 14 (18.7%)	99 (66.0%) 51 (34.0%)	0.85 (0.46 – 1.55)	0.56
Birth weight < 2500 gms > 2500 gms	63 (84.0%) 12 (16.0%)	108 (72.0%) 42 (28.0%)	2.11 (1.14 – 3.90)	0.01
Initiation of BF < 4 hrs > 4 hrs	69 (92.0%) 06 (08.0%)	131 (87.3%) 19 (12.7%)	2.40 (1.05 – 4.24)	0.02
Exclusive BF Present Absent	71 (94.6% 04 (5.4%)	129 (86.0%) 11 (14.0%)	1.14 (0.53 – 2.43)	0.72

The illness related factors are shown in Table 5.

Presence of PEM (Protein energy Malnutrition) (61.3%)(p=0.00), history of previous attacks (78.6%)(p=0.00), initiation of treatment after first 24 hours of illness (65.3%)(p=0.00), healthcare accessibility (health care facility farther than 2km) (62.6%)(p=0.00) were found to be significantly associated with severe pneumonia.

According to our study, immunization, history of ARI in family and history of measles were not found to be significant risk factors among children with severe pneumonia in comparison to children suffering from pneumonia.

Table 5: Illness related factor

Variables	Cases (N= 75)	Controls (N= 150)	Odds ratio (95CI%)	P value
Immunization				
Full	74 (98.6%)	147 (98.0%)	1.51	1.00
No/partial	01 (1.4%)	03 (2.0%)	(0.14 – 8.17)	
PEM				
Present	46 (61.3%)	51 (34.0%)	3.08	0.00
Absent	29 (38.7%)	99 (66.0%)	(1.67 – 5.70)	
H/o ARI in family				
Yes	21 (28.0%)	32 (21.3%)	1.43	0.26
No	54 (72.0%)	118 (78.7%)	(0.72 – 2.84)	
H/o measles				
Yes	02 (2.6%)	01 (0.6%)	4.09	0.25
No	73 (97.4%)	149 (99.4%)	(0.28 – 115.6)	
H/o previous attack				
Yes	59 (78.6%)	73 (48.6%)	3.89	0.00
No	16 (21.4%)	77 (51.4%)	(1.97 – 7.76)	
Initiation of Rx				
> 24 hrs	49 (65.3%)	69 (46.0%)	2.21	0.00
< 24 hrs	26 (34.7%)	81 (54.0%)	(1.20 – 4.09)	
Place of initial Rx				
Private	35 (46.6%)	37 (24.6%)	2.67	0.00
Government	40 (53.4%)	113 (75.4%)	(1.43 – 5.01)	

Healthcareaccessibility > 2 kms Within 2 kms	 47 (62.6%) 28 (37.4%)	 59 (39.3%) 91 (60.7%)	 2.59 (1.41 – 4.78)	 0.00

On multivariate analysis by binary logistic regression, presence of PEM, previous history of ARI, mode of delivery by ceasarian section, delayed initiation of treatment and failure to initiate breast feeding within 4 hours of birth were found to be strong associates of severe pneumonia.

Table 6: Binary logistic regression

Variables	Adjusted odds ratio	P value
PEM	4.01(2.34 – 6.89)	0.00
Previous history of ARI	2.92 (1.96 – 8.13)	0.00
Delivery by LSCS	2.86 (1.34 – 7.11)	0.00
Delayed initiation of treatment	2.01 (1.88 – 6.98)	0.00
Failure to initiate breast feeding within 4 hours	1.67 (1.18 – 8.56)	0.00

DISCUSSION: Socio-demographic factors: In socio-demographic factors variables like joint family, paternal and maternal education upto or less than primary and paternal occupation unemployed / unskilled were found to be statistically significant. Family plays an important role both in health and disease- in the prevention and treatment of individual illness, in the care of children and in the stabilization of the personality of both adults and children.

In our study, severe pneumonia was more common among children living in joint type of family, it may be due to the more family members and overcrowding which makes joint family a playground for communicable diseases. These diseases are known to spread rapidly in families because of the common environment which the family members share.

Income, education and occupation are the major components of most measures of social class and are generally positively correlated with health status. Diseases of respiratory system, eyes and skin, diarrhea have a higher incidence in lower classes which can be ascribed to the poor state of physical environment in which they live. Individuals in the lower social classes have been found to make less use of hospital facilities, consult the doctor less often and are less

likely to be aware and utilize preventive health services such as prenatal and postnatal care, general check ups and immunization services.

Housing conditions: In housing conditions, the association of kuchcha/ semi pucca type of housing, slum area, presence of overcrowding, inadequate ventilation and use of solid cooking fuel were found to be statistically associated with severe pneumonia.

Housing is a part of the total environment of man and being a part of it is to some extent responsible for the state of man's health and well being. A strong relationship is established between poor housing and respiratory infections. High morbidity and mortality rates are observed where housing conditions are sub- standard

In kuchcha/ semi pucca houses the walls and floors are permeable to water, remains damp, mud floors tend to break up and the cracks and crevices results in harbourage of dusts and insects, inadequate ventilation and these factors contribute to the ill health of its inmates.

In our study it has been found out that children living in kuccha/ semi pucca houses are three times more susceptible to develop severe pneumonia.

Ventilation controls indoor humidity and airborne contaminants, both of which either contribute to or act as health hazards. Inadequate ventilation can increase indoor pollutant levels by not bringing in enough outdoor air to dilute emissions from indoor sources and by not carrying indoor air pollutants out of home.

Use of biomass fuels (wood, crop residues, animal dung) coal and other media(kerosene) are predominant contributors to indoor air pollution. More than three- quarters of all Indian households are dependent on such fuels. These are burnt in simple stoves with incomplete combustion generating a lot of toxic products that adversely affect specific and non specific local defenses of the respiratory tract. The risk is highest for mothers and children due to longer stay indoors and close proximity during cooking[7] .This study shows that children living in houses where solid cooking fuel is used, have 4.33 times the risk of developing severe pneumonia when compared to children from homes where other fuels are used. Studies conducted elsewhere have also concluded that indoor air pollution is a major contributory factor in the development of respiratory tract infection [5][7] .

Slums are breeding grounds for social problems and many communicable diseases especially respiratory diseases. They exhibit high rates of disease due to unsanitary conditions, malnutrition and lack of basic health services.

Overcrowding is a health problem in human dwellings. It promotes the spread of respiratory infections among other communicable diseases. There are higher chances of spread of respiratory disease by droplet transmission which is one of the major modes of transmission especially in pneumonia. A few previous studies also have similar findings [4] [5] [9].

The variable of locality (rural/urban) was found to be statistically insignificant in our study, it may be due to the almost equal distribution of study subjects in rural and urban areas and may be also due to the small sample size.

Pregnancy and birth related factors: In our study ANC visits less than three, delivery by caesarian section , birth weight less than 2500gm, failure to initiate breast feeding within 4hrs were found to be statistically significant. These variables carry about 2 times risk of developing severe pneumonia.

Birth weight of an infant is the single most important determinant of its chance of survival, healthy growth and development. Most of the low birth weight infants become victims of PEM and infections. Low birth weight also reflects inadequate nutrition and ill health of the

mother. There is a strong and significant positive correlation between maternal nutritional status and the duration of pregnancy and birth weight.

Antenatal checkup aims at achieving, at the end of pregnancy a healthy mother and a healthy baby. In our study ANC less than 3 has emerged as one of the factors for development of severe pneumonia. This may be due inadequate nutrition and ill health of the mother and failure to identify the high risk cases. Improper ANC can increase the likelihood of delivery by caesarian section which may be either elective or emergency.

The earlier studies have not taken into consideration factors related to ante natal care and mode of delivery. In our study ANC registration, place of delivery, birth order less than 3, exclusive breast feed were found to be statistically insignificant. This may be due to the smaller study group wherein, most of the study subjects belonged to rural areas and that most of the deliveries, even though conducted at home, were under the supervision of trained dais.

Illness related factors: In our study, presence of PEM, history of previous attacks of ARI, initiation of treatment after more than 24 hours of onset of symptoms and health care accessibility (distance from home more than 2 Km) have been found to be statistically significant.

Health care accessibility can affect the outcome of the disease. The disease progresses faster in young children owing to their immature immune system. In our study children residing at places where health care accessibility is more than 2 Km and with initial treatment after more than 24 hours, are found to suffer more from severe pneumonia. In a study conducted earlier, delayed reporting to health care facility did not emerge as a risk factor [9].

As per treatment guidelines, children with severe disease require immediate admission and treatment to decrease morbidity and mortality.

In our study, immunization, history of ARI in the family and history of measles were found to statistically insignificant. This may be attributable due to the following: Inadequate awareness among parents about immunization schedule, symptoms of ARI and measles. Also, in our study we have made a comparison between children suffering from pneumonia and those from severe pneumonia whereas in other studies, comparison has been made with healthy children[5]or no comparison has been made[9].

Multivariate analysis revealed PEM to be a major risk factor in causing severe pneumonia in our study. In the previous studies too, it has been found to be a significant risk factor[5][4]. PEM is well known to cause decreased immunity among children and hence directly predisposes a child to be more susceptible for severe pneumonia.

Our study reveals that children with previous history of ARI had 2.92 times more risk of developing severe pneumonia against pneumonia. The previous studies also have similar findings. This may be attributable to the damage to the respiratory epithelium / parenchyma by previous ARI causing increased susceptibility to respiratory infections.

Delivery of children by Caesarian section had 2.86 times more risk of developing severe pneumonia when compared to children born by normal vaginal delivery. Studies conducted earlier[5][4] have not focused on this aspect as being a risk factor for pneumonia. However, in this study, analysis revealed that birth by caesarian section is a significant risk factor for severe pneumonia.

This may be attributed to delayed onset of breast feeding (more than 4 hrs after birth) in children born by caesarian section and increased use of respiratory resuscitation measures causing a higher risk for respiratory infections in children.

Infrequent and improper antenatal checkups can also contribute to increase in births by emergency caesarian section which is known to increase maternal and child morbidity. Delayed initiation of treatment (> 24hrs after the onset of symptoms first appeared) had a strong association with occurrence of severe pneumonia in our study.

Earlier studies have analyzed that patients required change of antibiotics 48 hrs after admission [9].

This highlights the importance of early initiation of treatment, the failure of which causes increased severity of symptoms and hence occurrence of severe pneumonia.

Previous studies conducted to reveal the risk factors of pneumonia have found that , lack of breast feeding[9] , discontinuation of breastfeeding[4] , no breastfeeding or breastfeeding less than 4 months[5] were significant risk factors for causing pneumonia .

In this study we have found the presence of delayed initiation of breastfeeding (by more than 4 hrs after birth) to be a significant factor in causing severe pneumonia among children. This can be attributed to the fact that babies breastfed for the 1st time after more than 4 hrs after birth were less likely to be colostrum fed than their counterparts being fed for the first time within 4 hrs after birth. Babies with delayed initiation of breast feeding were more likely to be given pre lacteal feeds like honey, water, jaggery, castor oil etc. Colostrum is known to have higher content of maternal antibodies and vitamins A, E and hence provides better immunity in children decreasing the risk of developing severe pneumonia.

CONCLUSION: In our study, PEM, previous history of ARI, delivery by LSCS , delayed initiation of treatment and failure to initiate breast feeding within four hours of delivery are significantly associated with severe pneumonia and hence these factors can be considered for screening severe pneumonia among children

REFERENCES:

1. WHO Article – World Pneumonia day 2009.
2. WHO Statistics on under 5 mortality 2008.
3. Park K. Environment and health In: Textbook of Preventive and Social Medicine. 20th edn. Ed. Park K, Jabalpur, Banarasidas Bhanot Publishers, 2009; pp 658.
4. Shah N, Ramankutty V, Premila PG, Sathy N (1994) Risk factors for severe pneumonia in children in south Kerala: a hospital-based case-control study. Journal of Tropical Pediatrics, 40:201–206.
5. S.Broor , R.M. Pandey, M.Ghosh, R.S.Maitreyi, Rakesh Lodha, Tanu Singhal & S.K. Kabra - Risk Factors for Severe Acute Lower Respiratory Tract infection in under five children , Indian Pediatrics 2001; 38: 1361-1369.
6. Murali Narayanan , AG Falade - Clinical Risk Factors for Death in Children with Pneumonia ; www.ichrc.org ,First published online: 13th October 2006.
7. Kirk R Smith, Jonathan M Samet, Isabelle Romieu, Nigel Bruce - Indoor air pollution in developing countries and acute lower respiratory infections in children; Thorax 2000;55:518–532.
8. Louis Niessen , Anne ten Hove , Henk Hilderink , Martin Weber , Kim Mulholland & Majid Ezzati - Comparative impact assessment of child pneumonia interventions , Bulletin of the World Health Organization 2009;87:472-480. doi: 10.2471/BLT.08.050872.

9. Karalanglin Tiewsoh, Rakesh Lodha, Ravindra M Pandey**,** Shobha Broor**,** M Kalaivani**,** Sushil K Kabra: Factors determining the outcome of children hospitalized with severe pneumonia: BMC Pediatrics 2009, **9:**15doi:10.1186/1471-2431-9-15
10. Maria Regina Alves Cardoso,Simon Nicholas Cousens,Luiz Fernando de Góes Siqueira, Fátima Maria Alves ,LuizAntônio V D'Angelo : Crowding: risk factor or protective factor for lower respiratory disease in young children?: BMC Public Health 2004, 4:19doi:10.1186/1471-2458-4-19
11. Park K. Epidemiology of communicable diseases , Acute respiratory diseases In: Textbook of Preventive and Social Medicine. 20th edn. Ed. Park K, Jabalpur, Banarasidas Bhanot Publishers, 2009; pp 154.

MICROBIOLOGICAL SURVEILLANCE OF AIR QUALITY IN OPERATION THEATRES - COMPARISON OF THE CONVENTIONAL SETTLE PLATE TECHNIQUES VS USE OF AN AIR SAMPLING DEVICE.

Dr. A. G. Prathab, Dr. C. Lalitha.

1. Professor, Department of Microbiology, MS Ramaiah Medical College and Teaching Hospital, Bangalore.
2. Assistant Professor, Department of Microbiology, MS Ramaiah Medical College and Teaching Hospital, Bangalore.

CORRESPONDING AUTHOR
Dr. A. G. Prathab, Professor,
Department of Microbiology,
MS Ramaiah Medical College. M.S.R Nagar, Bangalore,
E-mail: dragprathab.@yahoo.co.in,
Ph: 91-9845195014.

ABSTRACT: BACKGROUND: Air as a means of nosocomial transmission has always remained as a cause of concern for health care providers and especially personnel involved in infection control activities. Although there are no uniform consensus on either the standards for surveillance, methodology for monitoring or the levels of acceptable contamination, it still remains a fact that we need to have some criteria to monitor air quality in atleast the critical care areas like the operation theatres. **METHODOLOGY:** Air quality surveillance in the operation theatres was performed simultaneously using the settle plate technique and an air sampling device. A total of 9 operation theatres were subjected to 4 surveillance cycles with a minimum of 2 recordings in each theatre following standard protocols and accepted method used to calculate bioburden. **RESULTS:** A comparison was made between the two methods using the data available with 72 recordings . In the settle plate technique, the mean cfu /mm3 was found to be 17.11 and 22cfu/mm3 at less than 30cm and at a point more than 30cms of the operating table where as the corresponding means using the air sampler was137.83 and 164.11cfu/mm3 respectively which showed considerable statistical significance .**CONCLUSION :** The use of an air sampler would be more appropriate in monitoring the air quality in critical care areas ensuring a more stringent method of quality check without compromising on the the standard accepted norms and addressing the issue of patient safety with reference to infection prevention .

KEY WORDS: Air Quality, Operation theatres, Colony forming units, Settle plate method, Air sampling device, Infection prevention.

INTRODUCTION & BACKGROUND: It is a well known fact that air contains bacterial and fungal cells either as small individual particles or larger clumps and aggregates that can settle and serve as important sources of infection and the hospital is an important indoor environment responsible for spread of airborne pathogens. **(1-3)**

In this context, it is worth recalling the statement made by Riley that "the enclosed atmosphere of the hospital building and its human occupants constitute an ecological unit"(4)

It is also worth recalling that the critical care areas within the hospital including the operation theatres, critical care units, blood banks, transplant units and the emergency rooms can act as areas where there are various influencing factors that can contribute to patient

acquiring infection during the hospital visit. In fact, hospital acquired infections kill more patients every year than do AIDS, breast cancer or road traffic accidents put together. (5)
Among the hospital acquired infections, Postoperative infections constitute a third of all infections.(6).The air in the operation theatre environment is said to have a role in the causation of post-operative infections.(7).The resulting deep surgical infections may at times be complicated, difficult to treat ,may require reoperations and at times may even be life threatening.(8) Infection prevention measures that need to be practiced to avoid such situations therefore rests not only with the operating personnel but would also involve the entire infection control team and the role of the clinical microbiologist becomes critical in terms of monitoring air quality and giving inputs to the HICC and also the engineering and the technical support staff who help in maintaining the accepted standards in terms of air quality in the operation theatres. It is in fact estimated that a 13 fold reduction in airborne bacteria in the operating rooms would reduce wound contamination by around 50%.(9,10)

The complex hospital environment and the OT environment in particular therefore requires special attention to ensure a healthy indoor air quality and an efficient monitoring system in place to protect patients and health care workers against possible hospital acquired infections and occupational diseases.

The monitoring of bioaerosol contamination in critical care areas like operation theatres becomes even more important as it is subject to many variables which would influence the outcome in terms of air quality including mechanical ventilation, filtration, differential pressure control, directional airflow control, local exhaust ventilation and use of ultraviolet germicidal irradiation for disinfection etc; in addition to the usual problem of trafficking of personnel and deviation from accepted guidelines which would contribute to compromising on indoor air quality. A pioneering study by Charnley and colleagues showed that reduction of airborne contamination by improving OR ventilation drastically reduced the rates of post-operative infections.(15)

These issues bring into focus the need to monitor indoor quality of air by the use of sampling techniques although it still remains a fact that there are no specific regulatory requirements mandated by law to ensure adequate control of biocontamination in the so called “Clean rooms”.

In fact a study by Freiberg and colleagues (18,19) have proposed that sedimentation plates represent a technically easier method than air sampling and when correctly used is a more realistic indicator of airborne bacterial contamination near the operating table as air sampling captures small particles that might be removed by ventilation whereas sedimentation plates represent larger particles which resist removal and may pose a risk of an SSI.In fact tere are also opinions which suggest the use of using a combination of the settle plate and volumetric air sapling technique when surgical techniques or theatre dress are to be validated

There have also been recommendations that particle counting be used as a routine procedure instead of microbiological sampling for routine monitoring of air in OTs. However there have also been suggestions that there is no correlation between particle and bacterial counts in in conventionally ventilated OTs with HEPA filters, indicating that particles may not always be of microbial origin.

It is a fact that volumetric sampling produces quantitative data that are more readily conceptualized and seems more relevant to situations in which a pathogen is inhaled and has been used as a standard for rigorous studies. However settle plates are said to be more appropriate for settling organisms and such sampling can be performed without special

equipment or expertise. Unfortunately there are few side by side surveys of air sampling using volumetric and settle plate methods.(25)

In this context , a study was carried out to conduct air quality monitoring in operation theatres using the conventional settle plate method and an air sampling device and also to compare the test results obtained using the two methods which are the common methods used in most hospitals for surveillance of air quality in operation theatres .

METHODOLOGY: The air quality surveillance in the operation theatres were performed simultaneously using both the conventional settle plate technique and an air sampling device as per standard protocols. A total of 9 operation theatres were subjected to 4 surveillance cycles with a minimum of 2 recordings in each of the theatres.

The study was carried out in the operation theatres of the hospital before the start of the scheduled surgeries and adequate care was taken to ensure that there was no trafficking in these areas while the sampling procedures were completed. The air flow system was kept on for a period of 1hr before the entire procedure was carried out.(16) Standard op erating procedures (SOP) were followed at the operation theatres to monitor the bacterial load by the settle plate method and the air sampling device.(11)

In the settle plate method of sampling, Blood agar plates were appropriately labeled with respect to the date, time and period of exposure and placed at different areas in the operating rooms with the lids open for a period of 30 minutes and later closed. The plates were similarly also used to sample the air using the air sampler at the same sites as was done for the settle plates. Both the sets of plates after the procedure were closed and incubated at 37 degrees Celsius for 24hrs.

After incubation, the colonies on each plate were counted and recorded as the number of bacteria carrying particles settling over the area of the plate in a given period of time.
The level of bacterial contamination of air is usually expressed as the number of bacteria carrying particles per cubic millimeter or cubic feet. In studies concerned with airborne infections of man, the particles counted are those that carry bacteria capable of growth on blood agar during aerobic incubation for 24-48hrs at 37 degrees centigrade.(11)

The comparison between the air sampling technique and the settle-plate technique was determined and the number of microorganisms expressed as cfu/m3 was estimated for the settle plate technique using Koch's sedimentation method according to Polish standard PN89/2-04088/08,according to which,

Cfu/m3 = a.x1000 / p x.t.x 0.2 where
a= the number of colonies on the petri plate

p= the surface measurement of the of the plate used

t= the time of exposure of the petriplate.(13)

For the interpretation of results using the air sampling device, the number of colonies on the agar plate were counted and the positive hole conversion table was used to record the corrected no. of colonies (Probable Statistical total) and then if the sampled volume of air is 500 liters. ie. Sampling for 300 secs, the cfu was calculated per cubic mm as Probable statistical total using the positive hole conversion table/0.5. (21)

Descriptive statistics comprising of mean and standard deviation was used to describe the average number of cfu at two different locations within the ot.

Independent student's test was applied to compare the statistical significance in the differences of the mean cfu counts at the two different locations. Level of significance was fixed at 5%.

Picture - 1. Air -Filters – Operation theatres

Picture - 2. Outlet – Duct

Pictures – 3 & 4. Preparation of Culture plates – Settle plate & Air sampling device.

Pictures – 5 . Air sampling using the device.

Pictures – 6 . Air sampling using the device.

Picture : 7 - Air sampling device

Picture :7 - Test – Results – Left : Settle plate technique.

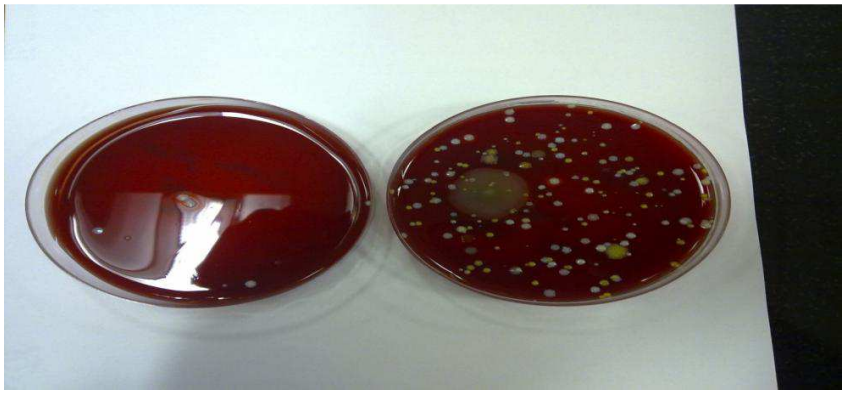


Table 1: Descriptive values of CFU count by different methods at two locations

Group 1 (Allocation more than 30 cms within the OT)				
	Minimum	Maximum	Mean	Std. Deviation
Settle plate – No.of colonies	4	18	9.19	3.143
Settle plate - Calculated Cfu / mm3	7	33	17.11	5.845
Air_sampler- No.of colonies	52	112	77.67	13.527
Correction factor-No.of colonies, based on Postive Hole conversion table	54	121	82.06	15.090
Air_sampler- Calculated- Cfu / mm3_	108	242	164.11	30.180
Group 2 (Allocation within 30 cms of the OT table in the OT)				
Settle plate – No.of colonies	4	12	7.08	1.763
Settle plate - Calculated Cfu / mm3	7	22	13.11	3.370
Air_sampler- No.of colonies	40	102	64.56	14.632
Correction factor-No.of colonies, based on Postive Hole conversion table	41	110	68.92	16.384
Air_sampler- Calculated- Cfu / mm3_	82	220	137.83	32.767

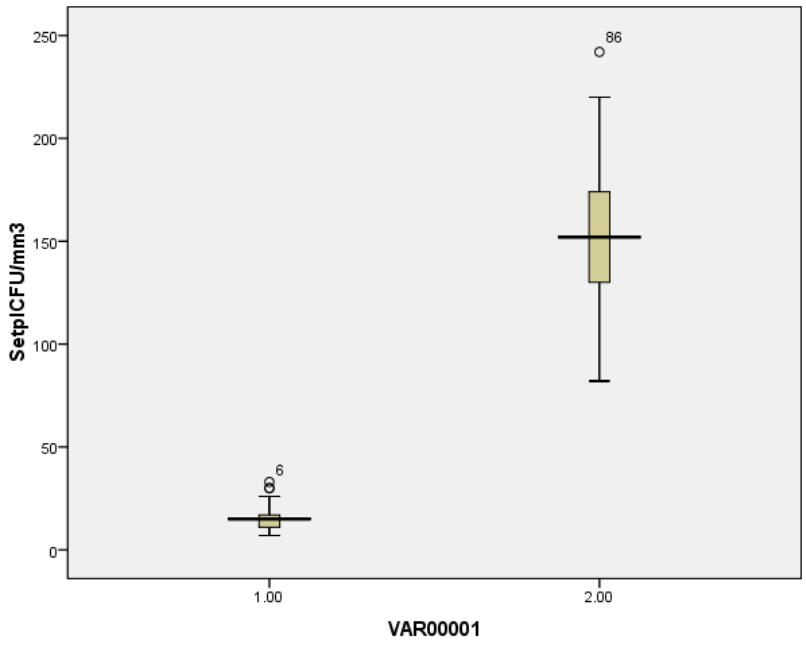
Table 2: Comparison of Mean Cfu counts using two different methods of surveillance of air quality :

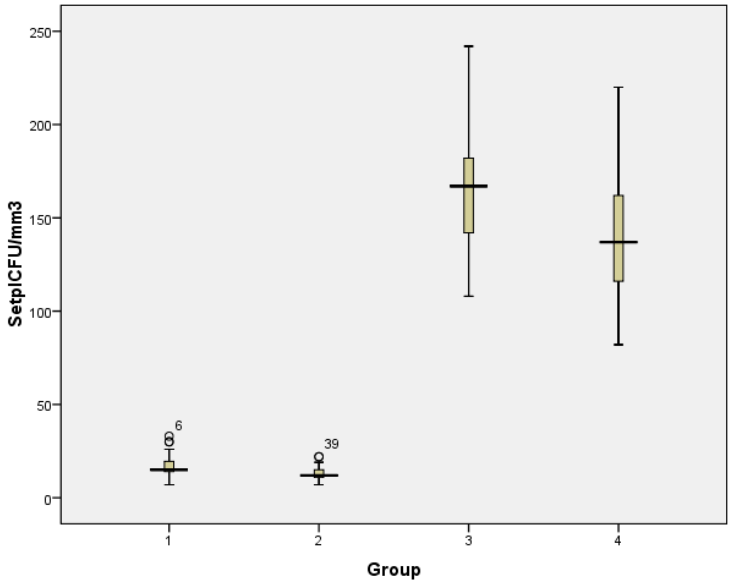
Group	Mean	Std. Deviation	Std. Error Mean
Settle Plate	15.11	5.147	.607
Air Sampler	150.97	33.961	4.002
N=72, Student t test, P<0.001			

From the above table it is observed that the mean CFU count using settle plate technique was 15.11 (5.14) where as the same using the Air sampling device was 150.97 (33.961). This difference in mean CFU count between the two techniques was found to be highly statistically significant (P<0.001)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		
					Lower Bound	Upper Bound	
1	36	17.11	5.845	.974	15.13	19.09	
2	36	13.11	3.370	.562	11.97	14.25	
3	36	164.11	30.180	5.030	153.90	174.32	
4	36	137.83	32.767	5.461	126.75	148.92	
Total	144	83.04	72.337	6.028	71.13	94.96	

From the above table the mean (SD) CFU is _72.337 cfu/mm3 . Only the difference in the mean CFU count at less than 30 cm and more than 30 cm in the OT using settle plate was not found to be statistically significant with all the other mean values differeing significantly using Analysis of Variance (ANOVA) p <0.05.





Groups :

1- Settle plate method at > 30 cms in the OT
2- Settle plate method at <30 cms in the OT
3- Use of Air sampler at >30 cms in the OT
4- Use of Air Sampler at < 30 cms in the OT

RESULTS AND DISCUSSION: Microbiological sampling of air in health care facilities remains a controversial issue because of currently unresolved technical limitations, concrete guidelines and policies and the need for substantial laboratory support. It has been a long debated issue that airborne sources of possible bacterial contamination of the environment in operation theatres can be potential threat for being an important cause in increasing the incidence of surgical site infections. This can contribute to the already existing burden of nosocomial infections in a health care set-up. The morbidity due to a surgical site infections and the additional health care costs can be taxing to the patients undergoing surgical procedures ,and it is the responsibility of the clinical microbiologist as a part of the infection control team to reassure the operating surgeons and the engineering department in charge of maintenance of the ventilation systems installed in these areas that the air quality remains within the acceptable norms. Following redesign and construction of a new theatre-complex ,air quality surveillance in the operation theatres were performed simultaneously using both the conventional settle plate technique and an air sampling device as per standard protocols and the bioburden was calculated using accepted norms.

The study was undertaken to perform air quality surveillance of the operation theatres following redesign and construction of the new OT complex and to determine the level of bacterial contamination using two sampling techniques – one a conventional method of settle plate technique which is considered to be out dated but still used to perform surveillance in many hospitals.

It is important to realize that monitoring the quality of air in critical care areas especially the operation theatres where the surveillance process helps in maintaining accepted levels of air quality also is said to have an influence on reduction in surgical site infections and

in turn on the rates of nosocomial infections prevalent in a health care set up. It helps in monitoring the capability of the air filters used in the OT's and also helps in assessing quality and making timely changes in measures that need to be adopted in order to maintain air quality in these areas.

The level of bacterial contamination of air is usually expressed as the number of bacteria carrying particles per cubic millimeter or cubic feet. In studies concerned with airborne infections of man, the particles counted are those that carry bacteria capable of growth on blood agar during aerobic incubation for 24-48hrs at 37 degrees centigrade.(11)

Although there are no rigid guidelines with respect to either the method or the level of air contamination in the operation rooms, it is generally accepted that the levels should be within 180cfu/m3 using the air sampling device and 35 cfu/m3 for the settle plate method.(14,15,20)

In the present study, the comparison between the air sampling technique and the settle-plate technique was determined and the number of microorganisms expressed as cfu/m3 .
Using the settle plate technique, it was estimated that the mean cfu /mm3 was found to be 17.11 and 22cfu/mm3 at less than 30cm and at a point more than 30cms of the operating table in the operation theatre where as the corresponding means using the air sampler was137.83 and 164.11cfu/mm3 respectively and was found to be highly statistically significant.

This shows the extent of difference in the test results using the two methods and conclusion in terms of certifying the areas as being microbiologically safe or within acceptable limits. The difference should be able to convince health care professionals, the personnel involved in maintenance of the filter systems and those dealing with the management and monitoring of Hospital infection control practices and policies and the authorities involved with infrastructure and hospital administration to definitely changeover to the current practice of monitoring air quality using the air sampling device rather than the traditional method of using settle-plates which still remains the practice in many health care centers.

Though settle plate method may be regarded as a crude measure of airborne contamination, in places without other facilities it can still provide a simple and cost effective way of enumerating the contamination rate of horizontal surfaces at multiple points. Although there is no need to routinely perform surveillance cultures in operation theatre's (22),there are evidences which suggest that there is a need to monitor the air quality which may help in reduction of post-operative complications due to infection. Air samplers allows sampling over a short time period comparatively and hence can indicate the occurrence of individual events like influx of excess personnel and unnecessarily opening the door. These events are lost while using settle plates since sampling is done for comparatively longer time periods. To date, there are no standardized methods available in the country for air sampling in operation theatres or for its frequency. . Routine bacteriological surveillance of operation theatres are nor advocated as results apply only to the moment and location they were obtained at(10,8,17).

At present, there is no international consensus on the methods, types of samples
(settle plate versus, volumetric air sampling)frequencies of sampling and tolerable limits of bioburden in Operation theatres. However it is recommended by most infection control practitioners that rather than taking routine surveillance cultures, bacteriological sampling may be required in situations of commissioning of new operation theatres either after new constructions or after engineering modifications, for investigation of epidemics of surgical site or for validation of changes of either products used or procedures followed in maintenance of operation theatres in terms of cleaning, disinfection or ventilation and for purpose of education.

It would also be useful to have a annual record of the airflows and the bacterial counts for each operating theatre which may be useful as a comparison in future.(24).

ACKNOWLEDGEMENT: Dr. Nandakumar, Associate Professor- Department of Community Medicine– For Statistical analysis of the study. E-mail-Id: bsnandakumar@gmail.com

REFERENCES :

1. 1.Riley,R.L.,1974.Airborne infection.Am.J.Med.,57:466-475
2. 2.Parker,M.T.,1973.Transmission in Hospital IV.International symp.Aerobiol.oostheck publ,co.utrecht,Netherlands:428-444
3. 3.C.,1970. Intramural spread of infection-airborne or not? Pro C.III Int,sympo Aerobiol.El.D.H.Silver Academic Press : 94-95
4. Riley,R.L.,1974.The ecology of indoor atmosphere airborne infection in hospital.J.Chronic Dis.,25:421-423
5. Childs, D. Hospital infection kills more than Cors,AIDS and Breast cancer.Available at:http://www.rense.general74/hosp.htm
6. Pench GL,Cheng AFB,Wang SL and Donnan S . Repeated prevalence surveys formonitoring effectiveness of hospital infection control. Lancet :1964:2:1021-1023.
7. Hart D. Pathogenic bacteria in air of operating rooms.Arch Surg:1938:37:4:521-530.
8. Dharan, S ., Pittet, D. Environmental controls in operation theatres. J. Hosp Infect, 2002; 51: 79-84
9. 9.Whyte,W., Hambraeus, A., Laurell,G., Hoborn, J. The relative importance of routes and sources of wound contamination during general surgery. II . airborne . J. Hosp Infect, 1992; 22: 41-54.
10. Mangram, A.J., Horan, T.C., Pearson,M.L., et al. Guideline for prevention of surgical site infection,infection control and hospital epidemiology.Am.J Infect Conrol, 1999;27: 97-134
11. Charnley,J., Efthekhar,N. Post-operative infection in total prosthetic replacement arthroplasty of the hip joint.Br J Surg,1969;56:641-649.
12. Friberg,B., Friberg,S.,Burman,L.G.,Inconsistent correlation between aerobic bacterial surface and air counts in operating rooms with ultra clean laminar airflows: Proposal of new bacteriological standard for surface decontamination.J Hosp Infect, 1999;42:287-293.
13. Friberg,B., Friberg,S.,Burman,L.G., Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation:an experimental study. J Hosp Infect, 1999;42:61-68.
14. Frank.S.Rhame., The inanimate environment,Text book of Hospital Infections-John.V.Bennet,Philip.S.Brachman,.,Lippincot-Raven publishers,4th edn:302
15. Purva Mathur., Text book of Hospital acquired infections,prevention and control,Lippincott Williams & Wilkins 1st Edn;2010:256
16. Mehta G. Microbiological surveillance of operation theatre. availablefrom:http://www.orthoteers.org/(S(yp4gi4eh11f1mm45pyosvuio))/mainpage.aspx?article = 372
17. C. Pasquarella*, O. Pitzurra† and A. Savino* Journal of Hospital Infection (2000) 46: 241–256.

18. Air petri sampling system – for monitoring the microbial quality of critical environments – Instruction catalogue, Himedia laboratories Private Limited.
19. ,
20. Parker MT. Hospital – acquired infections: Guidelines to laboratory methods, WHO Regional Publications, European Series No.4. (WHO Regional Office for Europe Copenhagen)1978,28.
21. Malathi Jambulingam[1], Suresh Kumar Parameswaran[2], Sagar Lysa[2], Margarita Selvaraj[1], Hajib N Madhavan[1] ., A study on the incidence, microbiological analysis and investigations on the source of infection of postoperative infectious endophthalmitis in a tertiary care ophthalmic hospital: An 8-year study.**,**Indian Journal of Ophthalmology,2010:58:4:297-302
22. Design and maintenance of health care facilities -Manual of "Infection Control Procedures" – N.N.Damani,2nd edn.,p 17-24.
23. Prevention of surgical site infections -Manual of "Infection Control Procedures" – N.N.Damani,2nd edn.,p 245-257.
24. Smyth,E.T,M.,Humphreys,H., Stacey,A.et al. Survey of operating theatre venlilation facilities for minimally invasive surgery in Great Britain and Northern Ireland: Current practices and considerations for the future.J Hosp Infect 2005;61:112-122
25. Design of wards and specialized units – Operation theatres, Manual of Hospital infection control-setting up a cost effective programme,Shaheen Mehtar,Oxford university press,1992, 139-140.

MICROFILARIA IN PREAURICULAR SUBCUTANEOUS NODULE ON CYTOLOGY– AN UNUSUAL PRESENTATION.

Kanade. U. S, Dantkale. S. S, Chaware. S. A, Narkhede. R. R, Gadhekar. M. S

1. Assistant Professor, Department of Pathology, Government Medical College, Latur, Maharashtra.
2. Associate Professor, Department of Pathology, Government Medical College, Latur, Maharashtra.
3. Assistant Professor, Department of Pathology, Government Medical College, Latur, Maharashtra.
4. Resident, Department of Pathology, Government Medical College, Latur, Maharashtra.
5. Resident, Department of Pathology, Government Medical College, Latur, Maharashtra.

CORRESPONDING AUTHOR
Dr. Rahul Narkhede,
Department of pathology, GMC,
Latur -413512, Maharashtra.
E-mail: narkhederahul17@gmail.com,
Ph: 0091 9403939169.

ABSTRACT: BACKGROUND: Bancroftian Filariasis is a tropical and subtropical disease caused by Wuchereria bancrofti and transmitted by the Culex mosquitoes. The diagnosis of it is conventionally made by demonstrating microfilariae in the peripheral blood smear. Microfilariae and adult filarial worm have been incidentally detected in fine needle aspirates of various lesions. We here report a unusual case of Bancroftian microfilariasis in 13 yr old asymptomatic male coming from endemic area with preauricular swelling in subcutaneous tissue. Our aim is to highlight the chances of finding microfilaria in cytology of an unsuspected case at an unusual site.
KEYWORDS: microfilaria, fine needle aspiration cytology, preauricular subcutaneous nodule.

INTRODUCTION: The medical literature documents filariasis back to 600 BC by Sushruta, recognizing the clinical manifestation of elephantiasis which was referred to as elephantiasis arabicum[1]. Filariasis is caused by slender thread like nematodes belonging to super family Filariodea.

Filariasis is endemic in India and South East Asia. Present estimate suggest that over 120 million people in 80 countries are affected by filariasis and more than 1.1 billion people live in areas where there is risk of infection.[2,3] .Despite the large number of people at risk and wide variety of tissues affected, it is unusual to find microfilariae in fine needle aspiration cytology(FNAC) smears.[2]

Individuals having circulating microfilariae are outwardly healthy but have the ability to transmit the infection to others through mosquito bites . Those with chronic filarial infection suffer severely from the disease but no longer transmit the infection.
Diagnosis of filarial infection is frequently made on clinical grounds in endemic areas, but demonstration of microfilariae in circulating blood is the only means by which one can make definitive diagnosis. [2,4]
We hereby report a case of preauricular subcutaneous nodule with microfilaria – an incidental finding on cytology.

CASE REPORT: A 13 yrs old asymptomatic male presented with preauricular swelling of 2x1 cm ,since 1month without any history of pain or fever associated with the swelling .

Peripheral smear did not reveal any significant finding. USG of the swelling was reported as preauricular abscess. X-ray finding and other investigations were normal and patient was nonreactive for HIV infection.

FNAC of the swelling was advised.

Patient did not have any signs and symptoms of filarial infection, and the disease was not clinically suspected.

FNAC of the preauricular swelling was done in aseptic conditions, by 23 gauge needle attached to 10ml disposable syringe. Slides were air dried, fixed, with ether alcohol and stained by hematoxylin and eosin.

CYTOLOGICAL FINDING : Fine needle aspiration cytology (FNAC) of the swelling revealed hemorrhagic aspirate with few scattered microfilariae in background of few lymphocytes, neutrophils and eosinophils . Morphologically the microfilaria showed presence of hyaline sheath(Figure 1), cephalic space length: breath ratio of 1:1 . Nuclei were spherical, regularly placed, appeared in row, well separated without any overlapping (Figure 3) and absent at cephalic end and tail tip.(Figure 2& 4).
On FNAC, diagnosis of microfilaria of Wuchereria bancrofti in subcutaneous nodule was given.

DISCUSSION: Filariasis is a major public problem in tropical countries, especially India, China, Indonesia and parts of Africa. Current estimates indicate that there are at least 6 million attacks of acute filariasis per year and more than 20 million people have one or more chronic filarial lesion.[2]The international task force for disease eradication has identified lymphatic filariasis as one of the six diseases considered eradicable or potentially eradicable.[5]

Clinically , filariasis can be of two major categories , - filariasis of skin and subcutaneous tissue and lymphatic filariasis. Onchocerca volvulus and Loa loa are most common organisms reported in former , and Wuchereria bancrofti and Brugia malayi are the 2 most common species in latter. [6] In the present case microfilaria of Wuchereria bancrofti was seen in subcutaneous nodule.

The life cycle of Wuchereria bancrofti is found in two hosts. Man is definitive host and mosquito is an intermediate host. [7] Adult worm resides in lymph node where the gravid female release a large numbers of microfilariae. These larvae pass through the thoracic duct and pulmonary capillaries to the peripheral circulation . [8]

The microfilariae of W. Bancrofti is detected by fine needle aspiration cytology (FNAC) at different sites like breast, thyroid, lymph node, liver, lungs and few cases have been reported in bone marrow and body fluids; but subcutaneous nodule is a very rare presentation. [7,9,10,11] and the appearance of microfilara in preauricular nodule can be considered as an rare site and to best of our knowledge no case has been reported at this site.

In our case subcutaneous nodule aspirates showed sheathed microfilaria, W. Bancrofti was confirmed by absence of nuclei in cephalic and tail end and was thus differentiated from W. Loa Loa.

Subsequent examination of night blood smear from patient failed to demonstrate microfilariae which is in accordance with the reports by other authors[2,12] thus suggesting that filaria can exist without microfilaremia. Blood eosinophil

counts were within normal range in our case, as also reported by Varghese *et al.*[12] Majority of cases in endemic regions neither show microfilariae in blood, nor any symptom. [2,3]

Patient was referred to clinician and he responded to Diethylcarbamazine (6mg/kg) for 21 days.

CONCLUSION: The main purpose of this case report is to raise the awareness that in tropical countries like India where filariasis is endemic , it should always be considered as a differential diagnosis of swelling at any site . Our presentation revealed that microfilaria may even be present at rare site like preauricular subcutaneous nodule. Careful examination of cytological smears is very important in prompt recognition of the disease and institution of specific treatment especially in unsuspected and asymptomatic cases.

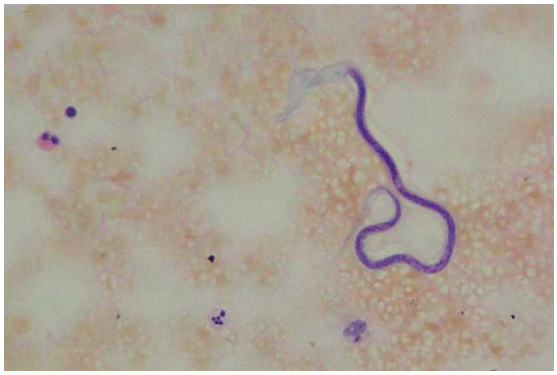


Figure 1: Microfilaria of Wuchereria bancrofti. The microfilaria is sheathed, with eosinophil in left corner.(H&E,40x)

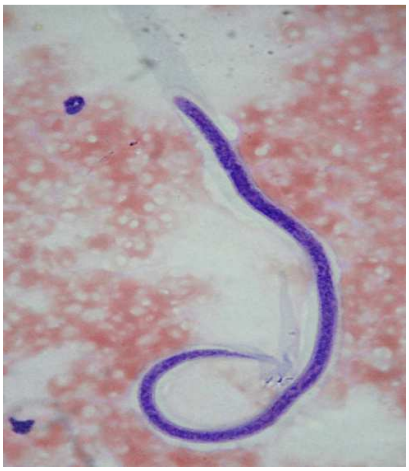


Figure 2: Microfilaria of Wuchereria bancrofti with few inflammatory cells in background. Note the cephalic and tail tip free from nuclei.(H&E,100x)

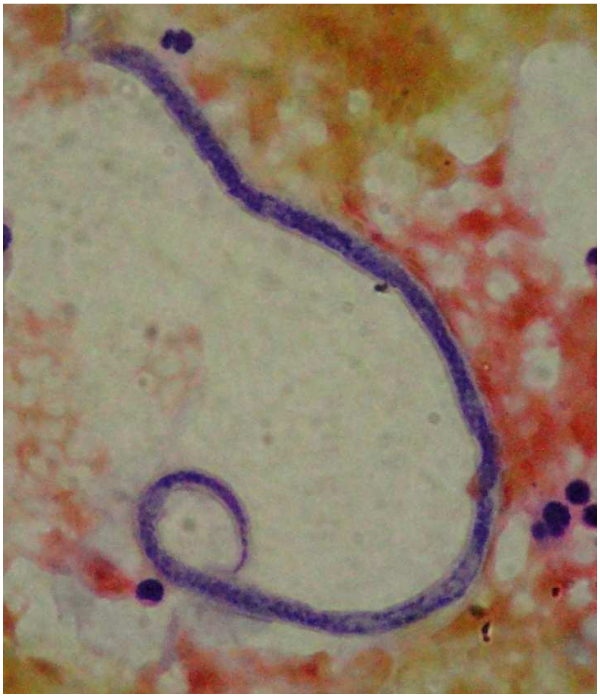


Figure 3: Microfilaria of Wuchereria bancrofti with spherical nuclei which are regularly placed without overlapping (H&E,100x)

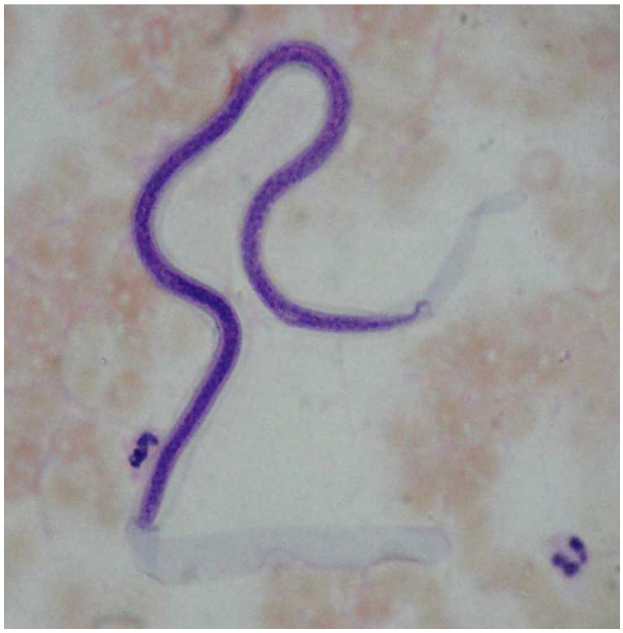


Figure 4: Microfilaria of Wuchereria bancrofti. The microfilaria is sheathed, its body is gently curved, and the tail is tapered to a point and nuclei do not extend to the tip of the tail (H&E,100x)

REFERENCES :

1. Rupinder Kaur, Kandathil Joseph Phillip, Kanwal Masih, Rajeev Kapoor, Cecil Johnny; Filariasis of the Breast Mimicking Inflammatory Carcinoma. Labmedicine 2009;40:683-85
2. Sivakumar S. Role of fine needle aspiration cytology in detection of Microfilariae: Report of 2 cases. Acta Cytol 2007;51:803-5
3. Park K. Text Book of Preventive and Social Medicine. 18ed Jabalpur, India2005: M/S Banarsidas Bhanot Publisher;. p. 211-6.
4. V Rawat, G Rizvi, N Sharma, H Pandey. An unusual presentation of Wuchereria bancrofti infection. Indian journal of medical microbiology ,letter to editor, 2009 ; 27 : 382-383
5. Sabesan S, Raju HK, Srividya A, Das PK . Delimitation of lymphatic filariasis transmission risk areas. Filaria J 2006;5:12
6. Mallick MG, Sengupta S, Bandyopadhyay A, Chakraborty J, Ray S, Guha D. Cytodiagnosis of filarial infections from an endemic area. Acta Cytol 2007;51:843–849.
7. Arora D R, Arora B, Medical parasitology, 2nd ed. SDR: Delhi; 2005. p. 184-90.
8. Kishore B, Khare P, Gupta RJ, Bisht SP Microfilaria of Wuchereria bancrofti in cytologic smears: a report of 5cases with unusual presentations Acta Cytol. 2008 Nov-Dec;52(6):710-2.
9. Valand AG, Pandya BS, Patil YV, Patel LG. Subcutaneous filariasis: An unusual case Report. Indian J Dermatol 2007;52:48-9
10. Kumar M, Shukla VK, Gupta S. Incidental detection of microfilariae in cytological smears: Clinical examples. J Trop Med Hyg 1991;94:110.
11. Dey P, Walker R. Microfilaria in fine needle aspiration from skin nodule. Acta Cyol 1994;38:114.
12. Varghese R, Raghuveer CV, Pai MR, Bansal R. Microfilariae in cytological smears: A Report of six cases. Acta Cytol 1996;40:299-30.

TOPICAL METRONIDAZOLE AND SUCRALFATE CAN REDUCE PAIN AFTER SURGERY AND PAIN ON DEFECTION IN POSTOPERATIVE HAEMORRHOIDECTORY.

S. K. Gahlot, S. K. Katiyar

1. Assistant Professor, Department of Anesthesiology, Major S.D. Singh Memorial Medical College & Hospital Farrukhabad.
2. Assistant Professor, Department of surgery, Rama Medical College Hospital & Research Centre, Kanpur

CORRESPONDING AUTHOR
Dr. S. K. Katiyar M. S,
FIAGES, 675, Singhpur,
Kanpur-208017,
E-mail: Drskkatiyar59@rediffmail.com,
Ph: 0091 09415044878.

ABSTRACT: Topical metronidazole (10%) and sucralfate (7%) cream has been previously demonstrated to decrease postoperative pain after hemorrhoidectomy. The aim of this study was to evaluate the effect of topical metronidazole(10%) and sucralfate (7%) cream in reducing postoperative and after defecation pain of hemorrhoidectomy. A total of 60 patients aged 25-65 Years were studied. Patient were divided into 2 group patient in group A received topical metronidazole (10%) and sucralfate (7%) in group B received placebo (petrolatum cream) pain was assessed using a visual analog scale preoperatively and postoperative hours 5 hrs, 10 hrs and days 1, 3, 7, 14 the use of narcotics, analgesics and complication were recorded. Patient in group A had significantly less postoperative pain than those in group-B upto 14 days ($p<.04$) in group-A after defecation pain was ranked significantly lower at day 3. Patient required lesser analgesics postoperatively on day 3 and 7 ($P<.04$) We concluded that topical (10%) metronidazole and (7%) sucralfate significantly reduce post hemorrhoidectomy pain and postoperative defecation pain compared with that of the placebo control group.
KEY WORDS –Metronidazole, sucralfate, Post hemorrhoidectomy, topical

INTRODUCTION: Pain is a major complication after a hemorrhoidectomy, pain management in patient is an important matter. In identifying approaches to reduce pain after a hemorrhoidectomy published studies have mainly focused on the choice of surgical techniques or prevention of secondary infection.

Both open and closed hemorrhoidectomy have been evaluated in terms of post surgical pain with antibacterial properties metronidazole has been used to diminish bacterial infection at surgery site.

In prior study, local anesthetics (bupivacaine) anti inflammatory drugs, nitrates, Lidocain aerosol have been evaluated for reducing pain after a hemorrhoidectomy these studies indicate some limitation with this medication. Such as short duration of action and serious side effects metronidazole and sucralfate in topical from has been more effective in reducing pain.

Topical sucralfate reduces pain post hemorrhoidectomy It strong adhere to damage tissue and acts as a physical barrier and protects damage tissue and facilitates healing and reduce pain.

Pain reducing with metronidazole is related to its antibacterial activity during days after hemorrhoidectomy but metronidazole's anti inflammatory action may be also beneficial for pain reduction in hours after surgery.

The purpose of this study was to evaluate the effectiveness of topical metronidazole (10%) and sucralfate (7%) in reducing postoperative pain and pain on defecation after an open hemorrhoidectomy.

MATERIAL AND METHODS: This prospective study was conducted on 60 patients admitted in surgical wards between July 2011 and June 2012. Double blinded study evaluated the role of topical metronidazole (10%) and sucralfate (7%) on post operative pain in 60 patients who underwent open hemorrhoidectomy for grades II, III and IV hemorrhoids.

Preparation of ointment- liquid paraffin was chosen as the best levigating agent and (10%) metronidazole and (7%) sucralfate ointment was prepared by incorporation methods in a petroleum jelly base.

The patients were randomized to either metronidazole or sucralfate or placebo treatment. Patients were recommended to apply 3cm of topical metronidazole (10%) and sucralfate (7%) or placebo ointment on surgery site three times daily after a sitz bath.

Post operative pain was evaluated using a self administered visual analog scale (VAS) score-o (no pain) to 10(very sever).

Pain scores were obtained with in 5 hrs, 10 hrs post surgery and at days 1, 3, 7, 14 pain on defecation was also recorded using VAS. Patients were asked about analgesic requirement and for any side effect.

STATISTICAL ANALYSIS: Quantitative data were compared between groups using t test qualitative data were compared using chi-squared test.

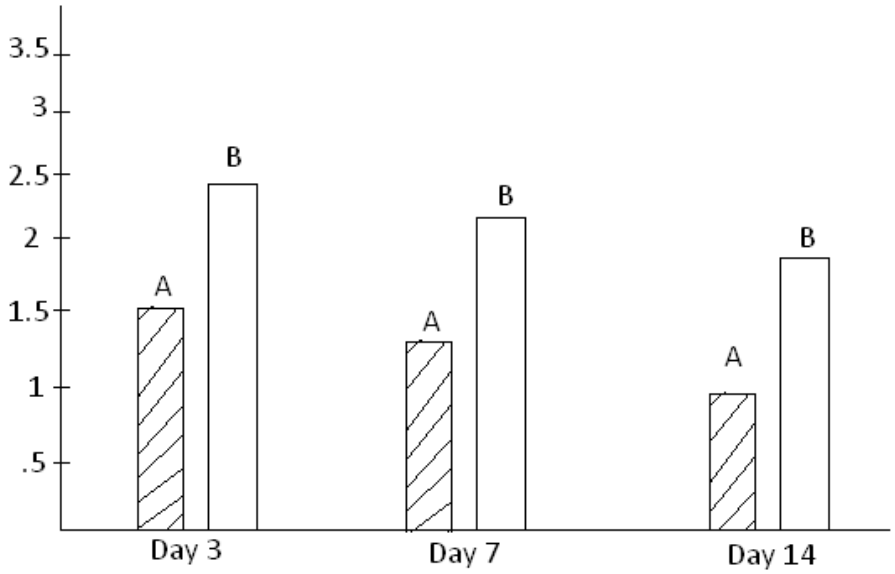
Table 1-Post surgery pain scores in group-A & group-B at 5 hrs, 10hrs, day 1, day 3 day 7, day 14 pain scores ranged from 0-No pain
10-sever pain

	5 hrs	10 hrs	1 day	3 day	7 day	14 day
Group A	5	3	3	2	1	5
Group B	9	7	5	4	3	2

Table 2- Pain on defecation group-A & group-B at day 1, day 3, day 7, day 14 pain scores ranged from 0-No pain
10-sever pain

	5 hrs	10 hrs	1 day	3 day
Group A	2	2	2	1
Group B	3	4	3	2

Figure 1- Post hemorrhoidectomy narcotic and analgesic consumption in metronidazole & sucralfate group-A and placebo group-B



RESULTS: Sixty patients were enrolled in the study. 32 patients received metronidazole & sucralfate and 28 patients received placebo both A& B groups were similar in age (45±15) years and (48±15) years, respectively. Most patients resided in rural area.

Patients in metronidazole and sucralfate group experienced significantly less pain at 5 and 10 hrs (P<.04) and (P<.01)

Pain on defecation was significantly lower in metronidazole & sucralfate group on day 3 P=.02 but no

Significant difference was observed on day 7 and 14 (P>0.5, P=.45)

Narcotic consumption in the metronidazole & sucralfate group was significantly less compared to placebo group at 10 hrs post surgery P<.05, additional analgesic was significantly less in metronidazole & sucralfate group on day 3 and 7 post surgery.

DISCUSSION: Post operative pain likely has two major components. Discomfort from incision and discomfort from tissue inflammation caused by infection. In primary hours and days after surgery inflammation is major cause of pain and during days after surgery infection is main causes of pain.

Metronidazole has been shown to reduces post operative pain after open hemorrhoidectomy in resent studies. The efficacy may be in part from bactericidal action in addition to its less under stood anti-inflammatory action.

Topical sucralfate significantly reduce pain at day 7 and 14 after hemorrhoidectomy by its protective action on damage tissue facilitating wound healing.

Compared with the placebo group lower narcotic and oral analgesic consumption in metronidazole & sucralfate group confirms the improved pain management following an open hemorrhoidectomy.

In present study metronidazole & sucralfate topical ointment was efficacious in reducing pain on defecation at 48 hrs after surgery which suggests that inflammation may be interfering with defecation in the initial days post surgery. Overall these results suggest that addition of topical metronidazole (10%) and sucralfate (7%) to conventional pain medication may be efficacious to reduce pain in patient after a hemorrhoidectomy.

REFERENCES:

1. Dis colon rectum 2008 feb:51(2)231-4.epub2007 Dec18 Gupta PJ, Heda ps; et al .
2. Belfour L, et al A randomized double-blind trial of the effect of metronidazole on pain after closed hemorrhoidectomy. Dis colon rectum 2002;45:1186-92.
3. Carapeti EA, Kamm MA, et al double blind randomized controlled trial of the effects of metronidazole on pain after day case hemorrhoidectomy. Lancet 1998;351:169-72.
4. Simelair R, cassute J.et al. Topical anaesthesia with lidocain aerosol in the control of postoperative pain. Anaesthesiology 1988;68:895-901.
5. Nicholson T, et al topical metronidazole (10%) decreases post-hemorrhoidectomy pain and improves healing. Dis colon rectum 2004;47:711-6.
6. Pryn Sj, Cross MM, Murison MS McGinn FP. Postoperative analgesia for hemorrhoidectommy; a comparison between caudal and local infiltration. Anaesthesia 1989;44:964-6.
7. Loder PB, Kamm MA, Nicolls RJ, Phillips RK. Reversible chemical sphincterotomy by local application of glyceryl trinitrate. Br J Surg 1994;81:1386-9.
8. Khubchandani TT, reed JF. Sequelae of internal sphincterotomy for chronic fissure in ano. Br J Surg 1989; 76: 431-4.

ASSESSMENT OF DELIVERY PATTERN AND FACTORS INFLUENCING THE PLACE OF DELIVERY AMONG WOMEN IN EAST KHASI HILLS DISTRICT OF MEGHALAYA.

Himashree Bhattacharyya, Star Pala.

1. Senior Resident Doctor, Department of Community Medicine, NEIGRIHMS, Shillong, Meghalaya.
2. Assistant Professor & I/ c, Department of Community Medicine, NEIGRIHMS, Shillong, Meghalaya.

CORRESPONDING AUTHOR
Dr. Himashree Bhattacharyya,
Department of Community Medicine,
NEIGRIHMS, Mawdiangdiang, Shillong,
Meghalaya- 793018.
E-mail: bhimashre@yahoo.co.in,
Ph: 91 9436985886.

ABSTRACT: BACKGROUND: Maternal health reflects major part of the health status of a particular region, state, country and the whole nation. Each year approximately 8 million women suffer from pregnancy related complications and over half a million women die of these complications. Despite so many efforts, institutional deliveries account only 40.8% of the total deliveries in India and only 24.4% in Meghalaya. Reproductive and health care services often fail to reach those most in need due to several reasons. **AIM OF THE STUDY**: 1.To assess the delivery pattern among women residing in the East Khasi Hills district of Meghalaya. 2. To study the factors influencing the place of delivery. **SETTINGS AND DESIGN**: Community based cross sectional study conducted in two urban and two rural areas in East Khasi Hills district of Meghalaya. **MATERIALS AND METHODS**: The household was taken as the sampling unit. The households were selected using simple random sampling. In the selected household all the females available who have given birth to a child in the last three years were interviewed using a pre tested Proforma. **STATISTICAL ANALYSIS:** Descriptive analysis and tests of statistical Inference (Chi Square test) was done. **RESULTS & OBSERVATION:** A total of 340 women were interviewed. Majority of women 156 (45.9%) were in the age group of 25-35 years. The total number of institutional deliveries was 218 (64.1%) and home deliveries were 122 (35.8%). Out of the total deliveries, 92(27.05%) of deliveries were conducted by untrained personnel and the rest 248 (72.9%) were conducted by trained persons. Some of the socio demographic factors which were observed to be associated with the place of delivery are the age of the women, educational status of both the women and her husband, per capita family income, parity of women and the area of residence. **CONCLUSION:** Increasing the proportion of safe and institutional deliveries not only require better health care services but also attention should be given to people's educational level ,socio economic status, general awareness as well as proper accessibility to the health care services.

KEY WORDS: Maternal, Delivery, Institutional, household, parity.

INTRODUCTION: Maternal health refers to the health of women during pregnancy, childbirth and the postpartum period. While motherhood is often a positive and fulfilling experience, for too many women it is associated with suffering, ill-health and even death. The major direct

causes of maternal morbidity and mortality include hemorrhage, infection, high blood pressure, unsafe abortion, and obstructed labor.[1]

Maternal health reflects major part of the health status of a particular region, state and the whole nation. Abnormal changes during pregnancy, events at the time and the outcome of the delivery is of major essence in deciding the future of the child. The vision of the World Health Organization (WHO) in 'Making Pregnancy Safer' is 'a world in which skilled care at every birth is ensured for all women'. [2]

Despite several decades of global health initiative focused on maternal health, maternal mortality has proven to be an intractable problem. The Millennium Development Goal Indicator of maternal health, the maternal mortality ratio has remained unchanged over the past 15 years, with an estimated mean annual decline of 0.4% since 1990-far short of the progress required to meet the Millennium Development Goal target of 75% reduction by 2015.[3]

Each year approximately 8 million women suffer from pregnancy related complications and over half a million women die of these complications. Of all maternal deaths that occur in developing countries, Africa and Asia have a lion's share of over 90%. [4]For every women who dies during pregnancy, approximately 30 more women suffer from injuries, infections and disabilities during pregnancy or child birth accounting at least 15 million women a year.[2]

In India, among the National Socio Demographic Goals for 2010, certain goals are directed towards safe delivery viz. to achieve 80% institutional deliveries and 100% deliveries by trained personnel by 2010. [5] To promote institutional deliveries, provision has been made under the current Reproductive and Child Health (RCH) programme to encourage round the clock delivery services at PHC's and CHC's, strengthened supply of drugs, skilled man power on contractual basis and transport facilities to assist women in need. [6]But despite so many efforts, institutional deliveries account only 40.8% of the total deliveries in India and only 24.4% in Meghalaya. [7,8]Reproductive health and basic health infrastructure and services often do not reach the remote areas and thus vast numbers of people cannot avail these services .Keeping in view all these factors the present study was conducted with the objective of assessing the delivery pattern and factors influencing the place of delivery in selected Areas of East Khasi Hills district of Meghalaya.

AIM OF THE STUDY:

1. To assess the delivery pattern among women residing in selected areas of East Khasi Hills, district of Meghalaya.
2. To study the factors influencing the place of delivery.

METHODS AND MATERIALS: A Community based Cross Sectional study was conducted in the two urban and two rural areas of East Khasi Hills district of Shillong. These areas were selected as they were the field practice areas of Community Medicine Department of NEIGRIHMS. The urban areas selected were Mawpat and Pynthorbah under Mawpat and Pynthorbah Urban Health Centre. The rural areas selected were Tynring A and Syeiong under Diengpasoh Primary Health Center.

The sample size was calculated by the formula $4PQ/ L^2$, where P=24.4% (Positive character), Q=1-P, L= Allowable error. Considering the percentage of institutional deliveries in Meghalaya as 24.4% from the NFHS III data (2005-2006) and taking 20% as allowable error the sample size was calculated to be 310. We however interviewed a total of 340 individuals in the study.

In the respective areas, the household was the sampling unit. The number of individuals selected from each area was determined by proportionate allocation. In the respective areas the households were selected using simple random sampling after obtaining a list of all the households in the respective area from the ANM. In the selected household all the females available who have given birth to a child in the last three years were interviewed. Data was collected on socio demographic characteristics of the women and reasons for selecting their place of delivery. If no female was present in the selected house at the time of visit, then next house was taken into consideration till the required sample size is fulfilled. If a women had delivered more than once in the last 3 years, then information regarding only the last delivery was obtained to eliminate recall bias. The respondents were informed about the purpose of the study. They were assured about confidentiality. A pre-tested questionnaire was used and the questions asked were in the local language about their demographic data, delivery practices and the associated factors. The p value of <.05 was considered significant. Data analysis was done by descriptive analysis and analytical statistics by Chi Square test using SPSS version 17.0.

RESULTS AND OBSERVATION:A total of 340 women were interviewed .Out of these women 145 (42.6%) women belonged to the age group 15-25 years, 156 (45.9%) women belonged to the age group 25-35 years and 39 (11.5%) women belonged to 35-45 years of age. With respect to the educational status 76(22.3%) women were found to be illiterate, 245(72.0%) women had done schooling and 19 (5.57%) women had done post schooling. With respect to the educational status of the women's husbands 33 (9.7%) were illiterate, 263 (77.4%) of husbands had done schooling and 44 (12.9%) had undergone post schooling. In this study, 95 (27.9%) of women had per capita family income of less than Rs 750, 165 (48.5%) of women had per capita income of Rs 750-1500, 62 (18.3%) of women had per capita income of Rs 1500-3000 and 18(5.3%) of women had a per capita income of Rs 3000-6000.Out of 340 women interviewed 179 (52.6%) of women were from urban area and 161 (47.4%) of women were from rural areas.

As per the type of delivery 300(88.2%) of women were found to have normal deliveries; 39(11.4%) of women had undergone Caesarean Section and only 1(0.2%) of women had forceps delivery.148 (43.5%) women were nullipara and 192 (56.5%) of women were found to be multipara. Out of 340 women studied 40 (11.8%) had complications at the time of delivery whereas 300 (88.2%) of them had no complications.

With regard to the place of delivery out of 340 women, 183 (53.9%) of women delivered in a government hospital, 35 (10.3%) of women delivered in a private hospital and 122 (35.8%) of women delivered at home. Thus the number of institutional deliveries were 218(64.11%) and home deliveries were 122 (35.88%).Out of the total deliveries, 68 (20.0%) of deliveries were conducted by Untrained Dais, 229 (67.4%) of deliveries were done by either staff nurse or doctor, 19 (5.5%) of deliveries were conducted by ANM and 24 (7.1%) of deliveries were done by Others like mother or relative of the women.

The socio demographic variables taken into consideration which may influence the place of delivery are age group of the women, education of the women as well as her husband, per capita income, parity of the women, area of residence- rural or urban.

Age of women and place of delivery: In the present study, a significant association was observed between age group of the mother and place of delivery. [Table 1]

Education and place of delivery: A significant association was also observed between mother's education and place of delivery [Table 2] A significant association was also observed between the husbands education level and place of delivery [Table3]

Income and place of delivery: Most of the mothers belonging to the lower income group had opted for home delivery. A significant association was observed between the per capita family income and the place of delivery [Table4]

Parity and place of delivery: A significant association was also found between parity and home delivery with most of the multi para women going for home delivery [Table5]

Residential area and place of delivery: 22 (18.1%) of women from urban areas delivered at home in comparison to 100 (81.9%) of women from rural areas. A significant association was observed between the area of residence classified into rural and urban and choice of place of delivery. [Table6]

In the present study a significant association was observed between the place of delivery and person conducting the delivery with most of the home deliveries being conducted by untrained persons. Almost all the hospital deliveries were conducted by trained nurses or doctors.[Table 7]

A significant association was observed between complications occurring at the time of delivery and place of delivery. [Table 8].The type of delivery and place are also associated with all the home deliveries being normal deliveries. [Table 9]

The primary reason given by the women for choosing home delivery were traditional attitude 38 (31.40%), Lack of transport 33(27.28%), less expenses 26 (21.48%), familiar surroundings 16 (12.39%), and 9 (7.43%) gave no reasons.

The primary reason given by the women for choosing private hospitals as their place of delivery were better care 14 (40.00%), more comfortable11 (31.42%), less complications 7 (20%) and 3(8.58%) women gave no reasons.

The primary reason given by the women for delivery in government hospitals are better care 66 (35.86%), government initiatives 54 (29.35%), comparatively less expenses 23(12.50%), more doctors 19 (10.32%) and no reason 21 (11.95%).

DISCUSSION: The present study was conducted to assess the delivery pattern and factors influencing the place of delivery among women in East Khasi Hills district of Meghalaya. Majority of women 156 (45.9%) were in the age group of 25-35 years and most of them 245(72.0%) had undergone schooling. The total number of institutional deliveries was 218 (64.11%) and home deliveries were 122 (35.88%). This is high compared to the figures of NFHS III where institutional deliveries were reported to be 40.8% in India and only 24.4% in Meghalaya. [7]This may be due to the reason that the study was conducted in East Khasi Hills district of Meghalaya where access to health care services is better compared to the rest of the state. This can also be attributed to the increasing number of programs related to maternal and child health over time since the NFHS III data was of the year 2005-2006.

Some of the socio demographic factors which were observed to be associated with the place of delivery in our study are the age of the women, educational status of both the women and her husband, per capita income, parity of women and the area of residence.

A significant association was observed between the mother's age and place of delivery with more mother's in the younger age groups going for institutional deliveries. In a study conducted by Carol Wanjira on delivery practices and associated factors among mothers seeking child welfare services in Kenya it was observed that younger mothers were more likely

to utilize skilled attendants during delivery than their elder counterparts. Mothers of the younger age groups are likely to be nullipara and may prefer institutional deliveries. Also the younger mothers may be more aware of the MCH services and benefits and therefore utilize the maternal health services better.

A significant association was also observed between mother's education and place of delivery. In the study conducted by Carol Wanjira on delivery practices among mothers in Kenya, a significant association was found between mother's education and place of delivery.[9] Another study conducted by Sushma Kumari Saini et al in Chandigarh also shows that women with higher education level delivered in the institutes in significantly higher percentage than their counter parts[10]. In a study on maternal education and utilization of maternal and child health services in India using NFHS data by P. Govindasamy and B.M. Ramesh it was shown that education emerges as the single most important determinant of maternal health-care utilization in India when the influence of other intervening factors is controlled.[11]

A significant association was also observed between the husband's education and place of delivery. A study conducted by Joseph Bobby et al in Bangalore city also showed a significant relation between the father's education and place of delivery[12]. This can due to the reason that if the people are educated they have more awareness about the advantages and benefits of a hospital delivery and also of the complications arising out of a unsafe delivery. Therefore they are likely to opt for a safe institutional delivery.

Most of the mothers belonging to the lower income group had opted for home delivery. A significant association was observed between the family per capita income and the place of delivery. A study on Socioeconomic inequalities in use of delivery care services in India conducted by Abdul Salam and S. A. Siddiqui using NFHS II data also revealed that both economic and educational status of women are positively associated with use of maternal health care facilities[13]. Similarly the study by Joseph Bobby et al also showed that families with a low per capita income of less than Rs. 2,500/- preferred home deliveries[12]. A study conducted by Rajendra. R. Wagle in Nepal also showed that low socio-economic status has been found as a predictor for place of delivery[14]. Most of the research consistently shows that high cost is an important constraint to service utilization particularly for the poor .Delivery in private hospitals as well as government hospitals can lead to expenses with regard to travel as well as hospital stay, visits by family members etc which may be difficult in families of lower income group.

A significant association was also found between parity and home delivery with most of the multi para women going for home delivery. A study conducted by Reajendra R Wagle in Nepal also showed a significant association between home deliveries and multiparity[14]. The reason for this can be that the women and the family members are more apprehensive if the women is a nullipara and will take more precautions for safe delivery of the first born child.

It was seen that 22 (18.1%) of women from urban areas delivered at home in comparison to 100 (81.9%) of women from rural areas. The place of residence (Rural or Urban) also had a strong relation to the place of delivery. The study conducted by Rajendra R Wagle in Nepal showed that the distance to the maternity hospital as having a causal role for place of delivery. Long distance from the maternity hospital was found to be significantly associated with home delivery [14].It was seen that women residing in rural areas had difficulty in hospital delivery due to lack of proper transport and other factors at the time. In a study by Amy J Kesterton regarding institutional delivery in rural India a strong association was observed between place of delivery and community access[15]. Hyam Bashour et al in a study on Syrian Women's preferences for birth place observed that the woman's area of residence was

statistically related to women's preference for a doctor's attendance at birth versus a midwife's attendance[16]. Thus, distance to the health facility can be a major barrier in health care utilization.

A marginally significant association was observed between complications occurring at the time of delivery and place of delivery. This is because hospital deliveries are conducted by trained persons and better equipped, so the chances of any complications occurring are lesser compared to home deliveries.

Out of 122 home deliveries, 55.8% were done by untrained dais, 24.5% were done by trained persons (ANM/doctor) and 19.7% were done by others. In a study on socio demographic correlates of place and person conducting the delivery in a rural community of Andhra Pradesh it was observed that untrained dais, trained dais and others conducted home deliveries in 43.5%, 40.3% and 16.2% of cases respectively. [17]The place of delivery also had a strong relation to the person conducting the delivery and the type of delivery. This was obvious as deliveries in hospital are always conducted by trained nurses or doctors and have the back up to conduct all deliveries whether Normal or CS in contrast to home deliveries which are always normal deliveries and mostly conducted by untrained personnel.

The major reason given by people for home deliveries is traditional attitude, Lack of transport, less expenses and familiar surroundings. The major reason for institutional deliveries was better care and government initiatives.

From our study it has been observed that Increasing the proportion of safe and institutional deliveries not only require better health care services but also attention should be given to people's educational level ,socio economic status , general perception, as well as proper accessibility to the health care services. Therefore appropriate steps should be taken for upliftment of the educational and socio economic status, better access to health care and changing the traditional attitude towards the deliveries.

Table 1: Distribution of place of delivery in relation to age of the women

Age	Home	Institutional	Total
	No (%)	No (%)	No (%)
15-25	55 (45.1%)	90 (41.3%)	145(42.6 %)
25-35	46 (37.7%)	110 (50.4%)	156(45.9%)
35-45	21 (17.2%)	18 (8.3%)	39(11.5%)
Total	122 (100.0%)	218 (100.0%))	340(100.0%)

(Chi Sq=8.508 at p<.05,d.f=2, Table value=5.99)

Table 2: Distribution of place of delivery in relation to educational status of women

Education status	Home	Institutional	Total
	No (%)	Number (%)	No (%)
Illiterate	56 (45.92)	20 (9.2%)	76(22.3)
School	65 (53.27%)	180(82.6%)	245(72.0)
Post School	1 (0.81%)	18 (8.2%)	19 (5.57)
	122 (100.0%)	218 (100.0%)	340(100.0%)

(Chi Sq=64.8 at p<.05,d.f.=2, Table value=5.99)
** Post School refers to HS, graduation, post graduation, any degree course undertaken after completing school.

Table 3: Distribution of place of delivery in relation to educational status of women's husbands

Education status	Home	Institutional	Total
	No (%)	No(%)	No (%)
Illiterate	17 (13.9%)	16 (7.3%)	33(9.7%)
School	101 (82.8%)	162 (74.4%)	263(77.4%)
Post School	4 (3.3%)	40 (18.3%)	44(12.9%)
	122 (100.0%)	218 (100.0%)	340(100.0%)

(Chi Sq=17.959 at p<.05, d.f.=2 Table value=5.99)

Table4: Distribution of place of delivery in relation to the per capita family income

Per capita family Income	Home	Institutional	Total
	No (%)	No (%)	No (%)
<750	72 (59.1%)	23 (10.5%)	95(27.9%)
750-1500	39 (31.9%)	126 (57.8%)	165(48.5%)
1500-3000	9 (7.4%)	53 (24.4%)	62(18.3)
3000-6000	2 (1.6%)	16 (7.3%)	18(5.3%)
Total	122 (100.0%)	218 (100.0%)	340(100)

(Chi Sq=94.42 at p<.05, d.f.=3, Table value=7.81)

Table 5: Distribution of place of delivery in relation to parity of women

Parity	Home	Institutional	Total
	No (%)	No (%)	No (%)
Nullipara	38(31.2%)	110(50.4%)	148(43.5%)
Multipara	84(68.8%)	108(49.6%)	192(56.5%)
Total	122(100.0%)	218(100.0%)	340(100.0%)

(Chi Sq=11.51 at p<.05, d.f.=1, Table value=3.84)

Table6: Distribution of place of delivery in relation to the place of residence of women

Area	Home	Institutional	Total
	No (%)	No (%)	No (%)
Urban	22(18.1%)	157(72.1%)	179(52.6%)
Rural	100(81.9%)	61(27.9%)	161(47.4%)
Total	122(100.0%)	218(100.0%)	340(100.0%)

(Chi Sq=90.42, d.f.=1, Table value=3.84)

Table7: Distribution of place of delivery with respect to person conducting delivery

Person	Home	Institutional	Total
	No (%)	No (%)	No (%)
Un Trained Dai	68(56.1%)	0(0%)	68 (20.0%)
Nurse/Doctor	11(9.1%)	218(100.0%)	229 (67.4%)
Trained dai/ANM	19(15.7%)	0 (0%)	19 (5.5%)
Others	24(19.1%)	0(0%)	24 (7.1%)
Total	122(100.0%)	218(100.0%)	340 (100.0%)

(Chi Sq=286.29 at p<.05, d.f.=3, Table value=7.81)

Table8: Distribution of place of delivery in relation to complications at the time of delivery

Complications	Home	Institutional	Total
	No (%)	No (%)	No (%)
Present	20 (16.4%)	20 (9.2%)	40(11.8%)
Absent	102 (83.6%)	198 (90.8%)	300(88.2%)
Total	122 (100.0%)	218 (100.0%)	340(100.0%)

(Chi Sq=3.92 at p<.05, d.f.=1, Table value=3.84

Table9: Distribution of place of delivery in relation to the type of delivery

Type	Home	Institutional	Total
	No (%)	No (%)	No (%)
Normal	122(100.0%)	178(81.6%)	300(88.2%)
CS	0(0%)	39(18.0%)	39(11.4%)
Forceps	0(0%)	1(0.4%)	1(0.2%)
Total	122(100.0%)	218(100.0%)	340(100.0%)

(Chi Sq= 25.22 at p<.05, d.f.=2, Table value=5.99)

REFERENCES:

1. World Health Organization-Maternal Health. Available at www.who.int/topics/maternal_health/en/
2. Garg Rajesh; Shyamsunder Deepti ; Singh Tejbir, Padda Avtar Singh -A study on delivery practices among women in rural Punjab. Health and Population: Perspectives and Issues. Vol.33(1); 23-33;2010
3. Kruk ME, Paczkowski M, Mbaruku G, de Pinho H, Galea S.Women's Preferences for Place of Delivery in Rural Tanzania: A Population- based Discrete Choice Experiment; Am J Public Health. 2009 Sep; 99(9):1666-72. Epub 2009 Jul .
4. UNICEF(2006). A call for quality.In: The State of the World's Children 2007: Women & Children- The double dividend of gender equality. The United Nations Children's Fund (UNICEF),26,127,p5\

5. National Commission on Population (GoI); National Population Policy 2000 Available at population commission.nic.in/npp-obj.htm.
6. RCH II; Programme Objectives and Strategies. Available at www.nohfw.nic.in/NRHM/PIP-69-10/ Rajasthan/RCH %20-text.pdf
7. National Family Health Survey-III; Key Indicators for India. Available at www.chsj.org/uploads/1/0/2/1/10215849/india.pdf
8. DLHS-MoH & FW; GoI; DLHS-3 (2007-2008). Available at www.jsk.gov.in/dlhs3/Meghalaya.pdf
9. **Carol Wanjira, Moses Mwangi, Evans Mathenge, Gabriel Mbugua and Zipporah Ng'ang'a.** Delivery Practices and Associated Factors among Mothers Seeking Child Welfare Services in Selected Health Facilities in Nyandarua South District, Kenya .BMC Public Health 2011, **11**:360 doi:10.1186/1471-2458-11-360. Available at http://www.biomedcentral.com/1471-2458/11/360.
10. Saini Kumari Sushma, Walia Indrajit. Trends in place of delivery among low income community in a resettled colony of Chandigarh, India. Nursing and Midwifery Research Journal, Vol 5, No 4, Oct 2009. Available at www. medind.nic.in/nad/t09/i4/nadt09i4p1333.pdf
11. Pavalavalli Govindasamy, Ramesh B.M.; Maternal education and utilization of maternal and child health services in India. NFHS subject reports No 5; Dec,1997; International Institute for Population Sciences; Mumbai; India.
12. Bobby Joseph, S.R. Sri Krishna, Jisha Philip and Belinda George. Preferences for home deliveries in a suburban community of bangalore city. Health and Population. Perspectives and Issues 25 (2): 96 - 103, 2002. Available at www.medind.nic.in/hab/t02/12/habt02i2p96.pdf
13. Abdul Salam, S A Siddiqui. Socioeconomic inequalities in use of delivery care services in India. J Obstet Gynecol India Vol. 56, No. 2 : March/April 2006 ,Pg 123-127
14. **Rajendra R Wagle, Svend Sabroe** and **Birgitte B Nielsen. Socioeconomic and physical distance to the maternity hospital as predictors for place of delivery: an observation study from Nepal** .BMC Pregnancy and Childbirth 2004, **4**:8 doi:10.1186/1471-2393-4-8. Available at http://www.biomedcentral.com/1471-2393/4/8
15. **Amy J Kesterton**[*], **John Cleland**, **Andy Sloggett** and **Carine Ronsmans.** Institutional delivery in rural India: the relative importance of accessibility and economic status BMC Pregnancy and Childbirth 2010, **10**:30 Available at :http://www.biomedcentral.com/1471-2393/10/30
16. Hyam Bashour et al, Syrian Women's Preferences for Bitrth Attendant and Birth place . PMC- Pub Med Central 2005; March; 32 (1): 20-26. Available at www.nebi.nlm.gov/pmc/articles/PMC 1457105/
17. K. Raghava Prasad, K. Nagaraj; Socio Demographic Correlates of Place of Delivery and person conducting the delivery in a rural community in Andhra Pradesh. J. Human Ecolo; 12(5): 357-362 (2001)

DETECTION & PREVALENCE OF EXTENDED SPECTRUM BETA - LACTAMASES AMONG ENTEROBACTERIACEAE SPECIES FROM VARIOUS CLINICAL SAMPLES AT KIMS, AMALAPURAM.

N. Padmaja, Anand Acharya, P. Nageswar Rao

1. Assistant Professor, Department of Microbiology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.
2. Associate Professor, Department of Pharmacology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.
3. Professor & Head, Department of Microbiology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.

CORRESPONDING AUTHOR
Dr. Anand Acharya,
KIMS Hospital, Amalapuram,
Andhra Pradesh.
E-mail: anand_kims@yahoo.co.in,
Ph: 0091 09963598050.

ABSTRACT: The present study was conducted in the Department of Microbiology, KIMS, Amalapuram, East Godavari from January 2012 to July 2012. Out of 100 different clinical samples, 50 were culture positive. Of the 100 samples collected, more were from post operative wound sepsis - 44 (44%), followed by cellulites - 20 (20%), Ulcers - 17 (17%), Injuries 15 (15%). Least number of cases are from burns - 4 (4%). Among 50 culture positive cases, 38 (76%) isolates belonged to Enterobacteriaceae family, followed by Pseudomonas aeruginosa - 8 (16%), followed by Staphylococcus aureus - 4 (8%). Among 38 of Enterobacteriaceae family isolates, 15 were ESBL producers. Among ESBL positive strains, more drug resistance was seen to Ceftazidime and Ampicillin (93.33%), followed by Ceftriaxone (86.66%), Aztreonam & Cefotaxime (80%).

INTRODUCTION: Hospital outbreaks of multi-drug resistant Enterobacteriaceae are now frequently caused by extended spectrum beta-lactamase (ESBL) producers. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over past years resulting in limitation of therapeutic options.

β - lactamase antibiotics are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use. The most common mode of acquisition of bacterial resistance to β - lactam antibiotics is the production of β – lactamase. However, new β - lactamases emerged against each of the new classes of β – lactamase that were introduced & caused resistance. The latest in the arsenal of these enzymes has been evolution of extended spectrum β - lactamases (ESBLs). These enzymes are commonly produced by many members of Enterobacteriaceae and efficiently hydrolyze oxyimino cephalosporins conferring resistance to 3rd generation cephalosporins like Cefotaxime, Ceftazidime & Ceftriaxone[4].

ESBLs pose a major problem for clinicians. Hence, this study is taken to identify the prevalence of these strains in hospital & to determine suitable preventive measures & treatment policies.

MATERIALS & METHODS: Different clinical samples collected from both inpatients & outpatients of KIMS Hospital, Amalapuram, during January 2012 - July 2012, constitute the material for this study.

A total of 38 Enterobacteriaceae species were isolated from 100 pus samples of different diseases including both sexes and all age groups.

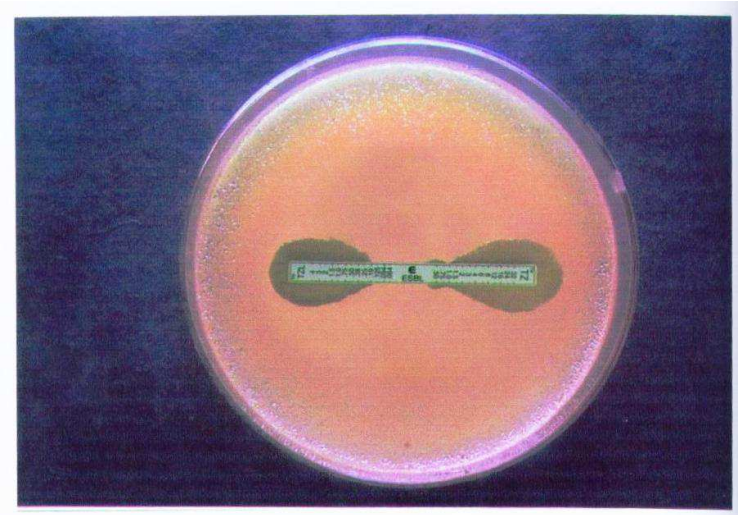
MATERIALS REQUIRED: Specimens were inoculated on to the media – Mac Conkey, Blood agar and incubated at 37^0 C for 18 - 24 hr and identified by Gram's stain, culture, biochemical tests and Antibiotic sensitivity test. For detection of ESBL, ESBL E Strip test is used.

METHOD: E-TEST ESBL SCREEN: (CORMICAN ETAL 1996)[14]: The strip carries two antibiotic gradients Ceftazidime (0.5-35 µg/ml) and Ceftazidime with Clavulanic acid (0.064 - 4 µg/ml). The E test method was carried out according to manufactures instructions.

Colonies from 18-24 hrs. cultures were emulsified in sterile normal saline, incubated for 4 to 6 hrs. to an inoculum turbidity equivalent to 0.5 Mc Farland standard. This suspension was swabbed over on to Muller hinton agar plate and it is allowed to dry for 10 – 15 mins at room temperature. An ESBL E strip was then applied to plate with sterile forceps and the plate was incubated at 37^0 C for 18 hrs. After incubation, the MICs were read according to the manufacturers guidelines and the ratio determined. (The MIC ratio ≥ 8 indicates ESBL production. It is considered Gold standard.[515]

INTERPRETATION OF TEST RESULT: The MIC value was read where the elliptical zone of growth inhibition intersected the E test strip. The MIC of Ceftazidime alone on one end of strip was compared with the MIC of Ceftazidime + Clavulanic acid combination at other end. A greater than fourfold reduction in the Ceftazidime MIC in the presence of Clavulanic acid was taken as positive for ESBL production[10].

For ESBL screening, the antibiotic discs used are Ceftazidime, Cefotaxime, Ceftriaxone, Aztreonam, Amoxycillin+Clavulanic acid, Ceftazidime+Clavulanic acid, Cefotaxime+Clavulanic acid and Imipenem. The incubated plates were read for zone of inhibition and results are interpreted comparing with the standard zone size given by Kirby-Bauer chart[9]



E STRIP TEST FOR ESBL PRODUCTION

QUALITY CONTROL:

Escherichia coli ATCC 25922 - Used as negative control.
In house ESBL producers are used as positive control.

RESULTS:

Out of 100 samples collected, high number of cases are from males 70 (70%) and low number of cases are from females 30 (30%).

S.No	Sex	No. Of Cases	%
1	Males	70	70 %
2	Females	30	30 %

Among 100 sample collected, more were from post-operative wound sepsis - 44(44%), followed by Cellulitis - 20 (20%), Ulcer - 17 (17%), Injuries 15 (15%), Burns 4 (4%).

S.No	Disease	No. of Samples
1	Post operative wounds	44
2	Cellulitis	20
3	Ulcer	17
4	Injuries	15
5	Burns	4

Of the 100 samples processed, 50 were culture positive. Of these, 38 (76%), isolates belonged to Enterobacteriaceae family, followed by Pseudomonas aeruginosa 8 (16%), followed by Staphylococcus aureus - 4 (8%).

Table: 3 Different types of Bacterial species isolated

S.No.	Bacterial Species	Culture Positive cases	Percentage
1	Escherichia coli	21	42 %
2	Klebsiella pneumonia	9	18 %
3	Proteus mirabilis	4	8 %
4	Citrobacter spp	2	4 %
5	Proteus vulgaris	2	4 %
6	Pseudomonas aeruginosa	8	16 %
7	Staphylococcus aureus	4	8 %

Of the 38 Enterobacteriaceae isolates, 15 are ESBL producers.

Table 4: Distribution of Bacteria producing ESBLs

S.No	Bacterial Species	Total isolates	ESBL Producers No.	%
1	Escherichia coli	21	8	38 %
2	Klebsiella pneumoniae	9	5	55.5 %
3	Proteus mirabilis	4	1	25 %
4	Citrobacter spp	2	1	50 %
5	Proteus vulgaris	2	0	-

(Note: Percentage of ESBL producers calculated as per the respective total isolates)

Among ESBL positive strains, more drug resistance was seen to Ceftazidime and Ampicillin (93.33%), followed Ceftriaxone (86.66%) Aztreonam & Cefotaxime (80 %). All ESBL producers showed full sensitivity to Imipenem.

Table 5: Comparison of Antimicrobial Resistance pattern of ESBL producers & Non ESBL producers.

S.No	Drugs	ESBL Positive (15)		ESBL Negative (23)	
1	Ampicillin	14	93.33 %	10	43.47 %
2	Gentamicin	3	20 %	9	39.13 %
3	Amikacin	4	26.66 %	4	17.39 %
4	Ciprofloxacin	4	26.66 %	3	13.04 %
5	Chloramphenicol	5	33.33 %	4	17.39 %
6	Co - trimoxazole	7	46.66 %	12	52.17 %
7	Aztreonam	12	80 %	8	34.78 %
8	Cefotaxime	12	80 %	2	8.69 %
9	Ceftazidime	14	93.33 %	3	13.04 %
10	Ceftriaxone	13	86.66 %	3	13.04 %
11	Imipenem	0	0	0	0

(Note: Percentage of positive and negative ESBL drug resistance pattern calculated as per respective antibiotics tested).

DISCUSSION: ESBLs are the main cause of resistance to beta lactam antibiotics in Enterobacteriaceae. As their occurrence has been increasing it becomes essential to evaluate their occurrence in population

The present study was undertaken to evaluate the pattern of drug resistance produced by isolates of Enterobacteriaceae family causing various diseases especially in hospitalized patients and to known the prevalence of ESBL production.

In the present study, out of 100 pus samples, rate of recovery of isolates belonging to Enterobacteriaceae family is 38. i.e. 76 %. In this study, males (70%) are more commonly affected than females (30%) due to the fact that males are more involved in out door activities & hence chances of infection or injury are more likely.

An outbreak of nosocomial infection due to ESBL isolates of Enterobacteriaceae family occur worldwide in intensive care units as shown by different workers, Yagi et al 2000, Silver et al 2001[4,6,12]. Risk factors associated with ESBL production include – prolonged hospital or intensive care stay, use of multiple courses of antimicrobial therapy, particularly extended spectrum cephalosporins. In the present study, ESBL production is more in Cellulitis cases due to prolonged hospital stay & indiscriminate use of multiple courses of antibiotics. Hence indiscriminate use of cephalosporins and broad spectrum antibiotics should be avoided.

CONCLUSION:

ESBL isolates of Enterobacteriaceae family is major problem world wide. In discriminate use of third generation cephalosporins to treat gram negative bacterial infection is partly responsible for the emergence of resistance to beta – lactam antibiotics. Strict adherence to the hospital antibiotic policy and good infection control practices can play a significant role in reducing the emerging drug resistance

REFERENCES:

1. A. Varaiya, J. Dogra, M. Kulkarni, P. Bhalekar - " Extended spectrum Beta lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in Diabetic foot infection - Department of Microbiology ", S.L. Raheja Hospital, Mahim West, Mumbai - 400016, IJMM, India - 7th August 2008.
2. Subha, S. Ananthan – " Extended spectrum Betalactamase (ESBL) Mediated Resistance to Third Generation Cephalosporins among Klebsiella pneumoniae in Chennai " - Indian Journal of Medical Microbiology, (2002) 20 (2) ; 92-95.
3. A.A. Dashit, P. West, R. Paton and S.G.B. Amyes – Characterization of extended-spectrum Beta-Lactamase (ESBL) - Producing Kuwait and UK strains identified by the Vitek system, and sub sequent comparison of the Vitek system, with other commercial ESBL - Testing systems using these strains" – Molecular Chemotherapy, Centre for Infectious Disease – January 2006.
4. Amit Jain & Rajesh Mondal – "Prevalence and antimicrobial resistance of extended spectrum Beta-Lactamase producing Klebsiella spp. isolated from cases of neonatal septicemia" – Indian J Med. Res, January 2007, PP 89-94.
5. Ankur Goyal, Amit Prasd, Ujjala Ghosal & K.N. Prasad - "Comparison of disk diffusion, disk potentation & double disk synergy methods for detection of extended spectrum beta lactamase in Enterobacteriaceae" – Indian Journal Med Res. 128, August 2008, PP 209-211.
6. Ashwin N Ananthakrishnan, Reba Kanungo, A. Kumar and S. Badrinath - "Detection of extended spectrum Beta-lactamase producers among surgical, wound infections and Burns patients in jipmer" - Indian Journal of Medical Microbiology (2000) 18 (4) : 160-165.
7. Bisson, G.Fishman, N.O. Patel, J.B.; Edel Stein, P.H ; Lautenbanch,E. '' Extended-spectrum beta-lactamase-producing Escherichia coli and Klebseilla species; risk factors for colonization and impact of antimicrobial formulary interventions on colonization", Prevalence, Infection and control, Thorofare, v.23, p.254-260,2002.

8. C. Rodrigues, P Joshi, SH Jain, M Alphonse, R. Radhakrishnan, ''A Metal-detection of Beta-lactamases in Nosocomial Gram Negative Clinical Isolates''- Indian Journal of Medical Microbiology, (2004) 22 (4) : 247-250.
9. Colonder, R.; Raz, R.; Chazan, B.; akran, W. ''Susceptibility pattern of extended-spectrum Beta-lactamase producing bacteria isolated from inpatients to five antimicrobial drugs in community hospital in Northern Israel'', International Journal of Antimicrobial Agents, Shannon, v. 24, p. 409-410,2004.
10. Cormican MG, Marshall SA, Jones RN. ''Detection of spectrum β-lactamases (ESBL) producing strains by E-test ESBL screen'' J. Clin Microbiol 1996: 1880-84.
11. MS Kumar, V. Lakshmi, R. Rajagopalam ''Occurance of extended spectrum Beta-Lactamases among Enterobacteriaceae SPP isolated at a Tertiary care institute'' - Indian Journal of Medical Microbiology (2006) 24 (3) : 208-11.
12. Yagi, T.; Kurokawa, H.; Shibata, N.; Shibayama, K.; Arakawa, Y. ''A Preliminary survey of extended-spectrum Beta-lactamases (ESBLs) in clinical isolates of Klebsiella pneumoniae and Escherichia coli''. FEMS Microbiology Letters, Amsterdan, v.184, p.53,2000.
13. Silva, J.; Gatica, R.; Aguilar, C.; Becerra, Z.; Ramos, U.G.; Velazquez, M.; Miranda, G.; Leanos, B.; Solorzano, F.. Echaniz, G. ''Outbreak of infection with extended-spectrum Beta-lactamase producing Klebsiella pneumoniae in a Mexican hospital''. Journal of Clinical Microbiology, Washington, V.39, p. 3193-3193, 2001.
14. 1996 Aug;34(8):1880-4. Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. Cormican MG, Marshall SA, Jones RN
15. Determination of minimal inhibitory concentration Jinnifer M. Andrews journal of antimicrobial chemotherapy(2001) 48 suppl.s1,5-16.

CHANGES OF SOFT TISSUE FUNCTIONS IN INVIDUALS HAVING FLUOROSIS IN RURAL BANKURA DISTRICT WEST BENGAL.

Debkumar Ray, Papia Mondal, Phalguni Chaktrabarti

1. Assistant Professor, Department of Biochemistry, B. S. Medical College, Bankura, West Bengal.
2. Demonstrator, Department of Biochemistry, B. S. Medical College, Bankura, West Bengal.
3. Demonstrator, Department of Biochemistry, B. S. Medical College, Bankura, West Bengal.

CORRESPONDING AUTHOR

Dr. Debkumar Ray,
Assistant Professor, Dept of Biochemistry,
16 /1, R. B. Ghosh Road,
Khosbagan Burdwan-713101,
E-mail: dr.debkumar@gmail.com,
Phone: 09476233354.

ABSTRACT: The present study was conducted to find out various health hazards related to high consumption of fluoride either by water or food in endemic areas of Bankura district,WB,India.The study revealed high levels of serum/urine fluoride content and relevent clinical outcome like mottling of teeth and skeletal deformity on prolonged exposure of fluoride. Consumption of fluoride causes lowering of haemoglobin and serum protein levels.

There are no significant changes of serum cholesterol whereas circulating testosterone is decreased without affecting the reproductive functions. The increased levels of serum transaminase, shows functional hepatic changes following prolonged exposure of fluoride. The changes in serum calcium, sodium and potassium causes electrolyte imbalance in the exposed individuals without any sex variation. A significant increase in the thyroxine (T 4) suggests an alteration in thyroid function, without any changes of thyroid stimulating hormone (TSH) and tri-iodothyronine (T3), thus the study revealed some deleterious effects of fluoride in the tissue functions of various vital organs of the endemic population.

KEYWORDS: Fluorosis, Soft Tissue changes, Hypothyroidism

INTRODUCTION: Fluorosis deleloped by intake of fluoride by consumable water/food and has been identified in India for six decades[1]. It is a slow, progressive crippling malady, affecting young and old, rural and urban populations with recently attained alarming dimensions. It is widespread in as many as 15 states of the Indian Republic, afflicting some 25 million people [2]. In West Bengal out of 18 districts 4 districts are worstly affected by Fluorosis[3]. Bankura is one of them where near about 1 lakh populations are affected by Fluorosis[3].Agricultural crops, water and food are contaminated with fluoride following high concentration of fluoride in the soil in endemic areas. The Geological Survey of India reveals that topaz, apatite, rock phosphate, phosphate nodules and phosphorite are widespread in the earth's crust in India and contain highest percentage fluoride [5]. As a result of the rich mineral content and high rainfall, fluoride leaches out and contaminates the water and the soil. Fluoride is known to affect the dental and skeletal systems. In addition, studies carried out in the past few years have shown detrimental effects on tissue functions of various organs mal models. Its effect on tissue functions on various vital organs on humans is less understood so our study is to find out the tissue changes on fluoride toxicity. We have carried out surveys in the fluoride endemic areas of Bankura (W.B) by the help of Department of Biochemistry, B.S. Medical College. & Hospital, Bankura, WB, India.

MATERIALS AND METHODS: Five hundred individuals covering fifty-two villages of Bankura district of West Bengal were initially examined. Individual record sheet having name, age, sex, address, source of consumable drinking water , depth of the well or pond, duration of stay in the village, food habits, dental changes and skeletal changes. Sample of consumable water and blood collected from fluoride exposed individuals. Fluoride levels measured from water and blood sample.

SELECTION OF CONTROL INDIVIDUALS: Similar consumable water and blood samples are collected from urban area of Bankura town, WB where fluoride content in water follows permissible limit recommended by Indian Bureau of Standards[6].

The following parameters are studied in Study and Control populations:

Fluoride content of drinking water and blood are determined with an Ion selective electrode, made by Orion Model 9609BNWF, and expressed as ppm (parts per million)[8]. Haemoglobin was determined by Sahli's method hemoglobinometer, and expressed in g%. Serum protein estimated by biuret method and expressed in g/dl [6].

Serum cholesterol determined by Pearson's method and expressed as mg/dl. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) are estimated by Reitman and Frankel"method [7] and expressed as IU (International Unit). Serum. Calcium, sodium and potassium levels were estimated by a Flame Photometer (Systronic digital unit type 125)[8] and expressed in mEq/L.

SERUM HORMONES: Serum Testosterone are assayed by the ELISA method [10] and expressed as ng/mL Serum. Tri-iodothyronine and thyroxine (T3 and T4) are determined by ELISA method and expressed as ng/mL. Thyroid stimulating hormone (TSH) serum levels are determined by the ELISA method and expressed in uU/mL. Serum catecholamines are determined by Von Euler and Hamberg method and expressed ug/mL serum [10].

RESULTS: Surveys conducted in 52 villages of Bankura(W.B) revealed symptoms of fluorosis in the majority of the individuals studied. 74% of the individuals showed slight to severe mottling of teeth. 59% had stiffness of spinal cord. Other skeletal problems such as stiff hands and fingers (60%), stiffness of legs and joints (65%) were also common and 93% of the cases studied having the habit of regular tea intake.

WATER FLUORIDE CONTENT: Water samples from various places of Bankura town showed fluoride levels within the permissible limit (recommended level of fluoride 0.6 ppm) whereas samples collected from endemic villages of Bankura revealed high fluoride contents, ranging in this survey (Table 1) from 1.0 to 6.53 ppm. In 52 villages 18 (35%) had fluoride content below 2 ppm and 26 (50%) had fluoride content within the range of 2-4 ppm while 8 (15%) had fluoride levels above 4 ppm. Bore water samples had higher fluoride concentrations than well water samples [11].

Tables 1-4 present that fluoride exposed individuals, when compared to controls, had significantly increased serum levels ($P < 0.001$) of fluoride, SGOT, SGPT, sodium and potassium, and serum cholesterol levels were essentially the same in both populations.

In this survey, there was an decrease in mean of serum testosterone levels in the fluoride exposed individuals, compared to controls.

SERUM FLUORIDE: In 80 samples analysed, 38% had fluoride concentration < 0.2 ppm; 47% showed fluoride levels in the range 0.2-0.4 ppm; while 15% showed above 0.4 ppm (Table 1). Fluoride concentration increased with age (Figure).

Haemoglobin: levels of the fluoride exposed group showed an insignificant decrease when compared to the control population (Table 2).

Serum cholesterol: levels are essentially the same for both fluoride exposed populations and controls (Table 2).

Serum protein and calcium: Levels of the endemic population showed a highly significant decrease ($p < 0.001$) as compared to the control (Tables 3 and 4).

Serum triiodothyronine (T3) and thyroid stimulating hormone (T5): There was no difference in levels in the fluoride exposed and control groups (Table 5).

Serum thyroxine (T4): levels showed a significant increase ($P < 0.001$) compared to control (Table 5).

Serum catecholamines: The serum adrenalin and nor-adrenalin levels increased significantly in fluoride exposed individuals ($P < 0.001$) compared to controls (Table 6).

TABLE- 1. Water and serum fluoride levels (ppm) in control and endemic population

Parameter	Control	Endemic Population
Water fluoride	0.638 ± 0.013	2.70 ± 0.18
Range	0.56 - 0.72	1.0 - 6.53
No of cases	15	52
Serum fluoride	0.04 ± 0.002	0.284 ± 0.032
Range	0.03 ± 0.05	0.131 ± 0.552
No of cases	15	76

TABLE- 2. Haemoglobin (g%), serum cholesterol (mg/dl) and testosterone (ng/mL)

Parameter	Control	Endemic Population
Haemoglobin	13.3 ± 0.339	12.89 ± 1.48
Range	11.8 - 16.4	7.8 – 16.0
No of cases	22	75
Cholesterol	155.22 ± 7.51	148.25 ± 3.90
Range	122.5 - 200	64.0 - 192.30
No of cases	15	40
Testosterone	6.42 ± 0.423	5.56 ± 0.49
Range	4.3 - 9.3	1.10 – 11.5
No of cases	15	35

TABLE- 3. SGOT, SGPT (IU/L) and serum protein (gm/dl) levels

parameter	Control	Endemic Population
SGOT	16.02 ± 1.2	29.38 ± 0.83
Range	12.0 – 24.0	23 - 41
No of cases	15	35
SGPT	11.7 ± 0.83	22.33 ± 0.73
Range	9 - 15	35
No of cases	15	35
Protein	8.64 ± 0.144	5.76 ± 0.89
Range	7.924 – 9.42	4.15 ± 6.98
No of cases	15	35

TABLE- 4 - Calcium, sodium and potassium levels (mEq/L)

parameter	Control	Endemic Population
Calcium	0.958 ± 0.045	0.595 ± 0.014
Range	0.71 – 1.15	0.40 - 0.79
No of cases	15	60
Sodium	1163.68 ± 28.84	1875.59 ± 30.8
Range	861 - 1500	1320 - 2450
No of cases	22	60
Potassium	129.35 ± 7.96	322.75 ± 42.38
Range	90 - 120	130 - 820
No of cases	22	60

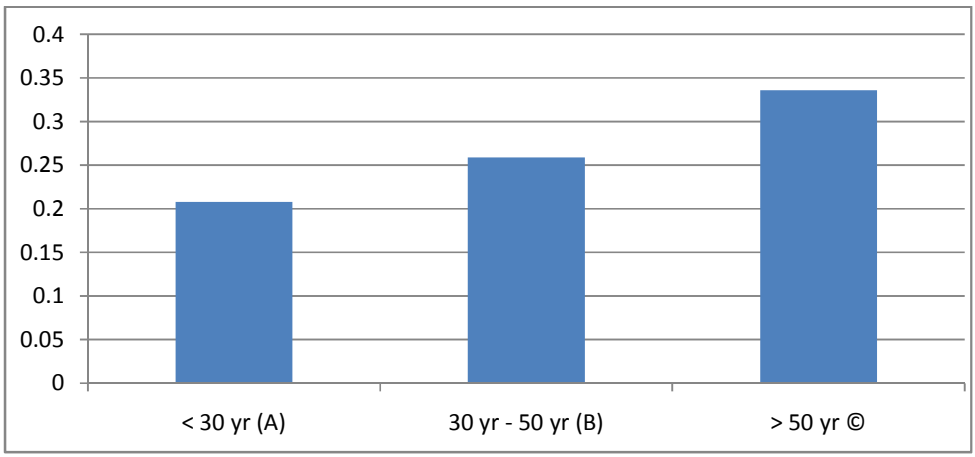
TABLE- 5. Serum T3, T4 and TSH

parameter	Control	Endemic Population
T3(ng/mL)	1.50 ± 0.135	1.528 ± 0.076
Range	0.7 – 2.1	1 – 3.7
No of cases	15	40
T4(ng/mL)	9.16 ± 0.63	14.77 ± 0.512
Range	5.4 - 13	7.2 - 20
No of cases	15	40
TSH(μU/mL)	2.56 ± 0.36	2.55 ± 0.37
Range	0.50 – 4.4	0.30 – 6.1
No of cases	15	55

TABLE- 6. Catecholamine levels (pg/mL) in serum

parameter	Control	Endemic Population
Adrenalin	220.67 ± 20.79	332.61 ± 20.54
Range	157.46 - 311.60	114.20 - 788.51
No of cases	15	50
Nor-adrenalin	164.51 ± 11.19	514.87 ± 35.27
Range	118.51 - 235.0	108.64 - 1053.07
No of cases	15	50

FIGURE. Age dependent variation in serum fluoride concentrations among endemic population. A: below 30 year. B: 30- 50 years. C: Over 50 years.



DISCUSSION: The study of various health hazards following prolonged exposure of fluoride in Bankura district of South Bengal revealed a wide occurrence of fluorosis, ranging from mild to acute[13].

The concentration of fluoride ions in plasma is directly related to the fluoride content of the drinking water [12]. This close relationship has been clearly demonstrated by several authors[7,9,12,14].

In this study, when the individuals were divided into three age groups (under 30 year, 30-50 year, and over 50 year), an increase in serum fluoride levels with age was observed (Figure). Plasma fluoride increased with age between 10 and 38 years. This difference in serum fluoride levels could be attributed to a difference in fluoride uptake by the skeleton. The young, growing skeleton, being low in fluoride, has a greater capacity for taking it up. In older people, the bone fluoride is higher and the plasma approaches equilibrium with it, hence it occurs with a rise in plasma fluoride with advancing years.

There are only a few reports in the literature of anemia in fluoride exposed individuals. Haemoglobin levels in the endemic villages were low, compared to those in the control population. Though the difference between mean values was not significant, individual values in the endemic population showed great fluctuation. Though fluoride is capable of causing anemia, the haemoglobin level is also governed by the individual's nutritional status[6].

Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation and by interfering with peptide chains on ribosomes. In the present study

protein levels in the endemic area were significantly decreased, which would adversely affect the growth of the affected individuals.

Conflicting reports have been published regarding fluoride toxicity and lipid metabolism [4]. The results of the present investigation revealed normal levels of serum cholesterol, thus ruling out the occurrence of hypo/hypercholesterolemia among fluorotic individuals in the early stages of the disease.

The circulating levels of testosterone in fluoride exposed individuals are also not altered significantly in males.

Chronic cases of fluoride exposed individuals need to be investigated in detail since the chances of atherosclerosis cannot be ruled out. Numerous investigators have reported calcification of arteries in association with skeletal fluorosis in high fluoride endemic areas. The decreased levels of serum calcium in the fluoride exposed individuals could be attributed to ectopic calcification in soft tissues. It could also be due to a decrease in the intestinal absorption of fluoride since fluoride is known to produce insoluble complexes with calcium. Further studies are necessary on endemic human populations to establish the role of fluoride atherosclerosis.

Calcium homeostasis is controlled by hormonal regulation of the thyroid and parathyroid glands[11]. In the present study, no significant change was observed in the levels of thyroid stimulating hormone (TSH) and T3 in the fluoride exposed individuals. An enhancement was observed in the levels of T4, which might be due to enhanced iodination to form this hormone rather than T3 which would result in its increased synthesis/release by the gland.

In fluoride exposed individuals the serum catecholamines were increased significantly, which would have a stimulatory effect on the sympathetic nervous system[9], thus influencing the hypothalamus gonadal axis and result in marked changes in reproductive functions. It would also affect the carbohydrate metabolism by accelerating the breakdown of glycogen.

The increased levels of serum transaminases in fluoride exposed individuals suggest alteration in liver function[5]. These levels increase several times if cellular damage occurs in the liver, so these enzymes are markers for assessing liver function.

The fluoride exposed group showed marked alteration in their serum electrolyte levels. Potassium and sodium levels increased significantly, compared to controls. Differential distribution of these two cations is essential for normal membrane function and integrity. Serum potassium is an indicator of cell damage. Increased levels suggest cell deterioration.

CONCLUSION: The present study revealed wide occurrence of fluorosis in the Bankura districts of West Bengal. Intake of high fluoride alters the normal body metabolism of the individuals. Further surveys are required in fluoride endemic areas, to reveal the magnitude of the problems caused by fluoride. The Government should take steps to supply safe drinking water in the villages affected by fluorosis.

REFERENCES:

1. Chinoy NJ. Effects of fluoride on physiology of animals and human beings. Indian Journal of Environment and Toxicology 1 (1) 17-32 1991.
2. Chinoy NJ. Effects of fluoride on some organs of rats and their reversal. Proceedings of the Zoological Society, Calcutta 44 (1) 11-15 1991.
3. Susheela AK. Technical information for training cum awareness camp for doctors, public health engineers and other officers on Prevention and Control of Fluorosis. Rajiv Gandhi National Drinking Water Mission, New Delhi 1991.

4. Nelson DG, Coote GE, Vickridge IC, Suckling G. Proton microprobe determination of fluorine profiles in the enamel and dentine of erupting incisors from sheep given low and high daily doses of fluoride. Arch Oral Biol. 1989; 34:419–429.
5. 5 Bronckers AL, Lyaruu DM, Bervoets TJ, Woltgens JH. Fluoride enhances intracellular degradation of amelogenins during secretory phase of amelogenesis of hamster teeth in organ culture. Connect Tissue Res. 2002; 43:456–465.
6. Reitman S, Frankel S. Photometric determination of SGOT and SGPT. American Journal of Clinical Pathology 28 56 1957.
7. Dean NA. Flame Photometry. McGraw Hill, London 1960.
8. Kemp HA, John R, Woodhead JS. Labelled antibody immunoassay. In: Butt WR (Ed). Practical Immunoassay. Marcel Dekker, New York 1984 pp 179-198.
9. Von Euler, Hamberg G. In: Oser BL (Ed). Hawk's Physiological Chemistry. McGraw Hill, Bombay 1965 pp 302-303.
10. Guy WS, Taves DR, Brey WS Jr. Organic fluoro-compunds in human plasma. Prevalence and characterisation. In: Fuller R (Ed). Biochemistry Involving Carbon Fluoride Bonds. American Chemical Society, Washington DC 1976 pp 117-134.
11. Singer L, Ophaug RH. Total fluoride intake of infants. Paediatrics 63 460-466 1979.
12. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th Annual Report. Clin Toxicol (Phila). Dec 2010;48(10):979-1178.
13. Kao WF, Deng JF, Chiang SC. A simple, safe, and efficient way to treat severe fluoride poisoning--oral calcium or magnesium. J Toxicol Clin Toxicol. 2004;42(1):33-40.
14. Kubota K, Lee DH, Tsuchiya M, et al. Fluoride induces endoplasmic reticulum stress in ameloblasts responsible for dental enamel formation. J Biol Chem. 2005; 280:23194–23202.

THROMBOCYTOPENIA IN RELATION WITH PLASMODIUM VIVAX MALARIA

K. Kuladeepa Ananda Vaidya, Pradeep Vernekar

1. Assistant Professor, Department of Pathology, SIMS & RC, Surathkal, Karnataka.
2. Assistant Professor, Department of General Medicine, SIMS & RC, Surathkal, Karnataka.

CORRESPONDING AUTHOR
Dr. K. Kuladeepa Ananda Vaidya,
Assistant professor, Dept of Pathology,
SIMS&RC, Srinivas Nagar, Mukka,
Surathkal, Karnataka - 575021.
E-mail: vaidyakuldeep@gmail.com,

ABSTRACT: Malaria is a major health problem in the tropics with increased morbidity and mortality. Plasmodium vivax is the most frequent and widely distributed cause of recurring malaria worldwide. Plasmodium vivax malaria is endemic infection in India and is commonly associated with haematological abnormalities, Thrombocytopenia is frequently observed in vivax malaria but the exact mechanism has not been elucidated. It has been increasingly observed that P. vivax malaria, which was otherwise considered to be benign malaria, with a low case-fatality ratio, can also occasionally result in severe disease as with P. falciparum malaria. This study was undertaken to correlate the presence and severity of thrombocytopenia with P. vivax malaria.
KEY WORDS: Plasmodium vivax, Malaria, Thrombocytopenia.

INTRODUCTION: Each year there are more than 250 million cases of malaria, killing between 1 to 3 million people [1]. Countries in which malaria had been previously eradicated or adequately controlled have recently seen a resurgence of malaria [2]. Hematological abnormalities have been observed in patients with malaria, anemia and thrombocytopenia being the most common [3]. Malaria is a common infection in most parts of India and is commonly associated with mild thrombocytopenia [4]. Profound thrombocytopenia is a well-recognized complication of falciparum malaria but has been less well described in vivax malaria.[5] Thrombocytopenia seen in complicated falciparum malaria is due to disseminated intravascular coagulation along with platelet endothelial activation, but the one seen in uncomplicated malaria like P. vivax has multifactorial etiology. Few postulated mechanisms are macrophage activation leading to platelet destruction, increased levels of cytokines, immunological destruction due to antiplatelet IgG, oxidative stress, shortened platelet life span in peripheral blood and sequestration in nonsplenic areas and partly due to psuedothrombocytopenia due to clumping of platelets [6]

MATERIALS AND METHODS: This is a prospective study conducted at a tertiary referral hospital at coastal Karnataka. Study was conducted over a period of 6 months. A total of 150 patients who were hospitalized after confirmation of diagnosis of malaria by examination of peripheral smear stained with leishmen stain [Fig1, 2] or by detecting parasite by fluorescent technique (MPFT). Smear has been considered negative if no parasite detected on examination under 100 high power fields. Platelet counts have been performed by automated haematology analyser. Patients with decreased platelet count (thrombocytopenia) were re-evaluated by

manual method. Thrombocytopenia was defined as platelet count of less than 150,000 cells/ cmm. Patients were divided into three subgroups based on platelet count. Thrombocytopenia was considered severe if <50,000 cells/cmm, moderate if 50,000-100,000 cells/cmm, and mild if 100,000 150,000 cells/cmm.

RESULTS: We studied 150 cases of acute vivax malaria; The commonest presenting manifestations were fever with chills and rigors. Out of 150 cases 133 cases had thrombocytopenia, which was the most common haematological finding. None had bleeding manifestations from any site. Anaemia was present in 20 cases and splenomegaly was seen in two of the cases. Thrombocytopenia was seen as an early laboratory finding in 133 cases at the time of admission with fever of not more than 5 days. Platelet counts reverted to normal on treatment. Other causes of thrombocytopenia were ruled out by complete history and physical examination and dengue serology wherever required.

Mild thrombocytopenia (1,00,000-150,000/cmm) was seen in 42.8% of patients positive for P. Vivax with thrombocytopenia, 6.8% patients had severe thrombocytopenia with platelet counts less than 50,000/cmm. (Table 3). The average duration of stay in the hospital for all patients was 5 ± 2 days.

Table1: Age and Sex distribution for P. Vivax

Age (in years)	Male	Female	Total
<10 years	-	1	1
10-20	45	7	52
21-30	58	-	58
31-40	21	-	21
41-50	12	2	14
51-60	2	-	2
61-70	1	-	1
Above 70	-	1	1

Table 2: P. Vivax with Thrombocytopenia

Thrombocytopenia	Number of patients
Present	133 (88.67%)
Absent	17 (11.33%)
Total	150

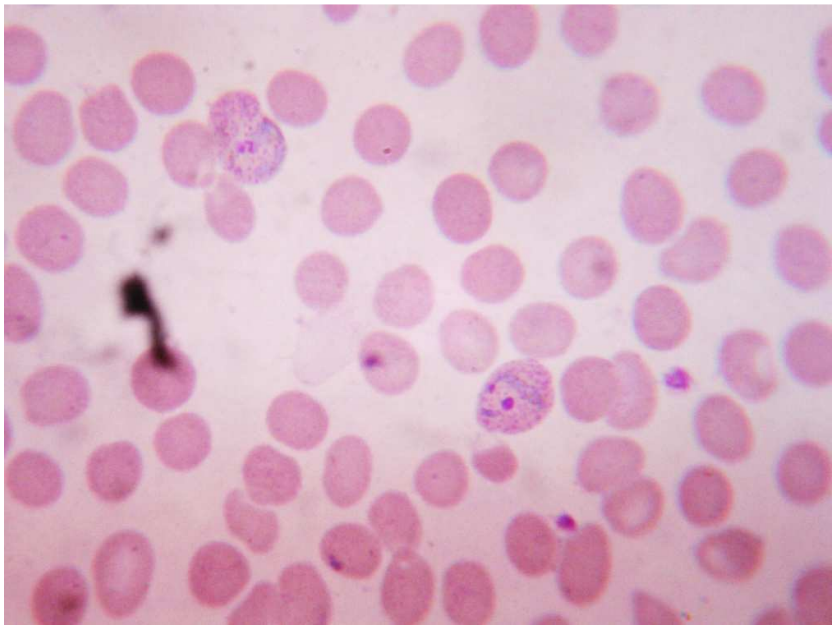
Table3: Severity of thrombocytopenia

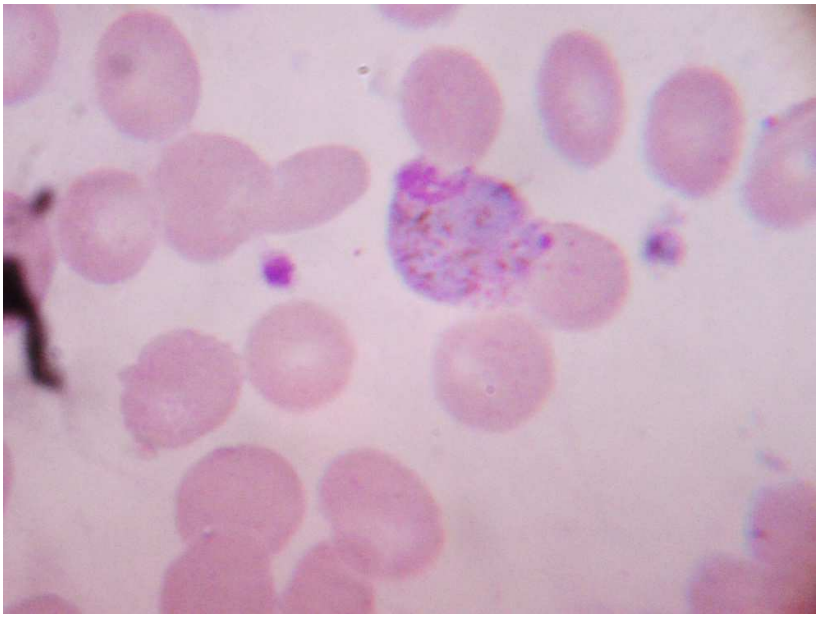
Severity	Number of cases
Mild	57 (42.8%)
Moderate	67 (50.4%)
Severe	9 (6.8%)

DISCUSSION: Malaria infects mankind globally. According to World Health Organization assessment, about 40% of the world population is at risk of developing malaria. About 300- 500 million people are infected with it. Every year about 2 million people die due to malaria and its complications. The highest mortality is in Africa, mainly in young children.[7]. Plasmodium vivax is the most widely distributed human malaria parasite with an at risk population of 2.5 billion persons. Although the exact burden of disease caused by *P. vivax* infection is still a matter of debate, this parasite causes approximately 100–300 million clinical cases each year.[8]. The trend of disease with plasmodium vivax malaria is changing. It is increasingly recognized that serious and life threatening complications can occur with vivax malaria. [9]

The mechanism of thrombocytopenia in malaria is not clearly known. Fajardo and Tallent in 1974 demonstrated P. vivax within platelets by electron microscopy and suggested a direct lytic effect of the parasite on the platelets [10]. Oxidative stress damage of thrombocytes has also been implicated in the etiopathogenesis based on the finding of low levels of platelet superoxide-dismutase and glutathione peroxidase activity and high platelet lipid peroxidation levels in malaria patients, when compared to those of healthy subjects [11]. Both non-immunological destruction [12] as well as immune mechanisms involving specific platelet-associated IgG antibodies that bind directly to the malarial antigen in the platelets have been recently reported to play a role in the lysis of platelets and the development of thrombocytopenia [13]. In clinical trials, recombinant – macro-phage colony stimulating factor (M-CSF) has been known to cause a reversible dose dependent thrombocytopenia. Elevated M-CSF levels in malaria, by increasing macrophage activity may mediate platelet destruction in such cases [14].

CONCLUSION: In conclusion, absence of thrombocytopenia is uncommon in the laboratory diagnosis of P. Vivax malaria. Thrombocytopenia due to P. vivax malaria is increasing in India but usually disappears with the treatment of disease. Thrombocytopenia in P. vivax has multifactorial etiology, and it has some diagnostic significance. This can be used for predicting presence of malarial infestation even in early stage of the disease. Thrombocytopenia should increase the suspicion of malaria, and multiple peripheral smears or more sensitive tests for detection of parasite should be performed.





REFERENCES:

1. Kahl U. Malaria kills over 1 million people every year. Genomic mapping of malaria parasite and mosquito raise hope for a vaccine as well as more effective drugs. Lakartidningen 2003; 100 (12): 1042-47.
2. McNeeley DF, Chu A, Lowe S, et al. Malaria surveillance in New York City, 1991-1996. nt J Infect Dis. 1998;2:132-6.
3. Khan SJ, Khan FR, Usman M, Zahid S. Malaria can lead to Thrombocytopenia. Rawal Med J 2008; 33: 183-5
4. Looareesuwan S, Davis J.G., Allen D.L., et al.Thrombocytopenia in malaria. Southeast Asian J Trop Med Public Health 1992; 23:44.
5. Martelo O.J., Smoller M., Saladin T.A. Malaria in Americansoldiers. Arch Int Med 1969; 123:383-7.
6. Katira B, Shah I Thrombocytopenia in Plasmodium vivax infected children, J Vect Borne Dis 43, September 2006, pp. 147–9
7. Phulpoto JA, Shaikh AH, Frequency of thrombocytopenia in malarial patients. Medical channel. 2010; 16(4):511
8. Kochar DK, Das A, Kochar S K, Saxena V, Sirohi P, Garg S, Kochar A et al Severe Plasmodium vivax Malaria: A Report on Serial Cases from Bikaner in Northwestern India. Am J Trop Med Hyg February 2009 vol. 80 no. 2 194-8
9. Narang GS, Singla N. Thrombocytopenia and other complications of Plasmodium vivax malaria. Curr Pediatr Res 2011; 15 (2): 117-119
10. Fajardo L.F, Tallent C. Malarial parasites within human platelets. JAMA,1974; 229:1205-9
11. Erel O, Vural H, Aksoy N, et al. Oxidative stress of platelets and thrombocytopenia in patients with vivax malaria. Clin Biochem 2001; 34 (4): 341-4
12. Looaresuwan S, Davis J.G, Allen D.L, et al. Thrombocytopenia in malaria. Southeast Asian J Trop Med Public Health 1992; 23 (1): 44-50.
13. Yamaguchi S, Kubota T, Yamagishi T, et al. Severe thrombocytopenia suggesting immunological mechanism in two cases of vivax malaria. Am J Hematol 1997; 56 (3):183-6
14. Lee S.H, Looaresuwan S, Chan J, et al. Plasma macro-phage colony stimulating factor and P-selection levels in malaria associated thrombocytopenia. Thromb Haemost 1997; 77 (2): 289-93

LINEAR LICHEN NITIDUS – A RARE CASE REPORT

U. S. Kanade, S. S. Dantkale, Madhuri. S. Gadhekar, H. T. Kamra
Dr. Rahul R. Narkhede.

1. Assistant Professor, Department of Pathology, Government Medical College, Latur.
2. Associate Professor and Head, Department of Pathology, Government Medical College, Latur.
3. Resident, Department of Pathology, Government Medical College, Latur.
4. Assistant Professor, Department of Pathology, Government Medical College, Latur.
5. Resident, Department of Pathology, Government Medical College, Latur.

CORRESPONDING AUTHOR
Madhuri S. Gadhekar,
Department of Pathology,
Government Medical College, Latur.
E-mail: drmadhurig@rediffmail.com,
Ph: 91 08275229512.

ABSTRACT: Pinkus first described Lichen nitidus a rare variety of dermatitis of unknown etiology in 1901. Its incidence was 0.034% in a study in blacks over a 25-years period. Various clinical variants of lichen nitidus includes- linear, confluent, vesicular, haemorrhagic, spinous follicular, perforating, generalised, palmar and plantar.

We hereby report a rare case of linear Lichen nitidus in a 17-year-old male presented with skin lesions spreading in linear fashion from anterior abdomen to back since 2 months. On examination, round, flat-topped, shiny papules, 2 to 3 mm in diameter presented over anterior abdomen wall extending towards back. Skin biopsy confirmed diagnosis of linear Lichen nitidus. Sections revealed vacuolar alternation of basal cell layer, diminished granular layer along with mixed-cell granulomatous infiltrate closely attached to the lower surface of the epidermis and confined to widened dermal papillae. The infiltrate composed of lymphocytes and epithelioid histiocytes. At places, rete ridges bend downward and inward and seem to clutch the infiltrate in the manner of "claw clutching a ball". Differential diagnosis of lichen striatus and lichen planus was ruled out on histopathology. Patient responded to treatment.

KEYWORDS: Lichen nitidus, Lichen striatus, Lichen planus.

INTRODUCTION: Pinkus first described Lichen nitidus {LN}, a rare variety of dermatitis of unknown etiology in 1901.[1] It is characterised by the presence of shiny, dome-shaped papules, which usually are asymptomatic. [2] Many clinical variants of LN have been reported. We hereby report a rare case of lichen nitidus, linear variant.

CASE HISTORY: A 17-year-old male presented with chief complaints of skin lesions since 2 months. Clinical examination revealed multiple, round, flat-topped, shiny papules, 2 to 3 mm in diameter over anterior abdomen wall spreading in linear fashion towards the back (fig. 1). There was no history of any prior medication, diarrhoea, constipation, joint pain, similar complaints in family members. Routine laboratory investigations were within normal limits. Clinical differential diagnoses were lichen striatus and lichenoid epidermal naevus. Skin biopsy was taken from papules with help of 5 mm biopsy punch. Histopathological examination showed epidermis with diminished granular layer, along with basal cell layer vacuolization. Focal granulomatous infiltrate was seen closely attached to the lower epidermis and confined to widened dermal papillae (fig. 2). The

infiltrate was composed of predominantly lymphocytes and few epithelioid histiocytes. At places, rete ridges were to extend downward and seem to clutch the infiltrate in manner of "claw clutching a ball" (fig. 3). The diagnosis of LN was made.

DISCUSSION: LN is chronic papulosquamous disorder characterized by multiple, 1–2 mm, flesh-colored, shiny, dome-shaped papules.[3] Its incidence is 0.034% in a study of skin diseases in blacks over a 25-years period.[4] Skin lesions classically involves the genitalia, upper extremities, chest and abdomen. Infrequently, the lower extremities, palms, soles, face, nails, and mucous membranes may be affected. Majority of cases are common in children and young adults. Various clinical variants of lichen nitidus are - linear, confluent, vesicular, haemorrhagic, spinous follicular, perforating, generalised, palmar and plantar.[5]

Our patient was 17 year old male presenting with shiny papules over anterior abdomen wall spreading in linear fashion towards the back representing a linear variant.

Few cases of LN are reported in association with other diseases such as atopic dermatitis, Crohns disease[6], juvenile chronic arthritis[7] and down syndrome.[8, 9] Familial cases have been described.[10] Our patient had no such association.

LN must be differentiated from lichen striatus, lichenoid epidermal naevus, lichen planus and keratosis pilaris, lichen spinulosus on microscopic examination.

The absence of irregular acanthosis, wedge-shaped hypergranulosis, and compact orthokeratosis, helped us to differentiate LN from lichen planus.

LN is distinguished from Lichen striatus, which shows superficial perivascular lymphocytic infiltrate and inflammatory infiltrate around hair follicles and eccrine glands.

Both keratosis pilaris and Lichen spinulosus show epidermal hyperkeratosis, hypergranulosis, and plugging of individual hair follicles with keratinous plugs, and thus discriminate them from LN.

No evidence of hyperkeratosis, papillomatosis and acanthosis ruled out lichenoid epidermal naevus.[11]

Aetiology of lichen nitidus remains unresolved. In view of similar ultrastructural findings and possible clinical coexistence, lichen nitidus, is considered by some authors, to be a variant of lichen planus. [12] However, the papule in lichen nitidus develop epidermal flattening and parakeratosis, unlike in lichen planus where it develops into acanthosis and hyperkeratosis. [13]

On follow up, there was remission of lesion after treatment with local potent steroids (fig.4).

Such rare cases may be easily missed, histopathology helps differentiation from other papulosquamous disorders, provide a definitive diagnosis and thus aid appropriate management.

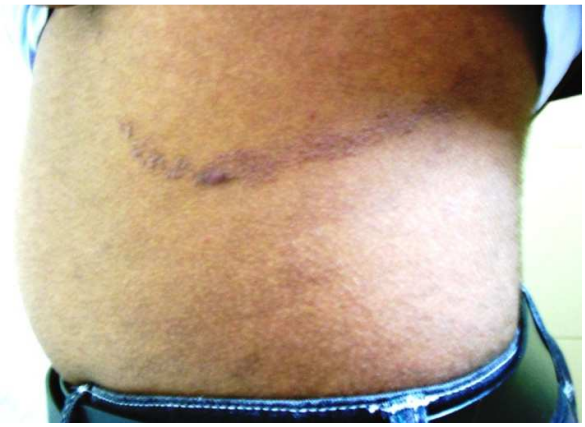


Figure1. Multiple papules present over anterior abdomen spreading in linear fashion towards the back.

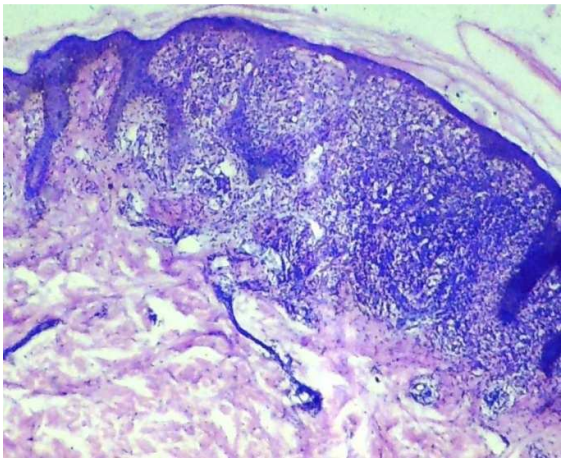


Figure 2. Rete ridges clutch the infiltrate in manner of "claw clutching a ball".

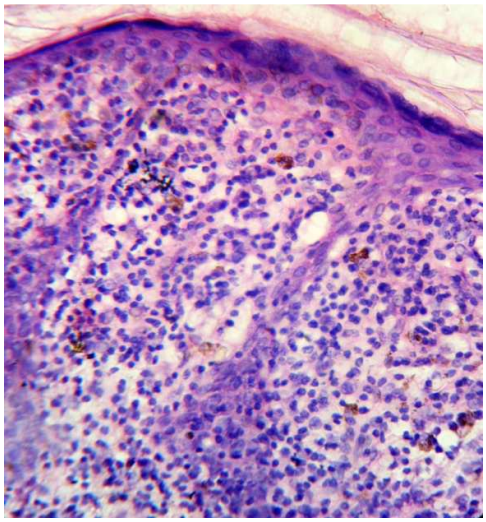


Figure 3. Dense lymphohistiocytic infiltrate in widened dermal papillae.

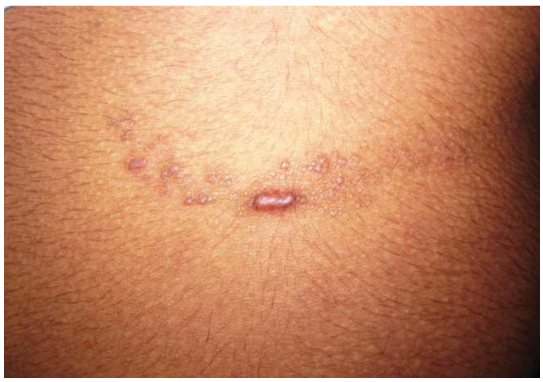


Figure 4. Patient on treatment with potent local steroids.

REFERENCES:

1. Chen W, Schranm M, Zouboulis CC. Generalised lichen nitidus. J Am Acad Dermatol. 1997; 36:630–1.[PubMed: 9092753].
2. David E. Elder, Bernett L. Johnson, Rosalie Elenitsas. Lever's Histopathology of the Skin. 9th Edition. Philadelphia. Lippincott Williams & Wilkins. 2005
3. Inamadar AC, Athanikar SB, Sampagavi W, Yelikar BR. Actinic lichen nitidus.Indian J Dermatol Venereol Leprol 2001;67:209-210
4. Hazen HH. Syphilis and skin diseases in the American Negro: personal observations. Arch Dermatol Syph. 1935; 31:316.
5. Black MM. Lichen planus and lichenoid disorders. In: Textbook of Dermatology (Eds: Champion RH, Burton JL, Ebling FJG) 5th edn, vol 3. Oxford; Blackwell Scientific Publication, 1992; 1996-1998.
6. Kano Y, Shiohara T, Yagita A, Nagashima M. Erythema nodosum, lichen planus and lichen nitidus in Crohn's disease: report of a case and analysis of T cell receptor V gene expression in the cutaneous and intestinal lesions. Dermatology. 1995; 190(1):59-63.
7. Bercedo A, Cabero MJ, Garcia-Consuegra J, Hernado M, Yaez S, Fernandez-Llaca H. Generalized lichen nitidus and juvenile chronic arthritis: an undescribed association. Pediatr Dermatol. Sep-Oct 1999; 16(5):406-7.
8. Henry M, Metry DW. Generalized lichen nitidus, with perioral and perinasal accentuation, in association with Down syndrome. Pediatr Dermatol. Jan-Feb 2009; 26(1):109-11.
9. Laxmisha C, Thappa DM. Generalized lichen nitidus with Down syndrome. J Eur Acad Dermatol Venereol 2006;20:1156-7.
10. Kato N. Familial lichen nitidus. Clin Exp Dermatol. Jul 1995;20(4):336-8.
11. Brownstein MH, Silverstein L, Lefing W Lichenoid epidermal nevus: "linear lichen planus" J Am Acad Dermatol. 1989 May; 20(5 Pt 2):913-15.
12. Stankler L. The identity of lichen planus and lichen nitidus. Br J Dermatol. 1967;79: 125–6.[PubMed: 6019108]
13. David E. Elder, Bernett L. Johnson, Rosalie Elenitsas. Lever's Histopathology of the Skin. 10th Edition. Philadelphia: Lippincott Williams & Wilkins; 2005 page 192

INTUBATION USING LMA CTRACH IN ANAESTHETIZED BUT UNPARALYSED PATIENTS – A PILOT STUDY

Geetanjali Chilkoti, Medha Mohta, Anjali Kochhar, Deepti Agarwal, A K Sethi

1. Assistant Professor, Department of Anaesthesia and Critical Care, Guru Tegh Bahadur Hospital and University College of Medical Sciences, Shahdara, Delhi.
2. Reader, Department of Anaesthesia and Critical Care, Guru Tegh Bahadur Hospital and University College of Medical Sciences, Shahdara, Delhi.
3. Assistant Professor, Department of Anaesthesia and Critical Care, Guru Tegh Bahadur Hospital and University College of Medical Sciences, Shahdara, Delhi.
4. Specialist, Department of Anaesthesia and Critical Care, Guru Tegh Bahadur Hospital and University College of Medical Sciences, Shahdara, Delhi.
5. Director Professor, Department of Anaesthesia and Critical Care, Guru Tegh Bahadur Hospital and University College of Medical Sciences, Shahdara, Delhi.

CORRESPONDING AUTHOR

Dr. Geetanjali Chilkoti,
A-1404, Jaipuria Sunrise Greens,
Ahimsa Khand, Indirapuram, Ghaziabad
E-mail: geetanjalidr@yahoo.in,
Ph: 91 9868399782.

ABSTRACT: BACKGROUND - Laryngeal Mask Airway (LMA) CTrach is a supraglottic device which is functionally identical to Intubating Laryngeal Mask airway (ILMA) which enables ventilation and allows real time visualization of endotracheal intubation. This study aimed at assessing the ease and efficacy of using LMA CTrach for orotracheal intubation in anaesthetized but unparalysed patients. **MATERIAL AND METHODS**: 40 patients of ASA Grade I/ II scheduled for elective surgeries requiring orotracheal intubation were studied. Anaesthesia was induced with fentanyl 1 $\mu g.kg^{-1}$ and sevoflurane. Neuromuscular blockers were not used. After CTrach insertion, various corrective manoeuvres were used to obtain the best laryngeal view. Two ml of 2% lignocaine was instilled over the vocal cords via a small sized catheter and tracheal intubation was performed after 2 minutes. The haemodynamic parameters were recorded. Any complications occurring following the CTrach insertion or tracheal intubation and those in the post operative period were also recorded. **RESULTS**: The insertion of CTrach was easy and well tolerated in all the patients. Thirty percent of patients required corrective manoeuvres. Tracheal intubation was successful in all 40 patients in first or second attempt. The problems encountered were hypotension following tracheal intubation in 20% patients and bucking over the tube at the time of CTrach removal in 17.5% patients. Hypotension persisted only for 2-3 minutes. In the post operative period, mild sore throat and pain on swallowing were complained by four patients each, which resolved within 24 hours.
CONCLUSION - The use of LMA CTrach to ventilate lungs and facilitate tracheal intubation in anaesthetized unparalysed patients appears to be a useful and safe technique as it does not require patient cooperation and use of neuromuscular blockers.
KEY WORDS: Equipment - LMA CTrach; endotracheal intubation; anaesthetics – inhalation - sevoflurane.

INTRODUCTION: Laryngeal Mask Airway (LMA) CTrach is a supraglottic device which is functionally identical to Intubating Laryngeal Mask airway (ILMA) but in addition has an

integrated fibreoptic bundle with Liquid Crystal Display (LCD) [1, 2, 3]. This system enables ventilation and allows real time visualization of endotracheal intubation [2, 3]. Thus LMA CTrach can be a useful airway adjunct for management of difficult airway situation. It has been used for tracheal intubation after administration of neuromuscular blocking agents; awake insertion of the device followed by intubation using high concentrations of sevoflurane; or awake intubation using topical anaesthesia [1, 2, 4, 6, 8]. All these methods have their own limitations. The administration of neuromuscular blocking agents may not be desirable in patients with anticipated difficult airway. High concentration of sevoflurane may lead to cardiovascular and cerebrovascular compromise in susceptible individuals [7] and the procedure in awake patients cannot be performed without patient cooperation. If intubation can be performed through LMA CTrach using moderate concentrations of anaesthetic agents but without muscle paralysis, it would be a useful technique in uncooperative patients with anticipated difficult airway. As this airway device has never been used in such situations, the present study was conducted to assess the feasibility of using LMA CTrach in anaesthetized but unparalysed patients requiring orotracheal intubation for elective surgeries.

MATERIALS AND METHODS: After approval from the institutional ethics committee, 40 patients of American Society of Anaesthesiologists (ASA) Grade I/ II scheduled for elective surgeries requiring orotracheal intubation were studied. Informed consent was obtained from all the patients before inclusion in the study. Patients having limited mouth opening ≤ 25 mm, oropharyngeal pathology or risk of regurgitation or pulmonary aspiration of gastric contents were excluded from the study.

The neck movements, Samsoon and Young modification of Mallampatti grading (MPG) and thyromental distance (TMD) were used to asses the airway of all the patients. The body mass index (BMI) and any other indicators of difficult airway, if present, were noted.

All the patients were kept fasting overnight and were given tab diazepam 10 mg in the previous night and 5 mg on the day of surgery 2 hours before anaesthesia. In the preanaesthetic room, inj. glycopyrrolate 0.2 mg and Midazolam 1 mg were administered intravenously. Standard intraoperative monitoring included continuous ECG, non-invasive blood pressure and pulse oximetry. Difficult airway cart was kept ready to manage any difficult airway situation. The LMA CTrach system was prepared after selecting appropriate size according to the manufacturer's recommendations (Size 3 for patients weighing 30-49 kg, size 4 for 50-69 kg and size 5 for more than 70 kg). Anaesthesia was induced with inj fentanyl 1 $\mu g.kg^{-1}$ and sevoflurane. Neuromuscular blockers were not used. The anaesthesia was considered adequate for CTrach insertion when the patient was unresponsive and the steady state end tidal concentration of 2% sevoflurane was achieved. The CTrach was inserted in the neutral head position by was introduced by one of the two senior anaesthesiologists who had the experience of using the airway device at least 20 times before the start of the study. The adequate spontaneous ventilation was confirmed by normal thoracic movements and capnography. After confirmation of the lung ventilation, the viewer was connected and the view of the laryngeal structures was noted according to the criteria listed in Table 1 [1]. Whenever the laryngeal view was partial or grade II, various adjusting or corrective manoeuvres were used to improve the view depending on the likely cause of failure (Table 2) [1]. Three types of corrective manoeuvres used were Down-Up-Down (DUD), Medial-lateral-medial (MLM) and Chandy's manoeuvres.

After the vocal cords were visualized a small sized suction catheter was passed through the CTrach which could be easily seen on the viewer. Two ml of 2% lignocaine was instilled over the vocal cords through the catheter. After waiting for 2 min, a lubricated endotracheal tube was

passed through the rigid anatomically curved airway tube of the CTrach.. During these two minutes, ventilation was assisted in all the patients. The correct tube placement was confirmed by direct visualization, chest auscultation and capnography. After the confirmation of the tracheal intubation, the CTrach was removed according to the manufacturer's instructions followed by the administration of neuromuscular blockers. The tracheal intubation was considered to have failed if trachea could not be intubated in three attempts. In these cases, it was decided to perform intubation by direct laryngoscopy using a Macintosh laryngoscope. In cases of failed direct laryngoscopy and intubation the plan was to use fibreoptic bronchoscope. Other rescue devices i.e. classic LMA and combitube were kept ready to deal with any ''Can not intubate, Cannot ventilate '' situation.

An independent observer noted the number of attempts of CTrach insertion and tracheal intubation, the time required to achieve ventilation through CTrach and the total time taken for the whole procedure. The time required to achieve ventilation was noted from the time of holding CTrach until the capnographic confirmation of adequate lung ventilation. The time taken for the whole procedure included the time required to achieve ventilation, optimize the laryngeal view, waiting period of 2 min for effective topical anaesthesia, tracheal intubation and removal of the CTrach. The ease of technique was also noted by the observer. It was assessed on a four point scale as easy, adjusting manoeuvers required, reinsertion required or failure at third attempt.

The heart rate, blood pressure and oxygen saturation were recorded before induction of anaesthesia, following CTrach insertion and after the tracheal intubation. Hypotension was defined as a fall in systolic blood pressure ≥ 20% of the preinduction value. Similarly a rise in systolic blood pressure more than 20% of the baseline value was defined as hypertension. Bradycardia and tachycardia were defined as heart rate less than 60 beats per minute and more than 100 beats per minute respectively.

Any complications occurring following the CTrach insertion or tracheal intubation e.g. coughing, bucking or bronchospasm; and those in the post operative period were recorded. The postoperative complications e.g. dysphagia, dysphonia, sore throat, nausea or vomiting were looked for and recorded at two and 24 hours after tracheal extubation.

RESULTS: We studied 40 patients, 11 males and 29 females, with mean age of 33.4 ±13.6 yrs and weight of 54.7 ±12.0 Kg. The surgical procedures performed were laparoscopic cholecystectomy, ileostomy closure and incisional hernia repair. The mean (SD) duration of surgical procedures was 89.6 ±35.6 min. There were 28, 10 and 2 patients with modified MPG I, II and III respectively. Thyromental distance was within normal limit in all except three patients where it was less than 6 cm. Out of these three patients, two patients were the same who had MPG III. In all these three patients the neck movements and mouth opening were adequate. The BMI of 36 patients were within normal limits whereas one patient had BMI > 35 $Kg.m^{-2}$ and the other three had BMI > 30 $Kg.m^{-2}$. The patient with BMI > 35 $Kg.m^{-2}$ also had reduced TMD with MPG III.

The LMA CTrach was easily inserted on first attempt and adequate ventilation could be achieved in all 40 patients. Only four patients had gag reflex during insertion which was self limiting. In 28 (70%) patients, the initial laryngeal view was grade I, whereas it was grade II, III and IV in 6, 4 and 2 patients respectively. Corrective manoeuvres were required to obtain the optimum view of vocal cords in these 12 (30%) patients. In 2 patients with immediate Grade IV view, secretions obscured the image for which the CTrach was removed, cleaned and reinserted.

We were successful in performing tracheal intubation using CTrach in all 40 patients. In 39 patients the trachea was intubated in first attempt whereas in one patient two attempts were required inspite of grade I view of the vocal cords. The mean times to achieve ventilation and to conduct the whole procedure were 26.8 ±15.2 sec and 4.3± 0.6 min respectively. As far as the ease of technique is concerned, the technique was easy in 28 patients, corrective manoeuvres were required in 12 patients and reinsertion of the CTrach was needed in 2 patients.

Haemodynamic parameters of the patients are shown in table 3. Mean heart rate increased significantly after insertion of the LMA CTrach and also after endotracheal intubation when compared with the baseline heart rate. On the other hand, there was a significant decrease in systolic and diastolic blood pressures following CTrach insertion as well as endotracheal intubation in comparison to the pre induction values. Hypotension was detected in 4 (10%) patients after CTrach insertion and in 6 (20 %) patients after endotracheal intubation. In all these patients hypotension persisted only for 2 – 3 minutes and was managed by rapid infusion of intravenous fluid. All patients maintained oxygen saturation above 96% throughout the procedure.

There was no patient movement at the time of intubation through the CTrach, however, 7 (17.5%) patients had bucking at the time of CTrach removal. These episodes of bucking were either self limiting or resolved following the administration of neuromuscular blockers. In the post operative period pain on swallowing and sore throat were complained by 4 (10%) patients each. These complaints were managed conservatively and resolved within 24 hours.

DISCUSSION: This is the first reported series of the use of LMA CTrach in anaesthetized but unparalysed patients. The main advantage of this technique over awake tracheal intubation is that patient cooperation is not required and thus it can be easily used in uncooperative patients with anticipated difficult airway. This also eliminates the recall in the post operative period of intubation procedure.

For anticipated difficult airway situations, awake fibreoptic intubation remains the technique of choice [6]. However supraglottic devices are useful alternatives provided the mouth opening is adequate and there is no oropharyngeal pathology [10, 11, 12]. The CTrach provides a patent airway conduit [12, 13] and the intubation can be facilitated under direct vision. The use of CTrach also gives the operator time to optimize the laryngeal view and patient's physiological parameters before intubation, as ventilation and oxygenation is continued throughout the intubation process [2, 3, 14]. Thus CTrach is a useful adjunct in difficult airway situations.

The performance evaluation studies of CTrach have shown the safety and efficacy of this device when used in paralysed patients with normal airway [1, 4, 5]. Liu et al reported 100% first attempt intubation success rate when CTrach was used in 84 normal paralysed patients [4]. It has also been successfully used in patients with potential [9, 11, 12] or unanticipated difficult airway [10].

The awake insertion of the CTrach followed by intubation under anaesthesia requires the inordinately high concentration of inhalational anaesthetic agents. Wender et al reported the awake insertion of CTrach in morbidly obese patients [6]. They attempted intubation once the end tidal concentration of sevoflurane reached 4-5% for at least 1 min. Haemodynamic changes were not discussed in this case series. However, the administration of high concentrations of sevoflurane can cause cardiovascular or cerebrovascular compromise in susceptible patients [7]. In addition, awareness remains the problem during awake insertion of the LMA CTrach.

We could ventilate and intubate trachea successfully using CTrach in all 40 patients. Thus the ease of ventilation and success of intubation using CTrach are comparable in awake patients and anaesthetized but unparalysed patients. However, awake intubation using CTrach

requires patient's cooperation and has the potential risk of recall and patient discomfort. Lopez et al reported the use of the LMA CTrach for awake intubation in 21 patients with difficult airway using sedation and topical instillation of lignocaine to anaesthetize the larynx [8]. They found CTrach easy to use for awake orotracheal intubation. However the incidence of recall was reported to be about 70%. Also corrective manoeuvres were required in a large number of patients (50%). In our series, only 30% patients required these manoeuvres; however we had included all patients irrespective of airway classification. In all three patients (two patients with MPG III and reduced TMD and one patient with reduced TMD only) with anticipated difficult airway, we were able to ventilate and obtain the best laryngeal view following simple corrective manoeuvres [1]. In all these three patients we were able to intubate the trachea successfully in first attempt. Obesity, a risk factor for difficult mask ventilation, did not affect the ease of ventilation, requirement of corrective manoeuvres or the success of first attempt intubation in the present study.

In our study, hypotension was detected in 10% of patients after CTrach insertion and in 20% after endotracheal intubation. The cause of hypotension is definitely inhalational induction with sevoflurane as LMA insertion is attempted once the steady state end- tidal concentration of 2% sevoflurane is achieved. The comparative high incidence of hypotension after tracheal intubation can be attributed to the prolonged duration for which sevoflurane is used to maintain adequate depth of anaesthesia that include waiting period of two minutes following LA instillation over the vocal cords. Hypotension persisted only for short duration and was managed only by rapid infusion of intravenous fluid.

This study is the first series reporting the immediate and postoperative complications following the use of LMA CTrach. The only immediate complication encountered at the time of CTrach insertion was gag reflex in 4 patients which was self limiting. Bucking over the endotracheal tube at the time of removal of the CTrach was observed in 7 patients, although no such problem was encountered at the time of intubation through CTrach. This high incidence of bucking was probably due to carinal stimulation at the time of CTrach removal because of inadvertent inward movement of tracheal tube. As CTrach removal is a blind procedure, advancement of the tracheal tube is usual at this time as one tends to exert slight downward pressure on the tracheal tube so that it does not slip out at the time of removal of CTrach. This problem can be prevented by more appropriate designing of the CTrach endotracheal tube with a visual trigger or mark 3 cm proximal to the endotracheal cuff [14]. It would help placement of tracheal tube to appropriate depth within the trachea and thus prevent the stimulation of carina at the time of removal of CTrach.

Recently CTrach has been incorporated in an algorithm for difficult airway management. Amathieu et al prospectively assessed a difficult airway algorithm that included two modified optical devices i.e. Airtraq laryngoscope and LMA CTrach [15]. They restricted the use of awake intubation to only few indications like restricted mouth opening ≤ 25 mm, severe cervical flexion deformity and history of previous impossible tracheal intubation. All the patients were anaesthetized and paralysed using succinylcholine or non depolarizing muscle relaxant. LMA CTrach was used for tracheal intubation following failed attempts with Macintosh and Airtraq laryngoscopes or as a rescue device in cases of impossible facemask ventilation. LMA CTrach achieved 100% success rate for ventilation and viewed tracheal intubation in this series.

LMA CTrach is available in four sizes i.e. 3, 4, 5 and 5L. Development of CTrach for paediatric patients is highly desirable as these are the potential candidates where awake intubation cannot be performed.

CONCLUSION: The use of LMA CTrach for facilitating endotracheal intubation in anaesthetized but unparalysed patients provides a high success rate for both ventilation and tracheal intubation along with the advantages of avoidance of awareness and ease of use in uncooperative patients. We suggest incorporation of a visual trigger on the endotracheal tube to be used with LMA CTrach and development of paediatric sizes. We also advocate thorough training and clinical experience with this new optical device so as to increase the success of its use in difficult airway situations.

1. **TABLE 1** Grading of vocal cord view [1]

Grade	View of laryngeal structure
I	Full view of the arytenoids and glottis
II	Partial view of the arytenoids and the glottis opening
III	View includes dark areas or blurred view but identifiable structures
IV	Red out indicates epiglottis or other tissues are blocking the view, white out indicates obstruction by secretions or lubricant and black out indicates insufficient light or insufficient depth of insertion into the larynx.

TABLE 2 Recommended adjusting manoeuvres to improve the grade of view [1]

Grade of view	Adjusting manoeuvres
I	None
II	Slightly shift the tip of the CTrach further Increase light intensity on the viewer
III	Perform suction through the channel for intubation Try to position the tip of the CTrach below the epiglottis under direct vision (Down-up manoeuvres)
IV	Down-up manoeuvre in case of red out image Reinsert the CTrach in case of white out image or Increase the light intensity on viewer in black out image.

3. TABLE 3 Haemodynamic parameters; values are mean (SD)

	Pre induction	After CTrach insertion	After intubation
Heart rate (bpm)	94.5 ±17.2	100.0 ±19.1*	106.0±18.1*
SBP (mmHg)	124.4± 14.2	111.8 ± 20.1*	107. 1± 17.7*
DBP (mmHg)	79.7(± 9.3	71.2±14.7 *	69.9± 14.9*

SBP: Systolic Blood Pressure, DBP: Diastolic blood pressure, *Significant difference from the pre induction values.

REFERENCES:

1 Timmerman A, Russo S, Graf B. Evaluation of the CTrach – an intubating LMA with integrated fiberoptic system. Br J Anaesth 2006; 96: 516-21.
2 Timmerman A, Russo S, Graf B. LMA CTrach™: Initial experiences in patients with difficult to manage airways. Anaesthesia 2006; 55: 528-34.
3 Brain AIJ, Verghese C, Addy EV, Kapila A, Brimacombe J. The intubating laryngeal mask: a preliminary clinical report of a new means of intubating the trachea. Br J Anaesth 1997; 79: 704-9.
4 Liu EH, Goy R, Chen F. The LMA CTrach ™, a new laryngeal mask airway for endotracheal intubation under vision: evaluation in 100 patients. Br J Anaesth 2006; 96: 396-400.
5 Liu EH, Goy RW, Lim Y, Chen FG. Success of tracheal intubation with intubating laryngeal mask airways: a randomized trial of the LMA Fastrach and LMA CTrach. Anesthesiology 2008; 108: 621-6.
6 Wender R, Goldman AJ. Awake insertion of the fibreoptic intubating LMA CTrach in three morbidly obese patients with potentially difficult airways. Anaesthesia 2007; 62: 948-51.
7 Deem SA, Bishop JM, Bedford RF. Physiologic and pathologic responses to intubation. In: Hagberg CA, ed. Benumof's Airway management, 2nd ed. Philadelphia: Mosby Elsevier 2007. pp. 193-214.
8 Lopez AM, Valero R, Pons M, Anglada T. Awake intubation using the LMA CTrach in patients with difficult airways. Anaesthesia 2009; 64: 387-91.
9 Dhonneur G, Ndoko SK, Yavchitz A, et al. Tracheal intubation of morbidly obese patients: LMA CTrach vs direct laryngoscopy. Br J Anaesth 2006; 97: 742-5.
10 Goldman AJ, Rosenblatt WH. The LMA CTrach in airway resuscitation: six case reports. Anaesthesia 2006; 61: 975-7.
11 Bjerkelund CE. Use of a new intubating laryngeal mask C Trach in patients with known difficult airway. Acta Anaesthesiol Scand 2006; 103: 508.
12 Goldman A, Rosenblatt W. Use of the fiberoptic intubating LMA CTrach ™ in two patients with difficult airways. Anaesthesia 2006; 61: 601-3.

13 Goldman A, Wender R, Goldman J. The LMA CTrach: a prospective evaluation of 100 cases. Anesthesiology 2006; 105: A 521.
14 Greenland KB. Fastrach TM tubes: modifying the design for use with the LMA CTrach ™? Br J Anaesth 2007; 99: 146-7.
15 Amathieu R, Combes X, Abdi W, Housseini LE, Rezzoug A, Dinca A et al. An algorithm for difficult airway management, modified for modern optical devices (Airtraque laryngoscope; LMA CTrach ™). Anesthesiology 2011; 114: 25-33.

EXTENDED SPECTRUM B-LACTAMASE PRODUCTION AMONGST GRAM NEGATIVE BACILLI ISOLATED FROM PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN EASTERN BIHAR.

Krishan Nandan, Sangeeta Dey, Dhananjay Kumar, Aninda Sen, Udayan Ganguly

1. Assistant Professor, Department of Microbiology, Katihar Medical College, Katihar. Bihar.
2. Professor, Department of Microbiology, Katihar Medical College, Katihar. Bihar.
3. Assistant Professor, Department of Microbiology, Katihar Medical College, Katihar. Bihar.
4. Professor, Department of Microbiology, Katihar Medical College, Katihar. Bihar.
5. Professor, Department of Microbiology, Katihar Medical College, Katihar. Bihar.

CORRESPONDING AUTHOR

Dr. Krishan Nandan, Assistant Professor,
Department of Microbiology,
Katihar Medical College,
Karim Bagh, Katihar. Bihar 854105,
E-mail: drknandan@gmail.com,
Phone: +91 9431259633.

ABSTRACT: **BACKGROUND:** Extended Spectrum β-lactamases (ESBL) are enzymes that have the ability to hydrolyze β-lactam antibiotics containing oxyimino group and are inhibited by β-lactamase inhibitors. These enzymes are responsible for resistance to penicillins, monobactams, third generation and in some instances fourth generation cephalosporins. ESBLs are encoded by transferable conjugative plasmids that often code for resistance to other antibiotics as well. **OBJECTIVE:** This prospective study aimed to determine ESBL production in Gram negative bacteria in patients attending a tertiary care hospital in eastern Bihar. **METHOD:** A total of 556 samples from patients attending inpatient and outpatient departments from May 2009 to April 2010 were included in the study. Samples were processed as per standard protocol and antibiotic susceptibility testing was done by modified Kirby–Bauer method. Isolates showing resistance to any third generation cephalosporin were subjected to Double Disc Synergy Test (DDST), Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and MIC reduction test for ESBL production. **RESULTS:** 42.62% of Gram negative bacilli were ESBL producers. 56.3% of Klebsiella pneumoniae strains were found to be ESBL producers whereas only 40.5% of *Escherichia coli* produced ESBL. PCDDT and MIC reduction test showed 100% correlation whereas the DDST failed to detect 13.5% of ESBL producers. All ESBL producers were sensitive to Imipenem and Cefoperazone/ Sulbactam. **CONCLUSION:** The present study gives us an indication regarding the occurrence of ESBL producing Gram negative bacilli in Eastern Bihar. The number of ESBL producers in this region is alarmingly large. It is therefore recommended that PCDDT be incorporated in all laboratories as a part of routine antibiotic susceptibility testing procedures as it is simple, reliable and reproducible test for detection of ESBLs.

KEYWORDS: Extended Spectrum β-lactamases, ESBL, DDST, PCDDT.

INTRODUCTION: Extended Spectrum β-lactamases (ESBL) are enzymes that have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (third generation cephalosporin and Aztreonam) and are inhibited by β-lactamase inhibitors like Clavulanic acid, Sulbactam and tazobactam[1]. Production of ESBLs lead to resistance to penicillins, monobactams, third generation cephalosporins like Cefotaxime, Ceftriaxone, Ceftazidime and in some instances fourth generation cephalosporins also[2].

Emergence of resistance to β-lactam antibiotics began even before the first β-lactam antibiotic penicillin was developed[3]. The first plasmid mediated β-lactamase, TEM-1 was reported in 1965 from an Escherichia coli isolate belonging to a patient in Athens, Greece named Temoniera [hence designated TEM][4]. Over the years, many new β-lactam antibiotics have been developed; however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics results in the emergence of new variants of β-lactamases[5]. The first report of plasmid-encoded β-lactamase capable of hydrolyzing the extended spectrum cephalosporins was published in 1983 from Germany[6]. Hence these new β-lactamases were coined as extended spectrum β-lactamases[7]. Over the past few decades, a number of new β-lactamases in clinical isolates of members of the family Enterobacteriaceae have emerged. The total number of ESBLs characterized exceeds 200 today[8]. ESBLs are encoded by transferable conjugative plasmids which often code for resistance to other antibiotics as well[7]. Being plasmid mediated, they are easily transmitted among the members of Enterobacteriaceae family, thus facilitating the dissemination of resistance not only to β- lactams but also to other commonly used antibiotics such as quinolones and aminoglycosides[9]. Major risk factors for colonization or infection with ESBL producing organisms are prolonged exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, catheterization, instrumental intervention and residence in an institution with high rate of use of third generation cephalosporins.[5]

This prospective study aimed at determining ESBL production in Gram negative bacilli in a tertiary care hospital in eastern Bihar. Detection of ESBL production is important because it ultimately determines the clinical outcome in patients infected with such strains. Studies on ESBL producing Gram negative bacilli have been reported from all parts of India however, to the best of our knowledge; no such studies till date have been reported from Bihar and Jharkhand.

MATERIAL AND METHODS: Clearance from Institutional Ethics Committee was obtained prior to carrying out this study. A total of five hundred and fifty six (556) samples viz. urine, pus, stool and pleural fluid from patients attending different inpatient and outpatient departments were included in the study. A brief clinical history of the patients regarding antibiotic intake, instrumentation and duration of hospital stay was taken. Specimens collected were inoculated on 5% sheep blood agar and MacConkey's agar. They were identified by standard biochemical tests.[10] Antibiotic Susceptibility Testing (AST) was done on Mueller–Hinton agar (MHA) plates by modified Kirby–Bauer disc diffusion technique using commercially available antibiotic discs (HiMedia, Mumbai). When the zone of inhibition of an isolate for any one or more of the third generation cephalosporin (Cefotaxime, Ceftriaxone, Ceftazidime & Cefoperazone) was less than or equal to the zone diameter recommended by CLSI (Clinical and Laboratory Standards Institute), the isolate was further tested by the PCDDT, MIC reduction test and DDST for ESBL production.[11]

DDST: Bacterial suspension to be tested was prepared in Mueller–Hinton Broth (MHB). After matching the turbidity to 0.5 McFarland's standard, the organism was inoculated on MHA as per guidelines for disc diffusion technique. Amoxycillin/Clavulanic acid disc was placed in the centre of the plate. Four antibiotic discs i.e. Cefotaxime, Ceftazidime, Ceftriaxone and Cefoperazone were placed at a distance of 20 mm (centre to centre) from Amoxycillin/Clavulanic acid disc and at 90° from each other. The plates were examined after

overnight incubation. Enhancement of inhibition zone for any of the four antibiotic discs towards Amoxycillin/ Clavulanic acid disc indicated the production of ESBL by the strain.

PCDDT: MHA plates were inoculated in the same manner as DDST. Four antibiotic discs viz. Cefotaxime, Cefotaxime/ Clavulanic acid, Ceftazidime and Ceftazidime/ Clavulanic acid were placed at a distance of 30 mm (centre to centre) from each other. Plates were examined after overnight incubation at 37°C. ESBL production was confirmed when there was an increase in the zone diameter by 5 mm or more when Clavulanic acid was added to the respective antibiotic. **[Fig. 1]**

MIC reduction test: Bacterial suspension to be tested was prepared in MHB and matched to 0.5 McFarland's standard. As a final concentration of 5 X 10^5 CFU/ml was required, bacterial suspension equivalent to 0.5 McFarland's standard was further diluted 1:100 in MHB.[12]

- Antibiotics in powder form were obtained from Sigma-Aldrich Chemicals Pvt. Ltd, Bangalore. Dilutions were prepared in the following range:
  - Ceftazidime : 0.25 – 256 µg/ml
  - Ceftazidime/Clavulanic acid : 0.25/4 – 256/4 µg/ml
  - Cefotaxime : 0.25 – 256 µg/ml
  - Cefotaxime/Clavulanic acid : 0.25/4 – 256/4 µg/ml

Antibiotic ranges were prepared one step higher than the final dilution range required to compensate for the addition of equal volume of inoculum. 100 µl of each antibiotic dilution was dispensed in their respective labelled wells. 100 µl of diluted bacterial suspension was added to each well. 200 µl of uninoculated and inoculated broth were also dispensed in wells in each row as sterility and growth control. After overnight incubation, the microtitre wells were examined. The lowest concentration of the antibiotic that showed no visible growth represented the MIC value of the organism. An isolate was confirmed as ESBL producer if there was ≥ 3 two folds (eight times) reduction in MIC of third generation cephalosporin on addition of Clavulanic acid as compared to third generation cephalosporin when used alone[12]. **[Fig. 2]**

RESULTS: A total of five hundred and fifty six (556) samples were received in the laboratory during the study period out of which one hundred and twenty two strains of Gram negative bacilli were isolated (21.9%). 62 strains were from indoor while 60 were from outdoor patients. Overall 52 (42.6%) of these isolates were found to be ESBL producers; 56.3% of Klebsiella pneumoniae were ESBL producers, followed by 50.0% of Klebsiella oxytoca and 40.5% of Escherichia coli. ESBL production was also seen in Pseudomonas aeruginosa (35.7%) and Proteus mirabilis (33.3%). **[Table 1]**

Most of the ESBL strains were isolated from the department of Surgery (38.5%) followed by department of Obstetrics & Gynaecology (28.9%) and department of Medicine (23.1%). Comparison of DDST and PCDDT with MIC reduction test showed that the PCDDT was superior to the DDST for detection of ESBL producers. PCDDT showed 100% correlation with MIC reduction test whereas DDST failed to detect 13.5% of ESBL producers.

ESBL producers were isolated more frequently in patients hospitalized for more than 14 days (44.8%) when compared with ≤ 7 days of hospitalization (20.7%). [**Table 2]**

All ESBL producers were resistant to Cefuroxime and Piperacillin. 90.0% of strains were resistant to Cefoperazone, 82.7% to Ceftriaxone, 76.9% to cefotaxime and 75.0% to Ceftazidime.

All the strains were sensitive to Imipenem and Cefoperazone/ Sulbactam combination. [**Table 3]**

DISCUSSION: The present study gives us an indication regarding the occurrence of ESBL producing Gram negative bacilli in Eastern Bihar. Unfortunately the number of ESBLs isolated in this region is alarmingly large (42.6% of all Gram negative bacilli). It was observed that majority of these ESBL producers were from indoor patients especially in those with prolonged hospital stay. Various studies have reported ESBL production to be as high as 61.7% and as low as 11.0%.[13,14,15]

Majority of ESBL producers were Klebsiella pneumoniae, this was followed by Klebsiella oxytoca and Escherichia coli. Many other studies have also reported that majority of ESBL producers were either Escherichia coli or Klebsiella pneumoniae.[13] [14,15] As was seen in this study, other authors have also reported ESBL production in Klebsiella oxytoca, Pseudomonas aeruginosa, Proteus mirabilis and Citrobacter freundii. [13,14,16,17]

The PCDDT was found to give good results as far as detection of ESBL was concerned, showing 100% concordance with the MIC reduction test. Similar findings were also reported by other workers.[7]

Presence of these organisms in the hospital environment is a man-made phenomenon due to over-use and misuse of 3rd generation cephalosporins and other broad spectrum antibiotics. Proper infection control practices and formulation of a hospital antibiotic usage policy is clearly indicated. The other main issue that needs to be addressed is the incorporation of tests for detection of ESBL as a routine in all Microbiology laboratories. The PCDDT was found to be a simple, reliable and reproducible test that showed 100% conformity with the MIC reduction test which is time taking, difficult to interpret and needs a high degree of precision. The PCDDT therefore has the potential to be incorporated in all laboratories, especially smaller ones that do not have the resources to avail automated systems, as a part of routine antibiotic susceptibility testing procedures.

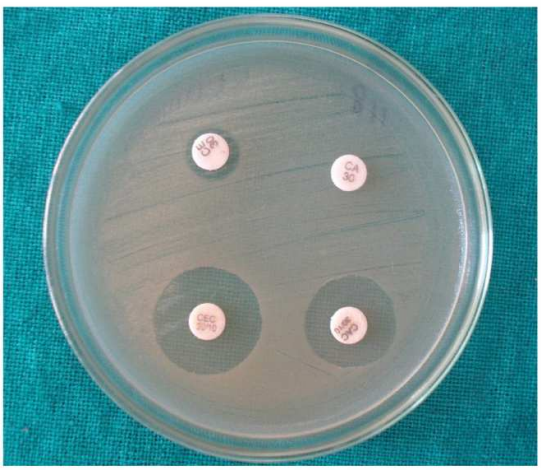


Fig.1 : Phenotypic Confirmatory Disc Diffusion Test showing enhancement of inhibition zone with corresponding disc containing clavulanic acid.

Fig.2 : Determination of Minimum Inhibitory Concentration by microdilution method.
CA: Ceftazidime CAC: Ceftazidime/clavulanic acid
CE: Cefotaxime CEC: Cefotaxime/clavulanic acid [arrows indicating MIC]
GC: Growth control SC: Sterility control

Table 1: Rate of isolation of ESBL producers among Gram negative isolates.

Isolate	ESBL (%)	Non-ESBL (%)	Total
Escherichia coli	30 (40.54)	44 (59.46)	74
Klebsiella pneumonia	09 (56.25)	07 (43.75)	16
Klebsiella oxytoca	04 (50.00)	04 (50.00)	08
Pseudomonas aeruginosa	05 (35.71)	09 (64.29)	14
Proteus mirabilis	02 (33.33)	04 (66.67)	06
Citrobacter freundii	02 (50.00)	02 (50.00)	04
Total	52 (42.62)	70 (57.38)	122

Table 2: Duration of hospital stay in relation with ESBL infection.

Duration of stay (days)	ESBL	Percentage	Non-ESBL	Percentage
≤7	06	20.69	22	66.67
8 – 14	10	34.48	06	18.18
>14	13	44.83	05	15.15
Total	29	100.00	33	100.00

Table 3: Antibiotic susceptibility pattern of ESBL and non-ESBL producing isolates.

Antibiotics	ESBL production	Resistant (%)	Intermediate (%)	Sensitive (%)	Total
Cefuroxime	ESBL	52 (100.0)	0 (0.00)	0 (0.0)	52
	Non ESBL	58 (82.9)	11 (15.71)	01 (1.4)	70
Ceftazidime	ESBL	39 (75.0)	13 (25.00)	0 (0.0)	52
	Non ESBL	18 (25.7)	10 (14.29)	42 (60.0)	70
Cefotaxime	ESBL	40 (76.9)	10 (19.23)	02 (3.8)	52
	Non ESBL	22 (31.4)	09 (12.86)	39 (55.7)	70
Ceftriaxone	ESBL	43 (82.7)	09 (17.31)	0 (0.0)	52
	Non ESBL	22 (31.4)	0 (0.00)	48 (68.6)	70
Cefoperazone	ESBL	47 (90.4)	04 (7.70)	01 (1.9)	52
	Non ESBL	20 (28.6)	10 (14.29)	40 (57.1)	70
Amikacin	ESBL	06 (11.5)	04 (7.70)	42 (80.8)	52
	Non ESBL	25 (35.7)	05 (7.15)	40 (57.1)	70
Levofloxacin	ESBL	14 (26.9)	07 (13.46)	31 (59.6)	52
	Non ESBL	27 (38.6)	11 (15.72)	32 (45.7)	70

Gentamicin	ESBL	36 (69.2)	01 (1.92)	15 (28.9)	52
	Non ESBL	41 (58.6)	09 (12.86)	20 (28.6)	70
Piperacillin	ESBL	52 (100.0)	0 (0.00)	0 (0.0)	52
	Non ESBL	24 (34.3)	21 (30.00)	25 (35.7)	70
Cefoperazone/ Sulbactam	ESBL	0 (0.0)	0 (0.00)	52 (100.0)	52
	Non ESBL	16 (22.9)	06 (8.57)	48 (68.6)	70
Piperacillin/ Tazobactam	ESBL	03 (5.8)	01 (1.92)	48 (92.3)	52
	Non ESBL	18 (25.7)	23 (32.86)	29 (41.4)	70
Imipenem	ESBL	0 (0.00)	0 (0.00)	52 (100.0)	52
	Non ESBL	0 (0.00)	0 (0.00)	100 (100.0)	70

REFERENCES:

1. Brooks GF and Carroll KC. Antimicrobial chemotherapy. In: Brooks GF, Carroll KC, Butel JS, Morse SA (eds). Jawetz, Melnick and Adelberg's Medical Microbiology, 24th ed. The McGraw-Hill Companies: New York: 2007; 162.
2. Russo AT and Johnson JR. Diseases caused by Gram negative enteric bacilli. In: Fauci AS, Kasper DL, Longo DL, Brawnwald E et al. (eds), Harrison's Principles of Internal Medicine, 17th ed, The McGraw Hill Companies: Boston: 2008; vol. I: 938.
3. Abraham EP and Chain E. An enzyme from bacteria able to destroy penicillin. Nature. 1940; 146: 837.
4. Datta N and Kontomichalou P. Penicillinase synthesis controlled by infectious R Factors in Enterobacteriaceae. Nature. 1965; 208:239–244.
5. Chaudhary U and Aggarwal R. Extended spectrum beta lactamases (ESBL) – An emerging threat to clinical therapeutics. Indian J Med Microbiol. 2004; 22(2): 75-80.
6. Knothe H, Shah P, Krcmery V et al. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection. 1983; 11: 315–317.
7. Agrawal Parul, Ghosh AN, Kumar Satish et al. Prevalence of extended spectrum β-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital. Indian J Pathol Microbiol. 2008; 51(1): 139-142.
8. Paterson David L and Bonomo Robert A. Extended spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005; 18(4): 657-686.
9. Varaiya A, Dogra J. Kulkarni M et al. Extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infection. Indian J Med Microbiol. 2008; 26(3): 281-282.

10. Forbes BA, Sahm DF, Weissfeld AS (eds). Overview of Bacterial Identification Methods and Strategies. In: Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, Missouri. 2007; 216-247.
11. CLSI. Performance standards for antimicrobial susceptibility testing: Eighteenth informational supplement, M100-S18, January. 2008; 28(1): 162-163.
12. Andrews JM. Determination of minimum inhibitory concentration. J Antimicrobiol Chemother.2001; 44: 5-16.
13. Deep and Usha Arora. Extended spectrum β-lactamases – mediated resistance among the family Enterobacteriaceae. Indian J Pathol Microbiol. 2007; 50(2): 458-461.
14. Sridhar Rao PN, Basavarajappa KG and Krishna G. Leela. Detection of extended spectrum beta-lactamase from clinical isolates in Davangere. Indian J Pathol Microbiol. 2008; 51(4): 497-499.
15. Kader Abdulrahman Abdulla and Kumar Angamuthu. Prevalence and antimicrobial susceptibility of extended spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a general hospital. Ann Saudi Med. 2005; 25(3): 239-242.
16. Aggrawal Ritu, Chaudhary Uma and Sikka Rama. Detection of extended spectrum β-lactamase production among uropathogens. J Lab Physicians. 2009; 1(1): 7-9.
17. Bhattacharjee A, MR Sen, P Prakash et al. Increased Prevalence of Extended Spectrum Beta lactamase producers in neonatal septicemia cases at a tertiary referral hospital. Indian J Med Microbiol. 2008; 26(4): 356-360.

A SYSTEMATIC APPROACH TO THE EVALUATION OF POST GRADUATE PRACTICAL EXAMINATIONS IN MEDICAL MICROBIOLOGY - AN OVERVIEW

A. G. Prathab, C. Lalitha, Shyam Prasad.

1. Professor, Department of Microbiology and Member, Medical Education Cell, M. S. Ramaiah Medical College, Bangalore.
2. Assistant Professor, Department of Microbiology, M. S. Ramaiah Medical College, Bangalore.
3. Professor, Department of Dermatology and Former Director-Medical Education Cell, M. S. Ramaiah Medical College, Bangalore.

CORRESPONDING AUTHOR

Dr. A. G. Prathab, Professor, Dept. of Microbiology,
MS Ramaiah Medical College, M. S. R. Nagar,
MSRH Post, Bangalore - 560054.
E-mail: dragprathab@yahoo.co.in,
Ph: 91 9845195014.

ABSTRACT: BACKGROUND: Assessment forms an essential part of any curriculum. There are several reasons why we need to assess student performance, most important of which is that it forms a basis for certifying student-doctors as Health-care professionals who practice in society, It also needs to be realized that assessment drives learning and therefore all the desired outcomes need to be taken into account while assessing .A well formatted blueprint will go a long way in fulfilling these objectives (1, 2).

The entire process of evaluation of post graduate practical examinations in Medical microbiology is done over a period of three days. The students have to perform a number of exercises with the end result of the tasks performed on a particular day yielding results on the next or the last day. Therefore it is not easy to maintain objectivity in assessment unless we have a concrete system of evaluation in place.

Hence, there was a felt need to prepare and try out a blueprint for examining post graduates in practical examinations which would bring about the desired outcomes in terms of maximum objectivity and testing of each individual component of the desired skill tested for.
The scheme when adopted for use in exams was found to yield satisfactory results in comparison to conventional methods of practice in evaluation.

This may also be used as a guide to plan and prepare similar documents according to the Syllabi and Guidelines followed by different universities in the subject specialty mentioned here or even for other subjects or courses.

KEYWORDS: Evaluation, Post Graduate, Practical Examinations, Medical Microbiology.

METHOD: The present system of practical examinations in microbiology involves the candidates performing various assigned tasks over a period of three days .Some of these exercises are carried out over a period of 2-3 days.[3,5]

The curricula of different universities in some instances do not specify in particular the distribution of marks for the different exercises in the practical examinations nor is there a uniform structured format which can be used for evaluation across universities.

The usual practice therefore is to award marks to the candidates after completion of exercises on the 3rd day based on the final reporting and presentation and comparing the

performance among the students. Some examiners follow a system of grading the performance of each individual exercise and finally tend to translate the grading to marks.

These methods are subjective and fail to assess the candidates based on the various aspects of the practical skills that need to be taken into cognizance during assessment.

The proposed scheme was prepared with an intention of overcoming the deficiencies Mentioned and making the entire process more objective.

The different exercises to be tested during the process of examinations were listed down and the tasks to be performed in each of them were broken down into individual components. The relevance and importance of each step was analysed and discussed by the panel of examiners and marks were accordingly allotted. Scope for adequate scrutiny of Knowledge, Skill and Attitude was taken into consideration while preparing the checklists.[4]

A set of three examiners were asked to assess the candidates as per the usual practice and one of the examiners used the checklist for assessment of the various exercises.

STRUCTURED SCHEME OF MARKING OF PRACTICAL EXERCISES FOR MD – Microbiology.[3,5]
TOTAL MARKS ALLOTED – 300 MARKS

1. Bacteriology – Short case (Pure Culture) – 30 Marks
2. Bacteriology - Long Case (Mixed Culture)- 50 Marks
3. Mycobacteriology : Ziehl-Neelsen Stain - 10 marks
4. Virology - ELISA – HIV/HBsAg/HCV – 30 marks
5. Immunology – 30 marks
6. Mycology - 30 marks
7. Parasitology - 30 marks
8. Serology - 30 marks
9. Slides and discussion - 30 marks
10. Microteaching Session / Pedagogy – 30 marks

DAY 1	DAY 2	DAY 3
Bacteriology-Pure culture	Report - Pure	Report - Mixture
Bacteriology - Mixture	Serology	Mycology- Slide culture
Mycology	Immunology	Slide discussion
Virology	Mycobacteriology	Pedagogy
Animal experiments	Parasitology	
	Slides	

1. Bacteriology : Short Case : 30 Marks[6]

1. Day 1- Preliminaries - 06 marks - 20%
2. Day 2- Isolation on plates - 03 marks -10%
3. Biochemical reactions -03 marks -10%
4. Antibiotic sensitivity testing - 03 marks -10%
5. Interpretation & Reporting - 03 marks -10%
6. Presentation - 03 marks -10%
7. Discussion – 06 marks - 20%
8. Time Management - 03 marks – 10

2. Bacteriology - Long Case: 50 Marks [6]

1. Day 1- Preliminaries - 06 marks - 12%
2. Day 2- Isolation on plates - 10marks -20%
3. Day 2 - Discussion – 06 marks - 12%
4. Day 3 – Biochemical reactions – 06 marks – 12%
5. Antibiotic sensitivity testing - 04 marks -08%
6. Interpretation & Reporting - 06 marks -12%
7. Presentation - 03 marks -06%
8. Discussion – 06 marks - 12%
9. Time Management - 03 marks - 06%

3. Mycobacteriology : Ziehl-Neelsen Stain : 10 marks[6]

1. Staining - 03 marks – 30%
2. Diagram – 02 marks – 20%
3. Reporting & Presentation – 20%
4. Discussion – 30%

4. Virology - 30 marks - ELISA – HIV/HBsAg/HCV[8]

1. Test Results –09marks – 30%
2. Interpretation – 06 marks – 20%
3. Presentation – 06 marks – 20%
4. Discussion – 09 marks -30%

5. Immunology-30 marks-ASO/CRP/RA [8] **and Animal Experiments- 15 marks each.**[6]

1. Reporting & Interpretation – 09 marks -30%
2. Discussion - 06 marks – 20%
3. Handling of Test Animal – 06 marks – 20%
   (Rabbit/ Mice/Guinea pig)
4. Discussion – 09 marks – 30%

6. Mycology : 30 marks.[7]

Day 1 – Interpretation & Reporting – 4.5 marks
Diagrams – 1.5 marks
Presentation – 3 marks
Discussion - 6 marks
Day 3 – Slide Culture – 06 marks
Diagram - 03 marks
Discussion – 06 marks

7. Parasitology : 30 marks.[7]

1. Focusing of Ova/ Cysts – 6 marks – 20%
2. Concentration technique – 3 marks – 10%
3. Egg Counting technique – 3 marks – 10%
4. Focusing of the Malarial / Blood Parasite – 6 marks – 20%
5. Diagrams & Report – 3 marks - 10%
6. Discussion – 9 marks (4.5 + 4.5) marks – 30%

8. Serology : 30 marks.[8]

Widal/VDRL/TPHA/RPR/Brucella Agglutination etc;

1. Technique – 6 marks – 20%
2. Test results – 7.5 marks – 25%
3. Interpretation & Reporting – 3 marks – 10%
4. Presentation – 3 marks – 10%
5. Discussion - 10.5 marks – 35%

9. Slides and discussion : 30 marks-10 slides-3 marks each

Pattern:
Bacteriology – 2 slides
Mycology - 2 slides
Parasitology – 3 slides
Virology – 2 slides
Immunology – 1 slide

1. Identification – 1.5 x 10 = 15 marks – 50%
2. Description - 0.5 x 10 = 5 marks – 16.6 %
3. Diagram - 0.5 x 10 = 5 marks – 16.6%
4. Discussion – 0.5 x 10 = 5 marks – 16.6%

10. Microteaching Session / Pedagogy – 30 marks.[4]

1. Introduces the subject – 3 marks
2. Presents topic in a logical sequence – 3 marks
3. Emphasises on important points – 3 marks
4. Appropriate use of A-V aids - 6 marks
5. Shifting emphasis – 3 marks
6. Solicits questions / doubts – 3 marks
7. Interactive session – 3 marks
8. Use of Innovation – Models /specimens etc; - 3 marks
9. Summarises effectively – 3 marks

DISCUSSION: As medical educators, we have an ethical obligation towards the society which Expects us to produce and send into the society quality and competent Health- Care professionals on whom they can depend upon in times of need to provide efficient health-care delivery.

This is linked to having a well defined, organized and systematic scheme of evaluation During conduct of examinations especially when systems adopt a summative evaluation process To determine the basic minimum requirements as well as the order of merit which is the norm in most of our universities.

The process of evaluation should include whether the predetermined educational objectives towards a said course have been achieved. For this to be complete, there needs to be a set of objectives and the appropriate measuring instrument which will facilitate in deciding whether the expected outcomes were achieved to the desired extent.

As teachers certifying outcomes of post graduate examinations, we need to have a blueprint of the process of evaluation which is reliable, valid, objective and feasible which can be subjected to improvement with constant review and constructive feedback from senior academicians, fellow colleagues, Peer group members and even the student community.[10]It also eliminates bias and inter-observer/ examiner bias during examinations and ensures that a quality system is maintained during the entire process of evaluation.

The entire process of evaluation of post graduates in Medical microbiology is done over a period of three days, having a large set of exercises with the end result of the tasks performed on a particular day yielding results on the next or the last day where it is not easy to maintain objectivity unless we have a system of evaluation in place.

Presently there is no uniform structured format which can be used for evaluation of practical examinations in Microbiology which comprehensively assesses all the different component skills expected of the student. The usual practice therefore is to award marks to the candidates after completion of exercises on the 3rd day based on the final reporting and presentation and comparing the performance among the students. Some examiners follow a system of grading the performance of each individual exercise and finally tend to translate the grading to marks.

These methods are subjective and fail to assess the candidates based on the various aspects of the practical skills that need to be taken into cognizance during assessment.

The present draft has been prepared with an intention of overcoming the deficiencies mentioned and making the entire process more objective. This format of evaluation if adopted for assessment during examinations will be worthwhile as it deals with an individual's achievement relative to himself than to others. It tries to test competence in that it not only checks the desired skills but goes onto assess the extent to which the skill is perfect. The assessment pattern attempts to look for the best rather than the typical performance. Time management has been given due consideration as a part of the evaluation process which is not accounted for routinely as it is equally important to not only possess the required skills but also carry out the desired assignment within a specified framework of time. The draft helps all the examiners to look for specific criteria during the process of assessment which makes the process more valid. The presence of a well structured assessment tool as a part of the curriculum will also be a motivating factor for the students undergoing the training programme to concentrate on essential skills for overall development.

The blueprint prepared may be used as a guide to plan and prepare similar documents according to the Syllabi and Guidelines followed by different universities in the subject specialty mentioned here or even for other subjects or courses.

REFERENCES:

1. Gipps CV: Beyond Testing: Towards a Theory. London, Faber Press 1994
2. Cohen-Schotanus J: Student assessment and examination rules. Med Teacher 1999, 21:318-321.
3. Rajiv Gandhi University Of Health Sciences,Karnataka -Regulations and curricula of Post-graduate Medical Degrre And Diploma Courses in Pre-Clinical And Para-Clinical Subjects – Volume- I & Volume- II-2006,Vol.I & II : 93-104
4. AnanthaKrishnan.N,Sethuraman.K.R.,Santhosh Kumar- Text book of Medical Education-Principles and Practice,2000,2nd edn,Pages-99-106,145-152.
5. MD- Curriculum &Scheme of examination http://www.Kims University.in/pdf/MD-Microbiology
6. J.G.Collee,J.P.Duguid,A.G.Fraser,B.P.Marmion.Mackie & McCartney Practical Medical Microbiology,13th edn
   Churchill Living stone,London,UK)1989: 141-181
7. Fran Fisher,Norma B.Cook,Fundamentals Of Diagnostic Mycology(W.B.Saunders Company)1998.1-254
8. James G. Cappuccino,Natalie Sherman.Microbiology,A Laboratory manual,7 th edn(Pearson education) 2007 :467-502
9. Gillespie.S.H,Hawkey.P.M,Medical Parasitology-A Practical Approach(Oxford University Press,Newyork)1995:253-264.
10. Southgate L,Grant J.Principles for an assessment system for post graduate medical Training (Internet).London:Post graduateMedical Education and training abroad:2004Sep14.Availablefromhttp://www.p.metb.org.uk/fileadmin/user/QA/Assessment/Principles_foran_assessment_system_v3 pdf

INFECTIONS BY MULTIDRUG RESISTANT ORGANISMS IN A NEUROSURGICAL AND NEUROPSYCHIATRIC CARE CENTRE AND THE CHANGING ANTIMICROBIAL SUSCEPTIBILITY PATTERNS.

R. Ravikumar, Jyothi E. K , Bornali Sarmah Dutta, Vidyasagar K , Nandan T. M, Ashalatha G

1. Professor and Head, Department of Neuromicrobiology, NIMHANS, Bangalore.
2. PhD Scholar, Department of Neuromicrobiology, NIMHANS, Bangalore.
3. Non-PG Senior Resident, Department of Neuromicrobiology, NIMHANS, Bangalore.
4. Non-PG Senior Resident, Department of Neuromicrobiology, NIMHANS, Bangalore.
5. Non-PG Senior Resident, Department of Neuromicrobiology, NIMHANS, Bangalore.
6. Post Doctoral Fellow in Neuroinfections, Department of Neuromicrobiology, NIMHANS, Bangalore.

CORRESPONDING AUTHOR

Dr. R. Ravikumar, Professor and Head,
Department of Neuromicrobiology,
NIMHANS, Bangalore-560029,
E-mail: ravikumarbly@yahoo.co.uk,
Ph: +91 9448073965

ABSTRACT: BACKGROUND: Infections contribute to major morbidity and mortality in the developing countries. Antimicrobial resistance is another major problem with the increasing infections and non availability of ideal antimicrobial agent. Despite the advances in antimicrobial therapy, infections remain a serious burden. This study was designed to assess the increasing infection rates and antimicrobial susceptibility patterns in neurosurgical and neuropsychiatric patients.

Over a period of three years and six months from January 2009 to June 2012, various samples were processed, isolated the infectious agent and identified. The different samples included samples like CSF, urine, pus, tracheal aspirates, shunt & catheter tips, tissue and blood. These were assessed for antimicrobial susceptibility patterns by conventional and automated culture and sensitivity systems. The results were then statistically analyzed.

This study found that in general, there is a trend towards increased rates of infections, the majority of which were caused by Multi Drug Resistant Gram Negative Bacilli (MDRGNB), followed by Candida species and Cryptococcus species, Methicillin Resistant Staphylococcus aureus (MRSA), and Mycobacterium tuberculosis. The infection rates, from January 2009 to June 2012, were 3.7% to 5.1% for MDRGNB, 2.89% to 4.24% for Candida, 2.93% to 3.13% for Cryptococcus, 2.8% to 2.1%(till June 2012) for MRSA and 1.3% to 5.7% for Mycobacterium tuberculosis. Also, a trend towards increasing resistance among the isolates to the carbapenem antibiotics was observed.

The increasing trend of infections is a cause for concern throughout the country. Indiscriminate use of antibiotics, hospital personnel not adhering to safety precautions etc are the main reasons for the increased infection rates. Newer therapeutic agents and strict antibiotic policies are required to effectively control the issue of emerging infections.

KEY WORDS: MDRGNB, MRSA, infections, carbapenems, resistance.

INTRODUCTION: The burden of health care associated infection in developing countries is high. Despite stringent precautions and policies, the infection rates are still rising and the control of infections remains an elusive goal. The prevalence of infections varies widely from region to

region in our country. In our country we are especially vulnerable to infections caused by resistant strains due to the overwhelmingly indiscriminate use and across the counter availability of antibiotics[1]. Development of carbapenem and multi drug resistant Gram negative bacterial strains have led to infections that are hard to treat and add to the physical, mental and financial burden for the patient and the community. A systematic, regional periodic synopsis of the prevalence of the various infections would not only help to study the changing trends of infections, but also to assess the effectiveness of the various control programs. The clinician needs to be aware about the patterns of infections and resistance and of the epidemiological observations in the various wards of the hospital, along with the observations and interpretations from the microbiology laboratory. Intercommunication and cooperation among the microbiologists and clinicians is absolutely essential for the effective treatment of infections.

The immense importance of microbiologic surveillance has been brought into focus by the emergence of newer pathogenic strains as well as multi drug resistant strains[2]. The etiological agents as well as the susceptibility patterns keep changing with time and control policies and it is essential that the data about these be refreshed so as to aid in the development of effective antibiotic policies and hospital infection surveillance programs. This will also help in putting an end to indiscriminate antibiotic prescription and thereby reduce emergence of resistant microorganisms.

The current study was conducted to assess the changing trends in the rate of various infections and the changing patterns of antimicrobial susceptibility in neurosurgical and neuropsychiatric care centre, over the last three and a half year period. In our centre, we commonly encounter neurological cases of chronic & acute pyogenic meningitis, immunocompromised cases with various neuroinfections, various neurodegenerative conditions etc; neurosurgical cases of trauma, shunt infections, post operative infections, hydrocephalus, malignancies etc; and neuropsychiatric conditions which include various psychological syndromes, infectious cases like neurosyphilis, substance abuse related infections etc. Thus, it provides an excellent set up to study various types of infections and their etiologies.

MATERIALS AND METHODS: The study was conducted over a period of three and a half years, from January 2009 to June 2012. All samples that came into the microbiology laboratory during this period were evaluated.

SAMPLES PROCESSED DURING THE STUDY: CSF, blood, tracheal aspirates, urine, tissue, pus, swabs, shunt & catheter tips etc.

COLLECTION: The samples were collected from the patients at the respective hospital wards and transported immediately in a sterile container to the microbiology laboratory.

PROCESSING OF THE SAMPLES:CSF specimens were subjected to gross examination and cell count. Urine and tracheal specimens were stained using Gram's staining technique to identify the Gram's character and morphology of bacteria. The routine samples included, tracheal aspirates, tissue, pus, swabs, shunt & catheter tips etc. These were cultured on Thioglycollate broth, Blood agar and Mac Conkey's agar and incubated overnight at 37^0C. Urine samples were cultured on cystine lactose electrolyte deficient medium (CLED) and blood agar and incubated as for the other samples[3]. Blood was cultured conventionally and also with automated BacT/ ALERT system.

After the overnight incubation, the culture plates were read and Gram's staining was done so as to derive the Gram's nature of the isolates. Based on the biochemical characteristics, the isolates were identified conventionally. Also, antibiotic sensitivity testing was carried out by conventional Kirby- Bauer disc diffusion method as per the CLSI guidelines[4].

All Gram positive cocci in clusters were tested for coagulase production. The Gram negative bacilli were tested for indole production, mannitol fermentation, motility and also tested on triple sugar iron agar medium for obtaining the fermentation pattern[3].

0.5Mc Farland's standards of inoculum were plated on to Meuller Hinton agar (MHA) to make lawn cultures on which Kirby Bauer disc diffusion for antibiotic sensitivity testing was carried out[4].

Vitek 2 compact 60 automated culture and sensitivity system was used to confirm the identification and antibiogram of the isolates[5, 6].

For the isolation of Mycobacterium, CSF was cultured on Lowenstein Jensen's (LJ) medium[3] and also by Mycobacterium Growth Indicator Tube (MGIT) automated technique[7]. The culture was read each week for 8 weeks before pronouncing as 'no- growth'. The antimycobacterial drug susceptibility was also tested using the MGIT system. Strains were genotyped to look for the presence mutant bands coding for Isoniazid and Rifampicin resistance, using Genotype MTBDRplus 96 assay.

India ink staining was done for the cryptococci for the demonstration of the capsule. Latex agglutination test (Cryptococcal antigen test- CRAg) for the identification of the capsular polysaccharide antigen was done for all doubtful cases. The samples were seeded onto the Sabouraud's Dextrose agar (SDA) medium and incubated at room temperature[8]. Incubation was continued for four weeks before declaring it as 'no growth'. Species level identification was based on the biochemical reactions which included caffeic acid test and urease test.

STATISTICAL ANALYSIS: The results were tabulated and the data was analysed using simple statistical tools like measures of central tendency like mean, and percentage calculations etc.

RESULTS: Over the three years and six months study, 35,543 samples were cultured on liquid and solid media for the isolation of the etiological agent. The year and sample wise distribution is given in Table 1. The rates of mycobacterial and cryptococcal isolation from CSF samples were as given in Table 2.

The study revealed that there is a trend, in general, towards an increased rate of infections during the study period. The results have been consolidated in the figures 1- 4 based on the samples from which the organisms were isolated.

Out of all the isolates, multi drug resistant Gram negative bacilli (MDRGNB) topped the list with the most number of isolates. In 2009 from routine samples it was 3.65% of all the samples processed. In the subsequent years, from 2010 to June 2012, the incidence rates were 1.12%, 3.81%, 5.08% respectively. Among the blood and CSF samples MDRGNB were found to be less in number, as expected considering their sterile nature. From blood, no MDRGNB were isolated during 2009. From 2010 to 2011 it was 0.69% and 0.14% respectively. Till the end of the month June 2012, no Gram negative isolates from blood samples were found to be multi drug resistant. CSF sample isolates were respectively 0.08%, 0.27%, 0.52% and 1.35% from 2009 to 2012.

MDRGNB were followed by Candida species, isolated mostly from urine samples. In 2009, it was 2.89% from routine samples, while CSF samples and blood samples had 0.05% and 0.19% respectively. There was a slight dip in the percentage isolation (2.67%) from routine

samples in 2010 when compared to the previous year. But steady increase was found in the subsequent years with 3.24% and 4.24% respectively, during 2011 and 2012. Blood isolates of Candida increased to 0.21% in 2010 but fell to 0.14% in 2011, and again increased to 0.25% in 2012(upto June). CSF isolates were 0.23% in 2010, 0.34% in 2011 and 0.06% in 2012. Refer Fig 1and 2.

CSF Cryptococcal isolates also showed an incidence of 2.93% in 2009, 2.76% in 2010, 3.73% in 2011 and 3.13% in 2012. Refer Fig 3.

MRSA had 2.77% isolation from routine samples during the first year of study. The subsequent years showed an incidence rate of 2%, 3.79% and 2.12% respectively during 2010, 2011 and 2012. From blood samples, the MRSA isolation rates were 0.39%, 0.35%, 1.44% and 0.25% from 2009 to 2012. From CSF samples, the rates were, from 2009 to 2012, 0.16%, 1.18%, 1.07%, and 1.03%. Refer Fig 3.

Mycobacterium tuberculosis isolates from CSF samples showed an incidence rate of 1.26%, 0.94%, 3.36%, and 5.67% over the three and a half year study period, ie from 2009 to 2012.

Most of the isolates from routine samples were Gram negative in nature. CSF sample isolates, on the other hand showed more number of Gram positive isolates. The strains of Gram negative bacteria isolated during the study period was predominantly Escherichia coli, Non fermenting Gram negative bacilli(NFGNB), Pseudomonas species, Klebsiella species, Enterobacter species etc. Coagulase negative staphylococci (CONS) were the predominant isolate from CSF samples. The isolates of Enterobacteriaceae are closely followed by the staphylococcal isolates.

During the period of the study, a change in the pattern of the isolate picture was not observed except that most of the MDRGNB were of non-fermenting nature. Most of the NFGNB strains were identified by Vitek 2C60 to be Acinetobacter species.

Most of the isolates were found to be resistant to Ampicillin. In the first line antibiotics against the Gram negative bacilli, Amikacin and Gentamycin were found to be the most sensitive. Among the second line of antibiotics, Imipenem had around 98% percent sensitivity. Piperacillin- Tazobactum combination was also found to have better efficacy towards the first line drug- resistant isolates. But, the study found that there is a shift towards increasing resistance to carbapenem antibiotics like Imipenem and Meropenem. More and more number of isolates was found to be Imipenem resistant as the study progressed. Further studies are required for statistical accounting of these data.

DISCUSSION: The rates of infections and the antimicrobial susceptibility patterns of different isolates depend on various factors. The chances of getting infections are high in individuals who are immunocompromised, in extremes of age, and in pregnancy[9]. Also, it depends on an individual's occupation, hygiene practises which inturn is heavily dependent on one's socioeconomic status, and public health awareness. Patients in a hospital are more prone to infections depending on their condition, place of stay (i.e. ICU, general wards etc), duration of stay etc[10].

Owing to the indiscriminate use of antimicrobial agents, which include self medication, use of inappropriate drug, not completing the course of the drug etc, has contributed heavily towards the emergence of the newer multi drug resistant strains. Unrestricted use of antimicrobial agents in animals/ animal feeds, fish, and on plants over a relatively long period of

time has resulted in an environment that has encouraged the development of resistance in many bacterial species[11,12].

Longer lasting infections also contribute towards development of resistance. In the community, all these factors along with the microbes' natural tendency to evolve, are responsible for the increase in rates of infections with MRSA, Vancomycin resistant Enterococcus, Gram negative strains carrying extended spectrum beta lactamases (ESBL) & metalobetalactamases etc.

With this retrospective study, we report our experience regarding the existing trends in incidence rates of infections with special reference to the rates of infections caused by MDRGNB and MRSA. Also, we report the rates of incidence and isolation of opportunistic fungal pathogens like Cryptococcus neoformans and Candida species. Cryptococcal strains were mainly isolated from immunocompromised individuals.

MRSA has become increasingly prevalent worldwide since it was first reported among hospitalized patients in a British hospital[13, 14]. MRSA has arisen as a major blood stream pathogen, as corroborated by the present study. Similar trend has been reported in the data from the West over the last two decades. MRSA is a major nosocomial pathogen and presents a serious issue in morbidity control among hospitalized patients[15]. MRSA strains are also highly prone to acquire resistance to other drugs to which these were earlier susceptible. In another unpublished study from our centre, it was found that none of the MRSA isolates were Linezolid resistant. But, it is important to have the knowledge of the existing susceptibility patterns and also, a strong surveillance system to screen for emerging and re-emerging patterns of resistance.

We also present in this study the corresponding data on Mycobacterium tuberculosis isolates from cerebrospinal fluid samples. The drastic increase from 3.13% to 5.08% isolation rate of the tubercle bacilli from the CSF samples can be attributed to the change in isolation method, from January 2012 onward. The isolation technique used, parallel to the conventional culture on LJ medium, was automated Mycobacterium Growth Indicator Tube system which employs Middlebrook's liquid medium for the isolation and also has better sensitivity than the conventional culture methods.

In the present study, most of the results obtained by manual/conventional methodologies were confirmed with automated culture and sensitivity systems. This would help in eliminating false positive results to a greater extend. One of the limitations of the present study is that, being a single centre study the results cannot be widely extrapolated. Also generalisation of surveillance data will be inaccurate as it depends on the institutional antibiotic policies, the type of patients and the community it is associated with. However, we believe that the data presented here would help in giving a greater picture about the need for strict antibiotic policies, hospital surveillance units, maintaining of universal safety precautions, efficient hygiene practises and spreading of awareness among the general public.

It is concluded that, there is increase in the rates of multi drug resistant isolates and therefore the infections caused by these organisms. Both Gram positive and Gram negative organisms are responsible for these infections and the opportunistic pathogens are responsible for increased morbidity among immunocompromised patients. Also, this study stresses on the requirement of strict antibiotic policies for hospital and other health care settings.

AKNOWLEDGMENT: The authors thank the support received from the Director/Vice Chancellor of National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore

and the assistance from the technical and supporting staff of the Department of Neuromicrobiology, NIMHANS.

Table 1: Year wise distribution of samples and etiological agents

Isolates	January to December						January to June	
	2009		2010		2011		2012	
	Isolate from routine samples							
	No	%	No	%	No	%	No	%
Candida	161	2.89	150	2.67	187	3.24	126	4.24
MRSA	154	2.77	112	2.0	219	3.79	63	2.12
MDRGNB	203	3.65	63	1.12	220	3.81	151	5.08
Total no of samples processed	5556		5604		5776		2970	
Isolates	Isolate from blood samples							
Candida	4	0.19	3	0.21	2	0.14	1	0.25
MRSA	8	0.39	5	0.35	20	1.44	1	0.25
MDRGNB	0	0	1	0.69	2	0.14	0	0
Total no of samples processed	2061		1445		1390		399	
Isolates	Isolate from CSF samples							
Candida	2	0.05	5	0.23	10	0.34	1	0.06
MRSA	6	0.16	26	1.18	31	1.07	16	1.03
MDRGNB	3	0.08	6	0.27	15	0.52	21	1.35
Total no of samples processed	3675		2200		2907		1560	

Table 2: Isolation pattern of Mycobacterium tuberculosis and Cryptococcus neoformans from CSF samples.

Isolates	January to December						January to June 2012	
	2009		2010		2011			
	No	%	No	%	No	%	No	%
Mycobacterium tuberculosis	30	1.26	23	0.94	54	3.36	57	5.67
Total no of samples processed	2378		2455		1607		1005	
Cryptococcus neoformans	80	2.93	82	2.76	80	3.73	42	3.13
Total no of samples processed	2729		2968		2146		1342	

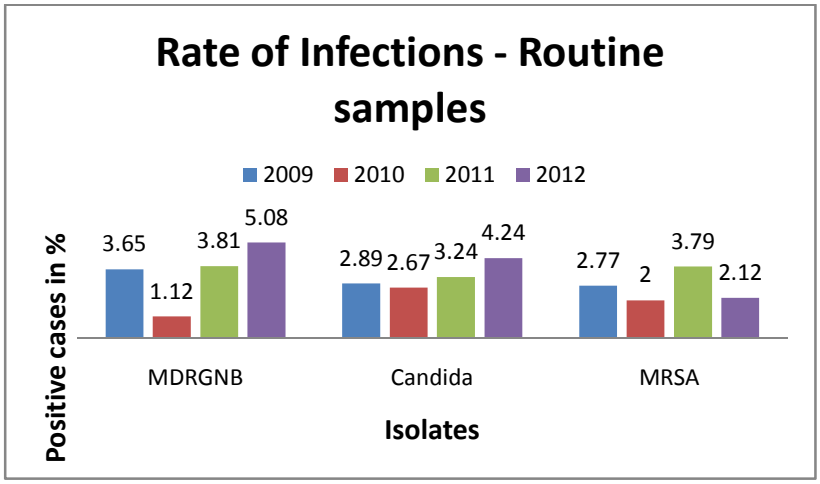


Fig1: Percentage of different isolates from the routine samples.

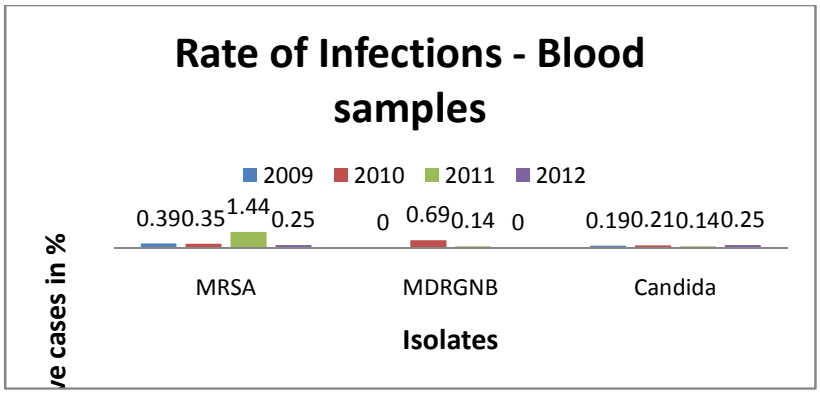


Fig 2: Percentage of different isolates from the blood samples.

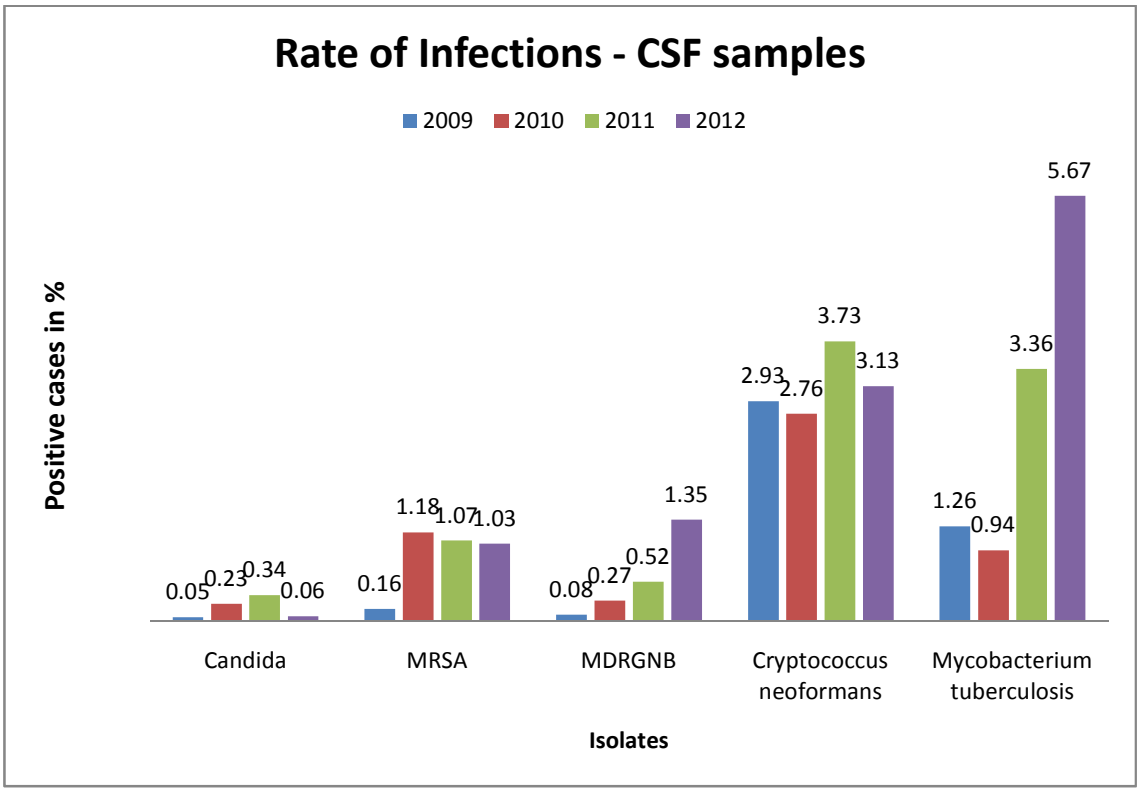


Fig3: Percentage of different isolates from Cerebrospinal fluid.

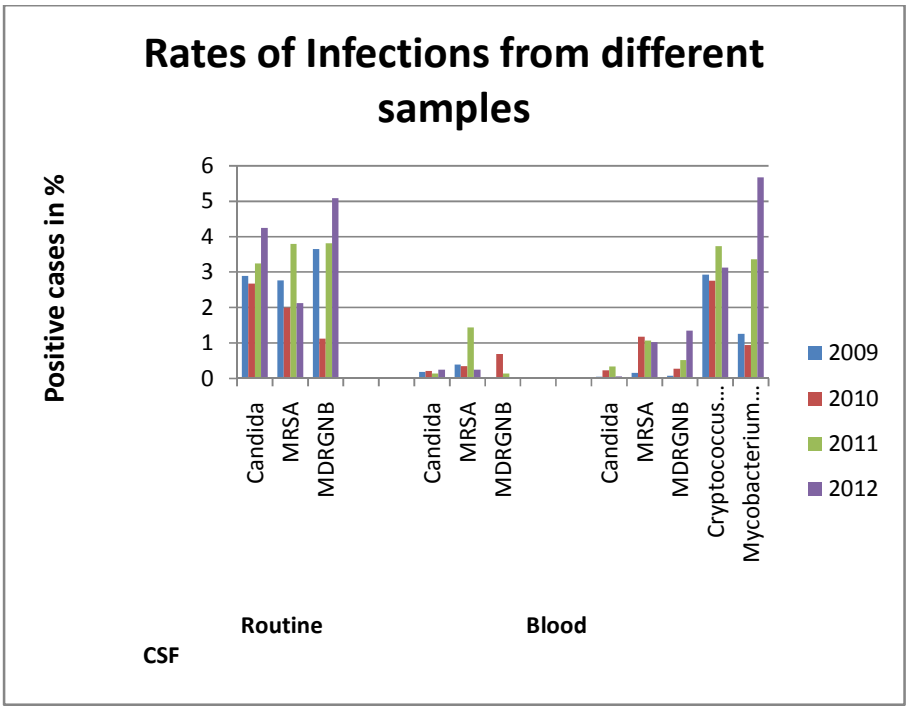


Fig 4: Percentage of different isolates from the various samples.

REFERENCES:

1. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S,Prasad KJ. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, India. Supplement to JAPI.2010;58.32-36.
2. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. J Biosci 2008; 33:593–603
3. Forbes BA, Sham DF, Weissfeld AS, editiors. Overview conventional methods for bacterial identification. Chapter B: Baliley and Scott's Diagnostic Microbiology, 10th ed. St.Louis: The CV Mosby Company; 1998.p167.
4. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. 9th Ed. CLSI Document M100-S22. 2012;32(3). Wayne, PA.
5. Chatzigeorgiou K, Sergentanis TN, Tsiodras S, Hamodrakas SJ, Bagos PG. Phoenix 100 versus Vitek 2 in the Identification of Gram-Positive and Gram-Negative Bacteria: a Comprehensive Meta-Analysis. J Clin Microbiol. 2011;49(9):3284–91.
6. de Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascual A. Use of Positive Blood Cultures for Direct Identification and Susceptibility Testing with the Vitek 2 System. J Clin Microbiol. 2004;42(8):3734–38.
7. 7.Bemer P, Palicova F, Rüsch-Gerdes S, Drugeon HB, Gaby E, Pfyffer GE. Multicenter Evaluation of Fully Automated BACTEC Mycobacteria Growth Indicator Tube 960 System for Susceptibility Testing of Mycobacterium tuberculosis. J Clin Microbiol. 2002;40(1):150–54.
8. Champa Saha D, Xess I, Biswas A, Bhowmik DM, Padma MV. Detection of Cryptococcus by conventional, serological and molecular methods. J Med Microbiol August 2009;58(8):1098-105.
9. Stefan H. Kaufmann E, Michael W. Steward.Topley and Wilson's Microbiology and Microbial Infections, Immunology, 10th Edition 2007.
10. Atul K Patel, Ketan K Patel, Kamlesh R Patel, Sanjiv Shah, Pratibha Dileep. Time trends in the epidemiology of microbial infections at a tertiary care center in West India over last 5 Years. Supplement to JAPI 2010;58:37-40.
11. Antimicrobial Resistance: An Ecological Perspective. Report from the American academy of microbiology; Based on an American Academy of Microbiology colloquium. July 16-18, 1999: San Juan, Puerto Rico.
12. Witte W. Impact of antibiotic use in animal feeding on resistance of bacterial pathogens. In Antibiotic Resistance: origins, evolution, selection and spread. John Wiley & Sons, Ltd. Chichester, West Sussex, England. 1997
13. Barber M. Methicillin resistant Staphylococci. J Clin Path 1961;14:385-93.
14. Colley EW, Mc Nicol MW, Bracken PM. Methicillin-resistant staphylococci in a general hospital. Lancet 1965; 191:595-97.
15. Tenover FC, Gaynes RF. The epidemiology of Staphylococcus aureus. In Fischett VA, Novick RP, Ferretti JJ, Portnoy DA, Rood JI , editors. Gram-positive pathogens. Washington D C: American Society for Microbiology. 2000:414–21.

LABORATORY DIAGNOSIS OF MALARIA, A REVIEW

Raghuveer[1]. C. V, Mangala Goneppanavar[2].

1. Medical Director and Dean, Srinivas Institute of Medical Sciences and Research Institute, Mukka Surathkal, Mangalore
2. Assistant Professor, Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Pillaiyarkuppam, Puducherry.

CORRESPONDING AUTHOR
Dr. Mangala G, Assistant Professor,
Department of Pathology, MGMC&RI,
Pillaiyarkuppam, Puducherry,
E-mail: mangalaanaghaa@gmail.com,
Ph: 0091 9443987586.

ABSTRACT: BACKGROUND: The gross number of malaria cases is on the increase worldwide mainly due to limitations of traditional methods for malaria diagnosis. Even though peripheral smear examination of thick and thin film is widely accepted for detecting Plasmodium, species identification and for parasite count; it has its own pitfalls. Sensitivity of quantitative buffy coat is 41-93%, specificity is >93% for P. falciparum whereas <52% for non-falciparum when parasitemia is 0.002%. With availability of optimal reagents Immunochromatographic tests are being manufactured with different combination of target antigens to suit local malaria epidemiology. OptiMAL has high sensitivity compared to microscopy and ICT but low when compared to PCR. Serological tests are more useful for screening blood donor in case of transfusion- induced malaria with low parasitemia not detectable by blood film. PCR is used for detecting drug resistant strains, genotyping of Plasmodium strains. Placental histology is the best method for diagnosing malaria in pregnancy.
KEY WORDS: Laboratory, diagnosis, Malaria.

INTRODUCTION: Half of the world's population is at risk from malaria accounting for 250 million cases per year causing 8,60,000 deaths.[1] Even though there is a downward trend in incidence of malaria in some countries,[2] the gross number of malaria cases is still on the increase [3] due to limitations of traditional methods. With emerging artemisinin-based combination therapies (ACT's) for malaria treatment, there is a need to look at efficacy and accuracy of various methods.[4] Wherever laboratory support is not available, malaria diagnosis and treatment is done based on 'malaria score' and chances of making an accurate diagnosis in such cases are probably highest in P. vivax infections while lowest in P. falciparum. In high endemic areas fever is often equated with malaria and treated, which may be correct in some percentage of cases during transmission season, but not so outside that season.[5] Contrary to this, there is misdiagnosis in non-endemic areas. Pitfalls of clinical diagnosis are i) over diagnosis of malaria ii) unnecessary morbidity and mortality as other illnesses due to underdiagnosis and undertreatment iii) over treatment and needless wastage of health resources spent on non-malaria cases iv) inappropriate prescription of anti-malarials contributing to anti-malarial drug resistance.[6]

Finding the parasite indicates infection, but does not necessarily imply disease. 'Threshold' above which infection generally means disease, is particularly difficult to define,

because it is i)variable from one endemic area to another ii)age dependent and iii)needs to be adjusted to local level of endemicity.[7] Various methods for malaria diagnosis are:.

I) Peripheral smear examination by light microscopy
II)Fluorescence microscopy techniques
III)Non-microscopic Rapid Diagnostic Tests

a) Immunochromatographic tests-detection of malaria antigen by HRP-2 and pLDH detection methods
b) Immunochromatographic dipstick assays used for diagnosis – ICT Pf, ParaSight F and

IV) Molecular methods.

I A) LIGHT MICROSCOPIC EXAMINATION OF PERIPHERAL SMEAR:

Collection of blood sample: i) Before patients receive anti-malarial treatment ii) Minimum of three samples collected at heights of fever. Parasites can be concentrated by centrifugation because late trophozoites, schizonts and gametocytes get concentrated in the buffy coat and help in identifying P. vivax, P. malariae or P. ovale but not P. falciparum in case of low parasitaemia. **THICK FILM:** Touch the drop of blood with glass slide and spread it evenly with the corner of another slide, to make a square or circular patch of moderate thickness. **THIN FILM:** Blood drop should be smaller for thin film than for thick film. Stain peripheral smear with Leishman's or Giemsa, although Giemsa is preferred in the tropics. Field's is commonly used for staining thin film.

Giemsa stain: Examination of thick film: Before reporting a negative result, at least 200 oil immersion visual fields at 1000 magnification should be examined on both thick and thin smears, which have sensitivity of 90%. A negative test DOES NOT rule out malaria.

ADVANTAGES OF THICK FILM:

1. Easy detection of parasites because of hemolysis
2. It is 20-40 times more sensitive than thin smear
3. Detection limit of 10-50 trophozoites/ul.

DISADVANTAGES OF THICK FILM:

1. Species identification not possible
2. Requires experience

INTERPRETATION OF THICK FILM: Young trophozoites – incomplete rings or spots of blue cytoplasm with a detached red chromatin dot. Late trophozoites of P. vivax - fragmented cytoplasm and Schuffner's stippling may be less obvious. Band forms of P. malariae are less characteristic but schizonts and gametocytes of these species retain their normal appearance, similarly gametocytes of P. falciparum also.

False positives:

1. Ghosts of hemolysed immature erythrocytes may be mistaken for Schuffner's stippling of P. vivax
2. Platelet clumps may simulate P. vivax

3. Vegetable spores, yeast may look like malarial parasites

4. Bacteria may contaminate the Giemsa solution and interfere with identification of malarial parasites.[5]

THIN SMEAR ADVANTAGES:

1. Species identification
2. Quantify parasitaemia
3. Assess for the presence of trophozoites, schizonts, gametocytes and
4. Detecting malarial pigment in neutrophils and monocytes.

Warhurst and Williams (1996) have discussed various methods for quantifying malarial parasites in thick smears. 1) Count the number of parasites in 200 WBC fields (asexual forms). Assuming that each microliter of blood contains 8000 WBC's, parasite count is multiplied by 40 to give the number of parasites /ul of blood. Since WBC count may vary by ten folds in malaria infected patients, accurate density determination requires repetition of WBC counts each time peripheral smear is made.[5] 2) Make thick smear with known volume of blood (5ul), count all the parasites in the smear and divide by 5 to get number of parasites/ul. Counting the parasites per WBC's is tedious and impractical in very high parasitaemia,[7] so parasitized red blood cells (RBC's) in thin blood films are counted as an alternative. The approximate number of parasites present in 1 ul of blood can be calculated by assuming that 1ul of blood contains $5x10^6$ RBC's, so 1% parasitemia will contain 1parasite/100 RBC and 50,000 parasites/ul of blood.[8] Alternatively counting the number of parasites / 1000 RBC's or again counting the number of parasites per 200 WBC and multiplying by a conversion factor can be done. A conversion factor of 4-4.5 million RBC's/ul is used to convert to a value of parasites/ul. Technicians use the following scoring method for 500-600 times magnification.

\+ …. 1-10 parasites / 100 thick film fields
++….11-100 parasites/100 thick film fields
+++….. 1-10 parasites/thick film field and
++++….>10 parasites/thick film field.[6]

Routinely check blood sample once in 12 hrs in falciparum malaria, to know the reduction in the number of parasites per ul of blood. In falciparum malaria parasitemia is > 50%, whereas in non-falciparum it rarely exceeds 2%. 1. To know the severity of malaria 2.To monitor the response to treatment and 3. To detect drug resistant falciparum malaria cases. Developmental stages of parasites help in assessing severity when falsely low parasite count occurs in falciparum due to sequestration of parasitized erythrocytes in tissue capillaries. Presence of more mature parasite forms (>20% of parasites as late trophozoites and schizonts) and >5% of neutrophils containing malarial pigment indicates more advanced disease and worse prognosis.[4]

I B) FLUORESCENT MICROSCOPY: To enhance the detection of parasites in peripheral smears, certain fluorescent dyes like Acridine Orange (AO) and Benzothiocarboxypurine (BCP) having an affinity for parasite nucleus can be used, both get excited at 490nm and exhibit apple green or yellow fluorescence. Rhodamine-123 is especially useful in assessing viable parasites because uptake relies on an intact working parasite membrane. QBC-II (Quantitative Buffy Coat) method

combines an AO coated capillary tube and an internal float, to mechanically expand the buffy coat together with the separation of lighter parasitized RBC's by centrifugation. Platelets concentrated below this buffy coat appear in upper RBC layer or within layer of platelets and WBC.[7] **ADVANTAGES:** 1.Rapid 2.Qualitative screening method: stains the different leucocytes and malarial parasites differently 3.By centrifugation large volume of blood is examined and Parasites are concentrated in narrow zone which makes visualization of parasites easier. **DISADVANTAGES:** 1. Non-specific fluorescence of nucleic acids of all cells especially platelets and Howell-Jolly bodies leads to the misinterpretation. 2. Requires microcentrifuge, fluorescent attachment and expensive capillary tubes. 3. Inferior to Romanowsky in identification of malaria species or for accurate parasite count.[5] When there are <100parasites/ul (0.002% parasitemia), sensitivity is 41-93%, specificity is> 93% for P. falciparum where as for P. vivax and other non-P. falciparum it is < 52%, especially during later stages of development because they become denser during this period and may be hidden in the mononuclear cell layer.[7]

PROCEDURE: Fill the QBC capillary with blood from EDTA stained end, wipe the outer surface, till blood flows to other end (AO stained end), repeat 10-15 times, hold horizontally so that column of blood moves away from AO stained end. Close this end with finger, plug other end with plastic closure, insert the float inside capillary using forceps, gently tap capillary so that flat moves down. Centrifuge in microcentrifuge at 12,000 rpm for 6-8 minutes, place in the groove of capillary holder. **OBSERVATION:** Using transmitted bright light, focus buffy coat under low power, and change light to epifluorescent to appreciate the red, yellow, green layers. Switch off fluorescent light, turn on the transmitted light. Put a drop of fluorescent oil on capillary, change objective to 60 x and focus the granulocyte layer, move the holder sideways and appreciate other layers.

INTERPRETATION: Schizonts, gametocytes - black pigmented structures in lymphocyte layer. Without changing the focus turn on the epifluorescent light and turn off the transmitted light, observe cells in the following order from closed end. Distinctly colored red cells, yellow to orange granulocytes, green lymphocytes and yellow plasma. Ring forms appear dull green with or without an orange dot at one side. Rings of P. vivax are larger than rings of P. falciparum. Double rings are of P. falciparum. Platelets appear as bright green small compact structures. P. vivax amoeboid forms - green irregular structures, schizonts - dull green round structures. Gametocytes of P. vivax - dull green large round structures mimicking small lymphocytes. To confirm turn to transmitted light, if black pigments are seen in these structures they are parasites and if not then they are lymphocytes. Gametocytes of P. falciparum - banana shaped green structures with central yellowish green pigment. Sometimes ring forms are seen in plasma layer also. In old blood sample mosaic pattern of RBC's in background with orange to yellow pigmented parasites seen. If capillary is examined after 24 hours two distinct colors of ring form can be appreciated.[9,10]

BCP intensely stains the nuclei of P. falciparum in contrast to poor staining of RBC inclusions and leucocyte nuclei, with >95% sensitivity and specificity for P. falciparum.[11]

II) NON-MICROSCOPIC RAPID DIAGNOSTIC TESTS: Assays based on detection of malaria antigen indicate current infection. Features of an ideal target antigen are: 1.It should be abundant in blood and other body fluids 2.Be malaria specific with no cross-reactions with other microorganisms 3.Should not persist after parasitemia disappears. Initial tests were based

on antigen-capture or antigen-competition format eg: ELISA After optimal reagents (i.e. monoclonal or polyclonal antibodies against specific malaria antigens) became available assays were simplified for the field use.[5]

Immunochromatographic (ICT) assay is done on small volume of blood 5-15ul using monoclonal antibodies impregnated on a test strip. Colored test line is obtained in 5-20 minutes.[12] Commercial tests are manufactured with different combination of target antigens to suit the local malaria epidemiology.[13] WHO recommendations for RDT's: 1.Results obtained from these test devices should be at least as accurate as results derived from microscopy 2.Sensitivity should be above 95% compared to microscopy 3.Sensitivity of detecting 100 parasites/ul should be 100% 4.Ability to distinguish viable parasites from parasite products like antigens or nucleic acids that are not associated with viable organisms 5.Able to predict treatment outcome or resistance to antimalarial drugs and 6. Should be able to assess parasite density.[14] Antigens released by parasitized cells include those specific to Plasmodium falciparum Histidine-Rich-Protein 2 (PfHRP-2), Plasmodium falciparum specific Lactate Dehydrogenase (Pf-pLDH), antigen specific to Plasmodium vivax (Pv-Pldh), antigens common to Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae [Pan-species parasite lactate dehydrogenase (pan-pLDH)] and Plasmodium aldolase.[7] It is located on parasite cytoplasm and on parasitized erythrocyte membrane being expressed throughout the erythrocytic cycle,[6,15,16] but its concentration varies as parasite moves from ring stage to late trophozoite stage.[17,18] Although predominantly seen in asexual stage, it can be seen in early P. falciparum gametocytes.[18,19] Current RDT's utilise Lateral-Flow Immunochromatographic (Dipstick) technology.

PROCEDURE AND PRINCIPLE: Here sample migrates as liquid across the surface of nitrocellulose membrane by means of capillary action.[15,20] Performance of final assay depends on source of the antigen used. Two types of antibodies, capture antibody and detection antibody are used, either of these can be monoclonal or polyclonal. Even if monoclonal antibodies are directed against same antigen, but if targeted for different epitopes of same antigen, it may exhibit quite different sensitivities and specificities[21] The capture antibodies are bound to membrane in an immobile phase, called so because they are fixed antibodies serve to extract and bind parasite antigen from migrating liquid sample. Detection antibodies are conjugated to an indicator like gold chloride in a mobile phase. This detection antibody-indicator(conjugate)-parasite antigen complex diffuses further across strip until it is bound to capture antibody reacts to another epitope of target antigen. Since capture antibody is in immobile phase and is fixed to narrow section of strip conjugate, signal is concentrated and becomes visible as cherry-red or purple colored line, if targeted antigen is present in the clinical sample. The excess of detection antibody-conjugate that was not bound by antigen and capture antibody, moves further until it is bound to goat anti-mouse antibody thereby generating control line. False positive Pf HRP-2 assay results occur for schistosomiasis caused by Schistosoma mekongi and for rheumatoid factor [22,23] Pf HRP-2 has been detected upto 4 weeks following clearance of parasitemia in patients and travellers.[24] RDT's combine a control line with one or more antigen detecting test lines: those with single test line are named two-band RDT's they target HRP-2 and detect Plasmodium falciparum only, while those with two and three test lines are called three and four band RDT's respectively because they detect other malarial parasites also (P. vivax, P. ovale and P. malariae) by using HRP-2 and Aldolase combined or Plasmodium lactate dehydroghenase (pLDH) as targets. Meta analysis of 21 studies on non-immune travellers with

suspected malaria revealed high sensitivity (88-99%) and specificity (95-100%) of HRP-2 tests for detecting P. falciparum. Positive 3-band HRP-2 test results detected malaria better than 2-band HRP-2 results. Negative HRP-2 based test results excluded malaria better than parasite lactate dehydrogenase based tests.[25] Most of the false negative results occurred at low parasite densities (<100 asexual parasites/ul or < 0.002% of red cells infected), while others occurred at high parasite densities as defined by WHO i.e. infection with > 5% red cells infected which are attributed to genetic variations of HRP-2, but Prozone effect (high dose-hook phenomenon) is also an alternative explanation. It is common in one-step immunoassays like agglutination tests, just dilution of the sample will correct this effect.[26] Since, HRP-2 persists in patients' blood for weeks after successful treatment pLDH is more appropriate for treatment monitoring.[27] Not all therapeutic regimens including chloroquine and quinine, are effective against eradicating gametocytes and sulfa-containing regimens may actually induce gametocytemia,[28] persisting gametocytes produce pLDH and it may remain positive despite clearance of asexual parasite forms.[29] However persisting HRP-2 is advantageous in detecting low-level fluctuating parasitemia in chronic malaria.[30] Mahadev et al compared ICT Pf/Pv with microscopy which revealed 97% sensitivity and 98.3% specificity for P. falciparum with PPV and NPV of 78 and 99.8% respectively. Several studies done at national and international level have revealed comparable results.[31] Rapid dipstick method (OptiMAL) has high sensitivity compared with microscopy and ICT malaria P. f./P.v.[32] but sensitivity is low compared with PCR.[33] Series of studies done in 2011 to determine how various monoclonal pLDH antibodies work in combination, emphasize use of different anti-pLDH antibody combinations to optimize immunochromatographic pLDH tests.[34]

III) SEROLOGICAL TEST:

A) Antibody detection-Indirect Fluorescence Antibody (IFA) and

B) Antigen detection-Immunochromatographic (ICT) Immunoassays.

USES:

i) Screening blood donors in case of transfusion-induced malaria where donor's parasitemia may be below detectable level of blood film.

ii) Patient with fever suspected of malaria whose repeated blood smears are negative

iii) Testing patients treated for malaria recently but whose diagnosis is doubtful.[35]

IFA test is based on the principle of detecting antibodies against asexual blood stage malaria parasites. Titre of > 1:20 are positive, < 1:20 are unconfirmed malaria and > 1: 200 titre are classified as recent infections. It is simple, sensitive but time consuming, expensive fluorescent microscope and trained personnel are required. Specific antibodies are produced after 1-2 weeks of infection and may persist for months to years in semi-immune patients from endemic areas with frequent re-infection. But in non-immune patient treated for single infection antibody levels fall more rapidly and may be undetectable by 3-6 months. Reinfection or relapse leads to secondary response with high and rapid rise in antibody titres.[36,37] False negative results can occur in early acute phase.

IV) MOLECULAR METHODS:

A) POLYMERASE CHAIN REACTION: Highly sensitive, specific, used for confirming uncertain species, detection of drug resistant markers and genotyping of Plasmodium strains.

PCR can detect as few as 1-5 parasites/ul of blood compared with around 50-100 parasites/ul of blood by microscopy or RDT. Also widely used for detecting Plasmodium Knowlesi in humans.[38,39] The 18S rRNA is used as gene target for differentiation of malarial species by nested PCR and reverse-transcription PCR. Other DNA targets like circumsporozoite protein gene is also evaluated for species-specific regions.[40,41] The detection limit using rt-PCR is 50 parasites/ml of whole blood, which is approximately 200 times lower than the detection limit of light microscopic diagnosis. Real-time Nucleic acid sequence-based Amplification (rt-NASBA) detects parasite RNS and is faster than PCR.[42,43]
Micrototal analysis systems (micro TAS) will be able to undertake all steps in analysis and applicable for environments that lack infrastructure. Having high sensitivity they accurately diagnose mixed infections, detect drug- resistant strains and make other genetic markers available.[44]

B) LAMP TECHNIQUE: Detects 18S ribosome RNA of Plasmodium falciparum. It is easy, sensitive, quick and cheaper than PCR. However reagents require cold storage.[45]

C) FLOW CYTOMETRY: accurate, precise and rapid method with application in malaria research.[46]

D) MASS SPECTROPHOTOMETRY: is based on principle of detecting heme from hemozoin which is a parasite-specific biomarker and has sensitivity of detecting upto10 parasites /ul of blood. It requires <1 minute, automated but not suitable for rural areas where electricity is a problem.[47,48]

V) POST-MORTEM DIAGNOSIS OF MALARIA: ICT is sensitive, specific, used especially for non-immune cases and also applicable in autolysed cases where microscopy is not possible. PCR can be used as confirmatory test, however one has to rely on histopathologic findings for malaria diagnosis in highly endemic areas where asymptomatic parasitemia is common.[49] Increased presence of malaria hemozoin pigment in peripheral phagocytes can be used to diagnose malaria in severe and fatal cases.

VI) DIAGNOSIS OF MALARIA IN PREGNANCY: Studies have compared results of peripheral blood film, placental blood film with placental histology for diagnosis of Plasmodium falciparum in pregnancy, which revealed that Placental histology is more sensitive (91%) than peripheral blood film (47%) or placental blood film (63%). So, placental histology is the best method for diagnosing malaria in pregnancy.[50]

REFERENCES:

1. World Health Organization. WHO releases new malaria guidelines for treatment and procurement of Medicines. 9 March 2010. Press release.
2. James C, Sungano M, Philip T and Nirbhay K “A synopsis of current malaria diagnosis trends”. Medical Journal of Zambia, 2009, vol 36 (2).
3. Breman JG, Alilio MS, and Mills A. Conquering the intolerable burden of malaria: What’s new, what’s needed: A summary. Am J. Trop. Med. Hyg 2004; 71(suppl 2): 1-15.

4. Kiszewski A, Teklehaimanot A. A review of the clinical and epidemiological burdens of epidemic malaria. Am J Trop Med Hyg 2004; 179 (suppl 2):128-135.
5. Marcel H. Diagnostic methods in malaria. In: Warrell DA and Gilles HM Eds. Essential malariology. 4th Ed. New York: Arnold publishers; 2002 :35-58.
6. Collee JG, Mackie TJ and McCartney JE. Practical medical microbiology. 14thEd. New York. Churchill Livingstone. 1996.
7. McKenzie et al.WBC counts and malaria. J Infect Dis, 2005; 192:323-330.
8. Moody A. Rapid diagnostic tests in malaria. Clinical Microbiology Reviews. January 2002, 66-78 p.15(1).
9. Wongsrichanalai C et al. Acridine Orange fluorescent microscopy and the detection of malaria in populations with low-density parasitemia. Am.J.Trop.Med.Hyg.1991.44:17-20.
10. Briggs et al. Malaria detection using VCS technology. Am J Clin Pathol, 2006; 126:691-698.
11. Cooke A.H. et al. Use of fluorochrome benzothiocarboxypurine in malaria diagnosis. Trans.R.Soc.Trop.Med.Hyg. 1992.87:549.
12. Chansuda Wongsrichanalai et al. A review of Malaria diagnostic tools: Microscopy and Rapid diagnostic tests (RDT's). Am J Trop Med Hyg. 2007; 77(6):119-127.
13. Bell D, Wongsrichanalai C, Barnwell JW. Ensuring quality and access for malaria diagnosis: how can it be achieved? Nat Rev Microbiol. 2006; 4: 682-695.
14. World Health Organisation. 2000. WHO/MAL/2000.1091. New perspectives in malaria diagnosis. World Health Organisation, Geneva, Switzerland.
15. Howard R.J. et al.1986. Secretion of a malarial histidine –rich protein (Pf HRP II) from Plasmodium falciparum -infected erythrocytes. J Cell Biol. 103:1269-1277.
16. Shiff C.J., Premji Z, and Minjas J.N. 1993. The rapid manual ParaSight -F test. A new diagnostic tool for Plasmodium falciparum infection. Trans.R.Soc.Trop.Med.Hyg.87:646-648.
17. Rock E.P. et al.1987. Comparative analysis of the Plasmodium falciparum histidine-rich proteins HRP-I, HRP-II and HRP-III in malaria parasites of diverse origin. Parasitology 95:209-227.
18. Hayward R.E., Sullivan D.J. and Day K.P.2000.Plasmodium falciparum: histidine-rich protein II is expressed during gametocyte development.Exp.Parasitol.96:139-146.
19. Tjitra E. et al.2001.Persistent ICT malaria P.f/P.v panmalarial and HRP2antigen reactivity after treatment of Plasmodium falciparum malaria is associated with gametocytemia and results infalse positive diagnosis of Plasmodium vivax in convalescence.J.Clin.Microbiol.39:1025-1031.
20. Bell D and Peeling R.W.2006. Evaluation of rapid diagnostic tests: Malaria.Nat.Rev.Microbiol.4 (Suppl.9): S34-S38.
21. Lee N et al.2006. Effect of sequence variation in Plasmodium falciparum histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria.J.Clin.Microbiol.44:2773-2778.
22. Eyal et al.2011.False positive Plasmodium falciparum Histidine-Rich protein 2 immunocapture assay results for Schistosomiasis caused by Schistosoma mekongi. Journal of Clinical Microbiology. 49(6):2331-2332.
23. Laferi H, Kandel K and Pichler H.1997.False positive dipstick test for malaria. N.Engl.J.Med.337:1635-1636.
24. Humar A et al.1997. ParaSightRF test compared with the polymerase chain reaction and microscopy for the diagnosis of Plasmodium falciparum malaria in travelers.Am.J.Trop.Med.Hyg.56:44-48.

25. Arthur M et al.2005.Meta-analysis:accuracy of rapid tests for malaria in travelers returning from endemic areas.Annals of Internal Medicine.142(10):836-846.
26. Philippe Gillet et al.2009. Assessment of prozone effect in Malaria Rapid Diagnostic Tests: Malaria Journal.8:271.
27. Moody A et al., 2000. Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the hospital for Tropical Diseases, London. Br J Haematol 109:891-894. Cross RefMedlineWeb of Science
28. Murray C.K. et al., 2008. Update on rapid diagnostic testing for malaria. Clinical Microbiology Reviews. 21(1):97-110.
29. Miller RS, McDaniel P and Wongsrichanalai C, 2001. Following course of malaria treatment by detecting parasite lactate dehydrogenase enzyme. Br J Haematol 113:558-559. Cross Ref MedlineWeb of Science
30. Bell DR, Wilson DW and Martin LB, 2005. False-positive results of a Plasmodium falciparum histidine-rich protein 2-detecting malaria rapid diagnostic test due to sensitivity in a community with fluctuating low parasite density. Am J Trop Med Hyg 73:199-203.
31. Mahadev SH et al., Role of ICT malaria Immunochromatographic test for rapid diagnosis of malaria. J Pak Med Assoc 2006;56(4):167-171.
32. Khan SA et al., Comparison of optimal malarial test with light microscopy for diagnosis of malaria. J Pak Medical Assoc 2004; 54:404-407.
33. Iqbal J et al., Comparison of OptiMAL test with PCR for diagnosis of malaria in immigrants. J Clin Microbiology 1999;37: 3644-6.
34. Opportunities for improving pLDH-based malaria diagnostic tests (Malaria Journal),1 August,2011, My Journals.org
35. Igbinosa et al., A sequential review on accuracy of detecting malaria parasitemia in developing countries with restriction on resources. J. Med.Med.Sci.2010;1(9)385-390.
36. Garraud O, Mahanty S and Perraut R. Malaria specific-antibody subclasses in immune individuals: a key source of information for vaccine design. Trends Immunol.2003; 24:30-35.
37. Seed CR, Kitchen A and David TM. The current status and potential role of laboratory testing to prevent transfusion-transmitted malaria. Transfusion Med. Rev.2005; 19:225-240.
38. Cox-Singh J et al., Plasmodium knowlesi malaria in humans is widely distributed. Clin Infect Dis.2008;46: 165-171.
39. 39) Luchavez J et al., Human infections with Plasmodium knowlesi, the Philippines. Emerg Infect Dis.2008;14: 811-813.
40. Snounou G et al., High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol. Biochem. Parasitol. 1993; 61: 315-320.
41. Tahar R, Ringwald P and Basco LK. Diagnosis of Plasmodium malariae infection by the Polymerase chain reaction. Trans. R. Soc. Trop. Med and Hyg.1997; 91:410-411.
42. She RC et al., Comparison of immunofluorescence antibody testing and two enzyme immunoassays in the serologic diagnosis of malaria. J Travel Med 2007; 14:105-111.
43. Chotivanich K, Silamut K and Day NPJ. Laboratory diagnosis of malaria infection-a short review of methods. Aust J Med Sci 2006; 27:11-15.

44. Peter G, Jutamaad S and Mathuros R. Microfluidic approaches to malaria detection. Acta Trop 2004; 89(3):357-369.
45. Han ET et al., Detection of four Plasmodium species by genus and species-specific loop mediated-isothermal amplification for clinical diagnosis. J Clin Microbiol 2007; 45:2521-2528.
46. Bhakdi SC et al., Re-evaluating acridine orange for rapid flow cytometric enumeration of parasitemia in malaria-infected rodents. Cytometry A. 2007; 71(9):643-5.
47. Scholl PF et al., Rapid detection of malaria infection in vivo by laser desorption mass spectrometry. Am J Trop Med Hyg.2004;71(5):546-51. 50)
48. Noppadan T et al., Malaria diagnosis: A brief review. Korean J Parasitol.2009; 47(2):93-102.
49. Nicole B. et al., Comparison of different methods for delayed post-mortem diagnosis of falciparum malaria. Malaria Journal 2009; 8:244.
50. Malaria diagnosis: Placental Histology best method for pregnant women. Malaria Weekly, June 2, 2003

COMPARISON OF CONVENTIONAL AND FLUORESCENT STAINING METHODS IN DIAGNOSIS OF PULMONARY TUBERCULOSIS AMONG HIV SEROPOSITIVE INDIVIDUALS

Dr. J. Suria Kumar. Dr. C. Chandrasekar. Dr. S. Rajasekaran

1. Assistant Professor, Department of Microbiology, Institute of Microbiology, Madurai Medical College, Madurai.
2. Superintendent, Government Hospital of Thoracic Medicine Tambaram Sanatorium, Chennai.
3. NACO National Consultant, (ART Quality Management), Chennai.

CORRESPONDING AUTHOR
Dr. J. Suria Kumar, M.D, Assistant Professor,
Institute of Microbiology,
Madurai Medical College, Madurai – 625020.
E-mail: suryjay@gmail.com, suryjay@yahoo.com,
Ph: 0091 9894010207.

ABSTRACT: BACKGROUND: The diagnosis of Tuberculosis (TB), with its high incidence and atypical manifestations in HIV, demands a reliable, rapid and cost effective diagnostic method. Fluorescent staining (Auramine phenol) carries advantage of easy interpretation of AFB compared to conventional Ziehl - Neelsen staining (ZN). **AIM:** To compare the efficacy between ZN and fluorescent staining in detection of AFB in sputum among the HIV seropositives.
MATERIAL AND METHODS: Adult HIV positive individuals, attending GHTM from March to May 2008, with clinical and/or radiological presentations suggestive of Pulmonary TB with no previous exposure to ATT were studied. From 225 clients, early morning sputum sample were subjected to Fluorescent, ZN staining and culture in LJ medium. Results analyzed to determine the sensitivity and specificity of ZN and Fluorescent methods, considering culture as gold standard. **RESULTS :** Of the 225 samples, 43 (19.1%) and 28 (12.4%) were smear positive by fluorescent and ZN stain respectively of which 27 (12%) samples were positive on both stains. The culture turned positive with 44 (19.5%) samples. Based on these reports, the sensitivity and specificity of fluorescent staining was 95.45% and 99.45% respectively and that of ZN stain was 63.64% and 100% respectively. **CONCLUSION:** Fluorescent staining is found superior to ZN staining and this concludes it is a better tool for screening PTB in HIV positives and needs further larger studies to recommend in public health program
KEY WORDS: Pulmonary Tuberculosis, HIV, ZN staining, Fluorescent staining, Auramine stain
MESHTERMS: Tuberculosis, Pulmonary, HIV

INTRODUCTION: In the year 1993, Tuberculosis was declared by World Health Organization (WHO) as a global emergency. Worldwide, one person out of three is infected with Mycobacterium tuberculosis – two billion people in total. TB currently holds the seventh place in the global ranking of causes of death[1]. India has about 1.8 million new cases of tuberculosis annually, accounting for a fifth of new cases in the world - a greater number than in any other country[2]. Tuberculosis is the most common HIV-related opportunistic infection in India, and caring for patients with both diseases is a major public health challenge. Tuberculosis can be expected to develop in more than half of those who are infected with HIV. At present about 5% of new tuberculosis cases in India are occurring in people with HIV infection[2]. HIV and TB form

a lethal combination, each speeding the other's progress. HIV increases not only the risk but also the rate of progression of recent or latent M. tuberculosis infection to disease. This reduces the time available for diagnosis and initiation of treatment. TB is the leading cause of death among HIV-infected persons. Alarmingly, fewer than half of TB cases in HIV-infected patients are diagnosed before death[3]. To complicate the scenario, the clinical presentation of pulmonary tuberculosis is atypical in late stages of HIV challenging the clinical diagnosis at primary and secondary care settings. The resurgence of TB in HIV demands for a reliable, rapid (to indentify the organism) and cost effective diagnostic method to facilitate early detection of tuberculosis for early intervention which is the need of the hour. Detection of *M. tuberculosis* by smear microscopy has highest priority in diagnosis of PTB and it correlates well with infectivity [4] and active clinical disease. The bacilli in the sputum can be detected by both the ZN stain and the Fluorescent stain. Each method is having its own sensitivity and specificity. Sensitivity of the ZN method varies from 20% – 80%. Fluorescent method is easy to interpret and the sensitivity is higher and recommended for large programmes[5]. Comparative studies of these microscopy methods in HIV patients are limited, and the present study will give a refined knowledge over the efficacy and accuracy of the two widely followed staining methods. The current study was undertaken 1) To perform both the conventional ZN and Fluorescent microscopy along with mycobacterial culture to diagnose Pulmonary TB in HIV seropositive individuals in the study population and 2) To compare the conventional and Fluorescent microscopy results with Mycobacterial culture results as standard.

MATERIALS AND METHODS: The study was conducted at the Government Hospital of Thoracic Medicine (GHTM), Chennai, India. The study population comprised of 225 consecutive adult HIV seropositive individuals having clinical or clinical and radiological suspicion of pulmonary tuberculosis, attending the HIV outpatient department (OPD) of GHTM, from the period of February 2008 to May 2008. Children and individuals with previous exposure to Anti-tuberculous drugs were excluded from the study.

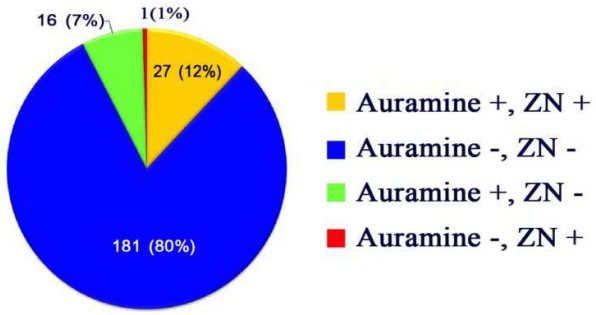
Sputum samples were collected as per the guidelines of Revised National Tuberculosis Control Program and from each early morning sputum sample two smears were made on clean glass slides, air dried and heat fixed. Of the two slides, one each was stained by ZN stain and auramine phenol stain. ZN stained smears were examined under oil immersion objective (x1000) of light microscope and Auramine phenol stained smears were examined by using 40x objective (x400) of fluorescence microscope. Simultaneously the specimens were submitted to culture after decontamination by Petroff's method. The specimen were inoculated on to Lowenstein – Jensen medium under aseptic precautions and incubated at 37^0C for eight weeks to look for the growth of the organism.

On identifying eligible clients from the OPD, the clients were oriented about the study. As per the practice of the hospital, elicitation of clinical history and proper clinical examination were done from which necessary data was collected in the data sheet created for the study along with the basic demographic data. Subsequently the lab results were compiled on each client's data sheet. The results were analyzed to determine the sensitivity and specificity of ZN and Fluorescent method, considering Mycobacterium culture as a gold standard by using Epi-info software.

RESULTS: Of the 225 samples studied, 181 samples turned negative by both of the staining methods as well as by culture. Of the remaining 44 samples which gave positive result by

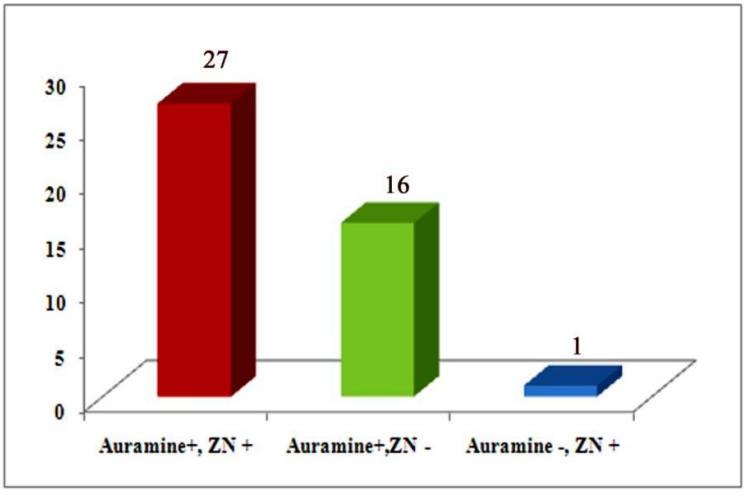
staining method, 43 samples (i.e. 19.1%) showed bacilli by fluorescent method and 28 (ie, 12.4%) samples were smear positive by ZN stain. And out of the 44 positive samples, 27 (12%) samples were positive by both of the stains.

Overall test results of the samples by staining methods



Of the 44 samples what gave positive result by the staining methods, all of them turned culture positive with 100% concordance. Taking the culture result as the standard for reference, the sensitivity and specificity of both the staining methods were calculated. The sensitivity and specificity of fluorescent staining was found to be 95.45% and 99.45% respectively and that of ZN stain was found to be 63.64% and 100% respectively. The p value, as calculated by the McNemar test, for the Fluorescent staining is 1.00 and for ZN staining is < 0.0001.

Chart depicting the comparison of positive results by the staining methods:



DISCUSSION: In this study, analysis was done on the lab results obtained by the staining and culture methods. The p value, as calculated by the McNemar test, for the Fluorescent staining is 1.000 which states that this difference is considered to be not statistically significant and for ZN staining are < 0.0001 states that the difference is significant compare with the gold standard. The sensitivity of Auramine staining is 31.6% higher than the ZN staining. This is well correlates with a similar study by Kivihya- Ndugga et al 2003[6], which shown the sensitivity of the Fluorescence microscopy is 37% higher than ZN staining. Another study by K Prasanthi et al, 2005[7] without using culture as a reference test showed that the sensitivity of Auramine staining

is 26% higher than the ZN staining in HIV positive individuals. But the specificities of both staining methods were almost similar (i.e. 99.5% with Auramine stain and 100% with ZN stain). These findings are supported by Karen R Steingart et al, 2006[8], stating that the mean specificity estimates of fluorescence microscopy and conventional ZN microscopy were similar. The major strength of the study was comparing the staining methods with culture which is considered as gold standard for the diagnosis of mycobacterial infection. The sensitivity and specificity of both the staining methods was calculated comparing the culture result, which adds to the strength to the evidence of the values. Very limited data are available on comparison of sputum microscopic findings with culture result, as reference standard in HIV setting. This study did not address the immune status of the patient, differences in the expertise of microscopists and cost effective issues which may be considered as the limitation of the study.

CONCLUSION: Considering the sensitivity and specificity of the Fluorescent staining in detection of Mycobacterium tuberculosis, with its additional advantage of easy visibility and identification of the organism, fluorescent stain can be considered superior to ZN staining and may conclude Fluorescent staining as a better tool for screening PTB in HIV positives. The relative shorter time consumption for identification of AFB adds further value to the fluorescent method especially for the labs handling high number of specimen. It may be concluded that before recommending for the large scale roll out of fluorescent microscopy in the TB program in India as a screening tool, similar study with larger sample size as well as further study to detect cost efficacy and efficiency costing exercise may be undertaken.

REFERENCES:

1. Global tuberculosis control: surveillance, planning, finances. WHO report 2006. Geneva: World Health Organization, 2006. (WHO/HTM/TB2006.362)
2. Steinbrook R. Tuberculosis and HIV in India. N Engl J Med 2007;356:1198-9
3. Markova R, Todorova Y, Drenska et al. 2009 Usefulness of interferon gamma release assays in the diagnosis of tuberculosis infection in HIV infected patients in Bulgaria. Biotechnol Biotechnol 23: 1103–1108
4. Urbanczik R. 1985. Present position of microscopy and of culture in diagnostic mycobacteriology. Zentralbl Bakteriol Mikrobiol Hyg [A], 260, 81–87.
5. Steingart KR, Ng V, Henry M et al. 2006. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis, 6, 664–674
6. Kivihya-Ndugga LE, van Cleeff MR, Githui WA, et al. 2003. A comprehensive comparison of Ziehl- Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. *Int J Tuberc Lung Dis* 2003; **7:** 1163–71.
7. Prasanthi K, Kumari AR. Effi cacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. 2005. *Indian J Med Microbiol* 2005; **23:** 179–81.
8. Steingart KR, Ng V, Henry M et al. 2006. Fluorescence versus conventional sputum smears microscopy for tuberculosis: a systematic review. Lancet Infect Dis, 6, 570–81.

UROPATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSTITAL

Yashavanth R, Ronald R, Anita K, Narendra N, Faseela T

1. Assistant Professor, Department of Microbiology, AJIMS, Kuntikana, Bejai, Mangalore.
2. Professor and HOD, Department of Microbiology, AJIMS, Kuntikana, Bejai, Mangalore.
3. Associate Professor, Department of Microbiology, AJIMS, Kuntikana, Bejai, Mangalore.
4. Professor, Department of Microbiology, AJIMS, Kuntikana, Bejai, Mangalore.
5. Lecturer, Department of Microbiology, AJIMS, Kuntikana, Bejai, Mangalore.

CORRESPONDING AUTHOR
Dr. Yashavanth Kumar Rai,
Assistant Professor, Dept Microbiology,
AJIMS, Mangalore,
E-mail: dryashwanthrai@gmail.com,
Ph: 0091 9986297656.

ABSTRACT: BACKGROUND: Urinary tract infections (UTI) are one of the most common infectious diseases in humans. Antimicrobial drug resistance is one of the major threats due to wide spread use of inappropriate and empirical antibiotic therapy. The present study highlights the organisms causing UTI and their antimicrobial susceptibility pattern. **MATERIALS AND METHODS:** A total number of 1266 urine samples were analysed in a tertiary care hospital. Urine samples were processed by calibrated loop technique, delivering 0.001 ml of urine and plated on to MacConkey's and Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^0C overnight. Appropriate biochemical tests were carried out to identify the uropathogens. Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents. **RESULTS:** Among this, 332(26.22%) culture positive isolates were identified and their antimicrobial susceptibility patterns were analysed. Among the culture positive isolates, Escherichia coli (54.21%)was the most common pathogen followed by Klebsiella spp (22.89%) and Enterococcus spp (6.92%). Majority of these isolates were resistant to Cotrimoxazole, Amoxycillin/ Clavulanic acid, Piperacillin, Norfloxacin, Ciprofloxacin and also to third generation cephalosporins. Escherichia coli isolates showed good sensitivity towards Amikacin, Piperacillin/ Tazobactum, Nitrofurantoin and Meropenem. Klebsiella isolates showed good sensitivity towards Amikacin, Levofloxacin, Piperacillin/ Tazobactum and Meropenem. Staphylococcus aureus and Enterococcus spp showed good response towards Linezolid, Netilmicin, Vancomycin and Teicoplanin. There is also increase in incidence of Extended Spectrum Beta Lactamase (ESBL) production among Escherichia coli (65%) and Klebsiella spp (55%). Study also showed that females (67.17%) are more vulnerable to urinary tract infections than males (32.83%). **CONCLUSION:** Due to excessive use of antimicrobials for all sorts of infections, uropathogens are increasingly showing resistance to antibiotics. Knowledge of uropathogens and their antimicrobial susceptibility pattern in a geographical region will help in appropriate and judicious antibiotic usage in a health care setup.
KEYWORDS: Urinary Tract Infection, Antibiotic Susceptibility Pattern, ESBL

INTRODUCTION: Urinary tract infections (UTI) are one of the most common infectious diseases in humans. Antimicrobial drug resistance is one of the major global threats.[1]Antibiotics are usually given empirically before the laboratory reports of urine cultures are available.

Inappropriate and empirical usage of wide spectrum of antibiotics, insufficient hygiene, immunosuppression and prolonged hospitalisation are some of the major etiological factors that increase the chances of UTIs.[2]Wide spectrum of organisms are implicated in its aetiology, the most common being Escherichia coli andKlebsiella spp followed by Gram positive organisms.[3]

Gram negative bacilli shows high incidence of resistance against Cotrimoxazole, Norfloxacin, Piperacillin, Fluoroquinolones and third generation cephalosporins.[4,5]Gram positive cocci shows high incidence of resistance against Ampicillin, Amoxycillin, penicillin, Amoxycillin/ Clavulanic acid and erythromycin. Antibiotic susceptibility pattern of uropathogens vary according to the geographical and regional locations. The knowledge about uropathogens and their resistance patterns is important for appropriate therapy and also for prevention of resistance amongst the microbes. In this context present study was carried out to know the common uropathogens and their susceptibility pattern in a tertiary care hospital.

MATERIALS AND METHODS: A total of 1266 clean catch midstream urine samples were collected in a sterile container from both outpatients and inpatients in our hospital during study period (January 2010 – December 2011). Urine samples were transported immediately to microbiology laboratory and processed. Urine samples were processed by calibrated loop technique delivering 0.001 ml of urine and plated on to MacConkey's and Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^0C overnight. For Gram negative bacilli more than 10^5 colonies per ml and for Gram positive cocci 10^3-10^5 colonies per ml of single organism were considered significant. Uropathogens were further identified by the morphological and biochemical characteristics.[6]

Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute(CLSI) guidelines.[7]Antibiotics for Gram negative bacilli included Amikacin(AK-30µg), Ceftazidime (Ca- 30 µg), Cefotaxime (Ce-30µg), Ceftriaxone (Ci-30 µg), Cefepime (Cpm- 30µg), Ciprofloxacin (Cf- 5µg), Cotrimoxazole (Co-25µg), Gentamycin (G -10µg), Levofloxacin(Le-5µg), Piperacillin(Pc-100µg), Piperacillin- Tazobactum(Pt- 100/10µg), Norfloxacin (Nx -10µg), Nitrofurantoin (Nf - 300µg), Ofloxacin (Of - 5µg), Meropenem (Mr-10µg), Aztreonam (Ao-30µg).Escherichia coli and Klebsiella isolates were tested for Extended Spectrum Beta Lactamase (ESBL) production by double disk diffusion method with ceftazidime and Ceftazidime Clavulanic acid combination as recommended by CLSI guidelines.[7, 8]Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 strains were used as controls.

Gram positive cocci were tested for Ampicillin (A-10µg), Amoxycillin (Am -30 µg), Cephoxitin (Cn-30µg), Clindamycin (Cd-2µg), Erythromycin (E-15µg), Linezolid (Lz -30µg), Netilmicin (Nt -30µg), Penicillin (P-10 units), Teicoplanin (Te-30µg), Vancomycin (Va-30 µg) (Hi Media, Mumbai). Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 strains were used as controls. The results were recorded and interpreted according to CLSI guidelines.

RESULTS: Out of 1266 urine samples, 332(26.22%) were found to be culture positive isolates. Among 332 culture positive isolates, 223 (67.17%) were obtained from females and 109(32.83%) were obtained from males as shown in Table1.The highest isolation rates was found in 20 – 69 age group as shown in Table 2.

Escherichia coli(54.21%) was the predominant uropathogen, followed by Klebsiella spp(22.89%), Enterococcus spp(6.92%), Staphylococcus aureus (5.21%), Acinetobacterspp (3.31%) and Citrobacter spp (3.01%) as shown in Table 3. The antibiotic resistance among uropathogens to the agents that had been recommended as the first line therapy is on rise. The Gram negative bacilli which were isolated showed high degree resistance pattern against Cotrimoxazole, Norfloxacin, Piperacillin, Ciprofloxacin and third generation cephalosporins. Escherichia coli isolates showed higher sensitivity towards Amikacin (91.18%), Nitrofurantoin (90.52%), Piperacillin- Tazobactum (96.44%) and Meropenem (100%). Klebsiella isolates showed good response towards Amikacin (92.61%), Levofloxacin (90%), Piperacillin-Tazobactum (95%), Meropenem (100%) as shown in Table 4. ESBL production among Escherichia coli isolates were 65% and Klebsiella isolates, 55%. Enterococcus spp showed good response towards Linezolid (100%), Vancomycin (100%), and Teicoplanin (87.5%). Staphylococcus aureus showed good response towards Linezolid (88.1%), Netilmicin (87.5%), Vancomycin (100%), Teicoplanin (100%), as shown in Table 5.

DISCUSSION: The changing trends in the aetiopathogenesis of urinary tract infections and increasing antimicrobial drug resistance are a matter of concern. Urethral catheterisation and instrumentation related UTI is the most common nosocomial infection.[9] Catheter related UTI increases morbidity, mortality and also cost of treatment for patients in a health care setup.[10] The indiscriminate, inadequate usage of antibiotics has contributed to the emergence of resistance strains.[11]Urine culture sensitivity is routinely done in suspected cases of UTI and empirical therapy should be started immediately and modified if required once the report of urine culture sensitivity is available.[12, 13]

The present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. Escherichia coli was the predominant pathogen followed by Klebsiella spp and Gram positive cocci. This study is in concordance with the studies done by Manjunath et al,[1] and Shigemura et al. [7] Our study shows that females (67.17%) are more vulnerable to UTIs than males (32.83%), which is similar to previous studies done by Manjunath et al,[1]and Dash et al.[14] Females are more prone to UTIs probably due to their short urethra and physiological changes.
Antimicrobial resistance of different uropathogens is one of the barricades that can interfere with an effective treatment. The present study indicates different antimicrobial susceptibility pattern among uropathogens. The broad spectrum activity of Fluoroquinolones and third generation cephalosporin has made them therapeutic options for UTIs. The organisms which belonged to Enterobacteriaceae family, showed high degree of resistance towards Cotrimoxazole, Fluoroquinolones and third generation cephalosporin, but these isolates showed good response against antibiotics like Amikacin, Nitrofurantoin, Piperacillin Tazobactum and Meropenem. This finding is similar to the studies done by Manjunath et al,[1]and Shigemura et al.[7] Our study also shows increase in ESBL producing strains among Escherichia coli (65%) and Klebsiella spp (55%), which is similar to the results of the studies done by Shiju et al,[8]and Shukla et al.[15] ESBL producing organisms pose a major problem in clinical therapeutics, so there is a need for judicious use of antimicrobial agents and their continuous Invitro monitoring should be carried out, so that misuse of extended spectrum cephalosporins can be avoided. Gram positive cocci showed good susceptibility patterns towards Netilmicin, Linezolid, Vancomycin and Teicoplanin.

This study furnishes the much needed information on the common uropathogens and their antibiotic susceptibility pattern in our region. In view of the emerging drug resistance

among uropathogens, therapy should be commenced after the culture and sensitivity has been performed. This would, not only help in the proper use of antibiotics but also prevent further development of bacterial drug resistance in community as well as in hospitals.

Table 1: Sex- wise distribution of UTI

GENDER	Number of Isolates
Male	109(32.83%)
Female	223(67.17%)

Table 2: Age-wise distributions of uropathogens

Age group	Male	Female	Total
0-9	3	18	21
10-19	10	35	45
20-29	15	58	73
30-39	16	36	52
40-49	23	23	46
50-59	13	23	36
60-69	23	20	43
70-79	3	8	11
80-89	3	2	5

Table 3: Clinical strains isolated from urine samples.

Uropathogens	No. of isolates	Percentage
Escherichia coli	180	54.21%
Klebsiella spp	76	22.89%
Acinetobacter spp	11	3.31%
Citrobacter spp	10	3.01%
Pseudomonas spp	8	2.40%
Provedencia spp	4	1.20%
Proteus mirabilis	2	0.60%
Enterobacter spp	1	0.30%
` Enterococcus spp	23	6.92%
Staphylococcus aureus	17	5.12%

Table 4: Antibiotic Susceptibility pattern(% sensitivity): Gram negative bacilli.

Uropathogen	Ak	Ao	Ca	Ce	Ci	Cpm	Cf	Co	G	Le	Mr	Nx	Nf	Of	Pc	Pt
E.coli	91.18	ND	36.99	33.38	32.34	35.88	31.20	28.31	58.67	60.94	100	23.77	90.52	53.47	23.74	96.44
Klebsiella Spp	92.61	ND	46.89	39.64	41.3	49.51	53.75	49.61	68.35	90	100	50.69	70	43.18	29.11	95
Acinetobacter spp	83.33	ND	71.42	66.66	66.66	ND	66.66	50	70	100	81.81	75	ND	50	50	81.81
Citrobacter Spp	79.16	ND	65	45	66.66	40	79.16	55	73.33	75	100	60	70.8	70	40	87.5
Proteus Spp	100	ND	50	50	50	50	50	50	ND	100	100	50	100	50	ND	100
Pseudomonas Spp	50	37.5	50	50	37.5	ND	37.5	37.5	ND	50	75	50	ND	37.5	62.5	87.5
Providencia Spp	100	ND	50	50	50	ND	50	25	ND	75	100	50	75	50	50	100
Enterobacter spp	100	ND	-	-	-	ND	-	-	ND	100	100	-	100	-	-	100

Table5: Antibiotic Susceptibility pattern(% sensitivity): Gram positive cocci

	A	Am	Ac	Cd	Cz	Cn	E	G	Lz	Nt	Nx	Nf	P	Va	Te
S.aureus	25	29.16	50	70.1	53.5	53.57	52.94	63.85	88.1	87.5	ND	ND	11.8	100	100
Enterococcus Spp.	14.28	20	36	ND	ND	ND	36	46.66	100	ND	40	60	20	100	87.5

*ND-NOT DONE.

REFERENCES:

1. Manjunath GN, Prakash R, Vamseedhar A, Kiran S. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and

community patients with urinary tract infections in Tumkur and Bangalore. Int J Biol MedRes 2011; 2(2):504-7.

2. Sweih NA, Jamaal W,Rotimi VO. Spectrum and antibiotic resistance of uropathogensisolated from hospital and community patients with urinary tract infections in two largehospitals in Kuwait. MedPrincPract2005;14:401-7.
3. Azad UK, Mohd SZ. Multidrug resistance pattern inurinary tract infection patients in Aligarh. Bio Med Res 2006;17(3):179 -81.
4. De Francesco MA, Ravizzola G, Peroni L,Negrini R, Manca N. Urinary tract infections inBrescia, Italy.Etiology of uropathogens and antimicrobial resistance of common uropathogen. Med SciMonit 2007;13(6):136-44.
5. Forbes BA,Sahm DF, Weissfeld AS. Overview of bacterial identification methods and strategies. Baliley and Scott's Diagnostic Microbiology,12th ed., chapter 13. St.Louis: Mosby; 2007.-.216-47.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing.21stInformation Supplement M100-S21.Wayne,PA, USA: Clinical and Laboratory Standards Institute,2011.
7. Shigemura K, Arakawa S, Tanaka K, Fujisawa M. Clinical investigation of isolated bacteria from urinary tracts of hospitalized patients and their susceptibilities to antibiotics. J Infect Chemother2009;15:18-22.
8. Shiju MP, Yashavanth R, Narendra N. Detection of extended spectrum beta-lactamaseproduction and multidrug resistance in clinical isolates of E.coli and K.pneumoniaein Mangalore. J ClinDiagn Res 2010; 4(3): 2442-45.
9. Stark RP, Maki DG. Bacteriuria in a catheterized patient. N Eng J Med 1984; 311:560-64.
10. Kunin CM, Douthitt S, Dancing J, Anderson J, Moeschberger M.The association betweenthe use of urinary catheters and morbidity and mortality among elderly patients in a nursinghome.Am J Epidemiol 1992;135(3):291-301.
11. Gruneberg GN. Antibiotic sensitivities of urinary pathogens,1971 – 1982.J AntimicrobChemother 1984;14:17–23.
12. Farrell DJ, Morrissey I, De Rubies D, Robbins M, Felmingham D. UK multicentre study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infection. J Infect 2003;46(2):94–100.
13. Gupta V, Yadav A, Joshi RM. Antibiotic resistance pattern in uropathogens. Indian J Med Microbiol 2002;20:96–98.
14. Dash N, Al-Zarouni, Al-Kous, Al-Shehhi F, Al-Najjar J, Senok A et al. Distribution and resistance trends of community associated urinary tract pathogens in Sharjah, UAE.Microbiology Insights 2008;1:41-45.
15. Shukla I, Tiwari R, Agarwal M, Prevalence of extended spectrum beta lactamase producingKlebsiellapneumoniae in a tertiary care hospital. Indian J Med Microbial 2004; 22:87-91.

EVALUATION OF SEROLOGICAL AND MOLECULAR METHODS OVER CONVENTIONAL METHODS IN DIAGNOSIS OF PULMONARY AND EXTRA PULMONARY TUBERCULOSIS

Deepika Verma, M. E. Siddique, Vineeta Mittal, Jagadamba Sharan, Sachin Kishore

1. Assistant Professor, Department of Microbiology, Era's Lucknow Medical College and hospital, Lucknow.
2. Professor, Department of Microbiology, Era's Lucknow Medical College and hospital, Lucknow.
3. Associate Professor, Department of Microbiology, Era's Lucknow Medical College and hospital, Lucknow.
4. Assistant Professor, Department of Microbiology, Era's Lucknow Medical College and hospital, Lucknow.
5. Assistant Professor, Department of Microbiology, Era's Lucknow Medical College and hospital, Lucknow.

CORRESPONDING AUTHOR

Dr. Deepika Verma,
C/O Dr. P. C. Verma,
A 74, Vaibhav, Suncity Vistaar,
Pilibit bye pass, Bareilly,
E-mail: dr_dipikaa05@rediffmail.com,
Ph: 0091 9305943057.

BACKGROUND: Despite advances in field of microbiology, diagnosis of Tuberculosis remains a challenge. Diagnosis of Extrapulmonary tuberculosis is more problematic due to low bacillary load in the specimen and difficulty in obtaining the specimen from the site of lesion in many cases. PCR and its modifications are a boon in diagnosis of tuberculosis in such cases. But serological methods like ELISA are still the first choice of small laboratories in India. **AIM**: Comparative evaluation of serological and molecular methods over conventional methods in diagnosis of extrapulmonary and pulmonary tuberculosis. **METHODS**: 170 different clinical specimens suspected of tuberculosis, (100 pulmonary and 70 Extrapulmonary) were examined by PCR using MPB 64 primer, culture and microscopy. All specimens were processed using USP methodology for inhibitors free PCR. TB Ig G, Ig M and Ig A was determined using PATHOZYME MYCO Kit. Response to ATT on clinical follow up was considered as gold standard. **RESULTS:** Total pulmonary specimens found positive by any of the four tests was 87 (out of 100) while that for extrapulmonary samples was 63(out of 70) . For **Pulmonary Specimens** the diagnostic accuracy of microscopy was 88.3%,for culture 88.3%,for ELISA 67.4% and for PCR 94.1%.For **extrapulmonary samples** the diagnostic accuracy of microscopy was 30.1%,for culture 49.2%,for ELISA 44.4% and for PCR 87.3%. **CONCLUSION:** As diagnosis by microscopy and culture are sensitive and specific, for pulmonary specimens, PCR should be kept reserved for clinically probable cases found negative by conventional tests. For Extrapulmonary specimens PCR can be used as an effective screening tool as conventional methods are mostly negative. ELISA was found to have no role in diagnosis of pulmonary TB. For Extrapulmonary TB ELISA can be used as an adjunct tool but results should be interpreted with utmost caution after full evaluation of the patient both clinically and radiologically.

KEY WORDS – ELISA, MPB 64 Primer, PCR, Tuberculosis

INTRODUCTION: The rapid and early diagnosis of tuberculosis is crucial first step for tuberculosis control program world wide especially in the wake of emergence of drug resistant TB and its related implications for HIV infected patients. Although the culture of *Mycobacterium tuberculosis* from the infected specimen remains the gold standard for diagnosing TB, the direct

microscopy for the detection of AFB in sputum continues to be most popular among all the methods currently employed. While the culture of mycobacterium takes 6-8 weeks to become positive [1,] the direct smear microscopy lacks sensitivity in extra pulmonary specimen, due to small number of organism[2] .Therefore there is need for more sensitive and specific tests for the rapid detection of tuberculosis. Many serological methods detecting Antibodies to various Antigens like 38 kDa (PhoS), 30/31 kDa (antigen 85, 19 kDa lipoprotein, 14 kDa, 16 kDa (ACR) and lipoarabinomanan (LAM) have been tested and among these 38 kDa antigen has shown the highest sensitivity and specificity.[3,4] Studies using this purified Ag in an EIA system found a high degree of specificity (91%) and sensitivity (72%) and concluded it to be a useful antigen for the serodiagnosis of tuberculosis.[5]

Recently molecular methods have revolutionized the diagnosis of tuberculosis. PCR is more sensitive as compared to conventional methods especially when the bacterial load is less in the sample and results can be obtained in one to two days. It also has its own drawbacks like false negative results due to PCR inhibitors in samples or absence of the target sequence and false positive results due to cross contamination[6].

The purpose of this study was to evaluate the utility of PCR and serological methods like ELISA over conventional methods like microscopy and culture separately in pulmonary and extrapulmonary samples from a pool of highly probable tuberculosis suspects referred to a tertiary care hospital.

MATERIAL AND METHOD: Research design

The study was conducted in Era's Lucknow Medical college and hospital, Lucknow. Clinical samples were taken from patients attending OPD's and admitted in wards of different departments of Era's Lucknow Medical college. A total of 170 patients suspected of tuberculosis were enrolled in the study, out of which 100 patients were pulmonary tuberculosis suspects while 70 patients were extra pulmonary tuberculosis suspects with no signs of pulmonary tuberculosis clinically. Out of 100 samples of pulmonary origin, only 86 specimens were found to be positive by one of the above tests and out of 70 specimens of extra pulmonary origin, 63 were found positive by one of above tests. Other 21 specimens which were negative by AFB staining, TB culture, ELISA and PCR were excluded from the study.

Two early morning sputum samples or gastric aspirates were collected from patients suspected of pulmonary TB. Extrapulmonary specimens like pleural fluid, ascitic fluid, cerebrospinal fluid, endometrial fluid, lymph node aspirate were collected from patients suspected of extra pulmonary tuberculosis.

SAMPLE PROCESSING, CULTURE AND PCR:

A) Pulmonary samples (sputum ,gastric aspirate)and pus

After the preparation of smear two samples of sputum or gastric aspirate were pooled and divided into 2 parts. One part was decontaminated by Modified Petroff's method for culture on L J media [7.]The other part was processed as per the protocol described using universal sample processing (USP) methodology for inhibitor free PCR[8.]

EXTRAPULMONARY SAMPLES: (Pleural fluid, Ascitic fluid, Cerebrospinal fluid, Endometrial fluid) After the preparation of smears the sample was divided into two parts. One part was centrifuged at 3000 g for 15 minutes and pellet was inoculated onto two slants of L.J. media and observed upto 12 weeks. No decontamination step was carried out prior to culture as these

samples are sterile. The other part was pelleted at a medium speed (5000 - 6000g) for 15 minutes and the pellets were processed in the same manner as that for sputum samples .Cerebrospinal fluid specimens were pelleted at 25000×g and given a USP wash followed by a water wash.

C) BLOOD: Antibodies to mycobacterium in human serum were detected by ELISA using Pathozyme- Myco IgM, IgG, IgA (Omega Diagnostic) and qualitative results were calculated according to manufacturer's protocol.

D) NIACIN TEST: Positive cultures on LJ slants were confirmed by microscopy for AFB and Niacin accumulation test using BBL Taxo Niacin test strips (Becton Dickinson, USA).

E) DNA EXTRACTION AND PCR DNA was extracted, by incubating the pellet in extraction buffer (1mg/ml proteinase K in 10 mM Tris- HCl pH8.0, 1Mm EDTA, 10% SDS). Proteinase K was inactivated by heating at 100^0C for ten minutes. DNA purification was done by addition of equal volume of Phenol: Chloroform (24:1) to the extracted DNA.[9] PCR was done using MPB64 primers (Hysel India (p) ltd) which are specific for Mycobacteria of the tuberculosis complex. Amplification reaction was typically performed in a 50µl reaction mix containing 1µl of forward and reverse primers, 1µl final concentration of dNTP, 0.4µl of Taq polymerase in 10X buffer and 2µl of extracted DNA.

For extrapulmonary samples amplification reaction was performed in a 25 µl reaction mix containing 0.5µl of forward and reverse primers, 0.5µl final concentration of dNTP, 0.4µl of Taq polymerase in 10X buffer and 5µl of extracted DNA .The sequences of the forward and reverse primers used were 5'-TCCGCTGCCAGTCGTCTTCC-3' and 5'-GTCCTCGCGAGTCTAGGCCA-3'. Forty cycles of amplification were performed using an initial denaturation step of 95^0C for five minutes, followed by denaturation at 95^0C for one minute, annealing at 55^0C for one minute and extension of 72^0C for one minute. A final extension was carried out at 72^0C for seven minutes[10].The 240 bp final segment was detected on a 2% agarose gel through ethidium bromide staining. DNA from *M. tuberculosis* strain H37Rv was used as a positive control. Appropriate negative controls were set up for each sample.

STATISTICAL ANALYSIS: Statistical analysis using Mc Nemar's Chi square test was performed for the calculation of sensitivity, specificity, Positive predictive value, negative predictive value and diagnostic accuracy. Response to Anti Tubercular Therapy on clinical follow up was considered as GOLD STANDARD.

RESULTS: A total of 170 samples were examined by microscopy, culture on LJ slants, and PCR using MPB 64 primer. Serum sample from each patient was subjected to serological testing by ELISA.

Response to ATT was found positive in 76 patients of pulmonary TB and 46 patients of extra pulmonary TB on follow up

PULMONARY TUBERCULOSIS: Out of 86, 56 (64.3%) were found to be positive by microscopy, 67(77%) by culture, 54(62%) by ELISA and 77(88.5%) by PCR. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of tests under consideration are shown in table 1. PCR was found to be most sensitive test with highest

diagnostic accuracy while ELISA was found to be least sensitive test with least diagnostic accuracy.

EXTRAPULMONARY TUBERCULOSIS: Out of total of 63 positives in clinically suspected patients of extra pulmonary tuberculosis 2(3.2%) were found to be positive by microscopy, 14 (22.2%) by culture, 34 (53.9%) by ELISA and 48(76.1%) by PCR.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of tests under consideration are shown in table 2. Out of all the four microbiological tests performed PCR was found to be most sensitive and its diagnostic accuracy was highest while microscopy was least sensitive and diagnostically least accurate.

DISCUSSION: In search of a need of a simple, sensitive and specific test for tuberculosis which would improve or replace the sputum smear microscopy, demonstration of specific Antibodies has repeatedly been focused. ELISA has been used as a rapid diagnostic Test for TB in low-income countries, which has over 90% of the global burden of TB cases.

Several molecular techniques have emerged during the last decade for the detection of specific DNA of M. tuberculosis in clinical specimens. Not only the molecular methods decrease the turn around time, but also are more patient friendly as result can be obtained by a single specimen. Also probability of confirming diagnosis of TB in paucibacillary cases is higher with PCR than with the conventional techniques. Besides it also helps the clinicians to initiate ATT (Anti Tubercular Treatment) in the initial stages of TB especially in paucibacillary infection. The present study demonstrates the utility of PCR and ELISA in pulmonary and extra pulmonary specimens separately.

Out of 86 samples of pulmonary origin, PCR detected 77(89.5%) , while conventional tests could detect 56(72.7%) by microscopy and 67(87%)by culture. This difference was not found to be statistically significant (P value 0.321) revealing the fact that microscopy and culture still remain the basic tools for diagnosis of pulmonary tuberculosis in a resource constrained country like India and PCR should be kept reserved for probable cases negative by conventional tests. PCR alone was positive in 10 (61.1%) among 18 samples found negative by conventional bacteriological techniques and were declared not to have tuberculosis using the diagnostic paradigm of the RNTCP. This aspect has great potential in the laboratory diagnosis of tuberculosis particularly in paucibacillary cases.

Nine such individuals were traced and clinical information gathered within a year of first presenting the clinic. Eight individuals took ATT and subsequently a clinical improvement was found in them, while one individual who refused to take ATT was found to be immediate family contact of a tuberculosis case. One patient was lost during follow up. (These two cases were not included in evaluation of sensitivity and specificity in our study.)

In our study in patients of pulmonary TB, PCR was found to be more sensitive than culture and microscopy. The sensitivity of PCR was found to be 92.2% and specificity 70% respectively, as compared to that of microscopy whose sensitivity was 73.6% and specificity 100% and culture whose sensitivity was 86.8% and specificity was 100%.The decreased specificity of PCR in our study could be due to the fact that in our study 3 samples which were found positive by PCR were not considered true positive in the criteria of gold standard as they did not take ATT subsequent to our report. However on clinical follow up these patients were found to be highly probable on history due to close family contact. If these 3 PCR positive cases are also considered to be cases of tuberculosis, specificity of PCR would become 100%. The

higher sensitivity and specificity of PCR have also been reported by other workers. While Kavita Modi Parekh et al [10] have found 91.5% sensitivity and 86% specificity over culture and microscopy, Jose Manuel Querol et al [11] have reported 100% sensitivity and 97% specificity.

EXTRAPULMONARY TB: In case of extra pulmonary TB, the sensitivity and specificity of PCR was more (93.4% and 70.5%) than that of microscopy (4.3% and of 100%) and culture (30.4% and 100%). Again, It is worthwhile to mention here that that low specificity in our result as compared to microscopy and culture was due to the fact that 2 samples which were found positive by PCR in our study were not considered true positive in the criteria of gold standard as all these patients did not take ATT (I died and 1 lost during follow up). However examination had shown evident cervical lymphadenitis not responding to usual antibiotics in both patients.

Out of total 63 samples of extra pulmonary origin, in our study, PCR detected 100% of specimens which were positive by conventional methods and 33(68.7%) of the 48 samples negative by conventional methods and were declared not to have tuberculosis using the diagnostic paradigm of the RNTCP. Thus PCR could detect 68.7% more cases as compared to conventional methods. This difference was found to be statistically significant (p value <0.001) indicating the great utility of PCR in extra pulmonary samples. Out of these, 31 individuals took ATT and improved during treatment except one patient with extensive pleural effusion who died during the course of treatment and one patient was lost during follow up. Only 31 cases, who took ATT of the 33 cases found positive by PCR alone were considered as true cases of TB.

Our results were in accordance with the results of several workers B Sekar et al [12], Mandir verma et al [13], Parmeet Kumar et al [14] who have reported similar sensitivity and specificity of PCR in detecting extra pulmonary tuberculosis.

Our in–house PCR assay was based on amplification of a gene MPB 64 which is specific for Mycobacterium tuberculosis complex. MPB 64 gene encodes for the immunogenic protein found only in culture filtrates of *M. tuberculosis* and occasional isolates of M. bovis BCG. Although IS 6110 is the most widely used primers but studies from Tuberculosis research centre, Chennai in south India found 42.7 % isolates either lack or have a few copies of IS 6110. [15,16] . Other workers [17,18] have reported similar results. The PCR tests targeting this sequence will not be applicable to such cases in India and this indicates the need to have alternate gene targets for diagnosis in these situations. Considering the above points, primers targeting the MPB 64 gene were used in our study.

The USP (Universal Sample Processing) Methodology described by Chakravaroty et al [8] was used in the present study. This method is simple and enabled the isolation of inhibitor – free, high quality mycobacterial DNA from a variety of clinical material in an environmentally friendly and inexpensive manner. This solution contains a chaotropic agent GuHCl (Guanidinium hydrochloride). The unique properties of the mycobacterial cell wall renders these organisms selectively resistant to the action of GuHCl while cells of other bacteria and eukaryotic cells are disrupted upon exposure to it.

Several Enzyme-Linked Immunosorbent Assays (ELISAs) have been tried to achieve the rapid and easy diagnosis of pulmonary tuberculosis. The kits used in our study were PATHOZYME-MYCO IgM, PATHOZYME-MYCO IgG and PATHOZYME-MYCO IgA containing recombinant forms of two antigens from the Mycobacterium tuberculosis complex: r38 kDa, expressed in and purified from Escherichia coli and the lipoarabinomanan (LAM; a common lipoglycan component of the mycobacterial cell wall purified from M. tuberculosis.

Out of 86 patients of pulmonary TB, ELISA was found to be positive in 51(59.3%) cases out of 68 (79%) patients which were positive by microscopy and culture. In addition ELISA was positive in 5 more patients found negative by conventional tests out of which only one responded to ATT. The other 4 patients were false positives later identified as respiratory diseases other than tuberculosis. Similarly in case of extra pulmonary tuberculosis, out of 15 samples positive by conventional tests, ELISA was positive in 11 samples. In addition ELISA was positive in 23 more patients out of 48 found negative by conventional test. However response to ATT was seen only in 11 patients out of these 23. The other 11 patients were false positives later identified with diseases other than tuberculosis. This may be due to high prevalence of TB in our country where large number of population is exposed to mycobacterial antigen (subclinical specific infection or infection with environmental mycobacteria) which may mount a significant and sustained antibody response. This sort of results has also been reported from other workers from different parts of our country [19,20] .In such a Scenario there can be no utility of the serodiagnostic tests in the diagnosis of pulmonary tuberculosis. In our study also no statistical significance was seen between ELISA and conventional methods (p value 0.321) in diagnosis of pulmonary TB.

However in diagnosis of extra pulmonary tuberculosis, a statistically significant difference (p value < 0.001) was seen between ELISA and conventional methods .But these tests should be interpreted with utmost caution and an extensive evaluation of antibody tests should be made taking into account all the aspects of the disease considering the significant and sustained antibody response caused by sub-clinical specific infection or infection with environmental mycobacteria as mentioned above.

CONCLUSION: As positivity of PCR in comparison to conventional methods was statistically insignificant in our study, we found that the utility of PCR was more for early diagnosis in patients of pulmonary tuberculosis and should be kept reserved for clinically probable cases found to be negative by microscopy. Moreover results of PCR should be interpreted in conjunction with the clinical and radiological data owing to its low specificity. In suspected patients of extra pulmonary tuberculosis PCR can be used as a screening tool as the diagnostic accuracy of PCR was much more than that of conventional methods which are mostly negative.

Determination of TB IgM, IgG and IgA antibody has no role in diagnosis of pulmonary tuberculosis. In diagnosis of extra pulmonary tuberculosis ELISA can be used as an adjunct tool but positive results should be interpreted with utmost caution after full evaluation of the patient both clinically and radiologically.

Activities leading to discovery of new antigen with immunodiagnostic potential need to be intensified and the techniques for antigen detection will continue to have edge over antibody detection methods due to obvious reasons.

Also, more than one PCR system targeting more than one gene should be used in diagnosis of TB by PCR to achieve 100% sensitivity and specificity.

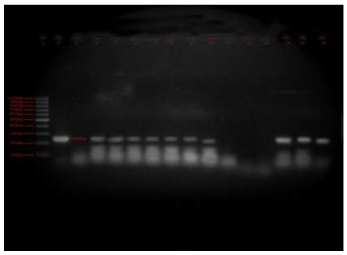


Table 1: Analysis of Different Tests on Samples of Pulmonary Tuberculosis Showing Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy

DIAGNOSTIC TEST	SENSITIVITY% C I (%)	SPECIFICITY% C I (%)	POSITIVE PREDICTIVE VALUE % C I (%)	NEGATIVE PREDICTIVE VALUE % C I (%)	DIAGNOSTIC ACCURACY %
Microscopy	73.6 (65.2-83)	100 (65.5-100)	100 (92.1-100)	33.3 (17.9-52.8)	88.3
Culture	86.8 (79.4-94)	100 (65.5-100)	100 (92.1-100)	50 (27.8-72.1)	88.3
ELISA	68.4 (61.1-81.9)	60 (8-64.6)	92.8 (77.8-88.8)	20 (12.5-35.8)	67.4
PCR	92.2 (83.2-96.7)	70.0 (35.3-91.9)	96.2 (87.8-98.9)	77.7 (26.1-79.5)	94.1

TABLE 2
Analysis of Different Tests on Samples of Extra Pulmonary Tuberculosis Showing Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy

DIAGNOSTIC TEST	SENSITIVITY % C I (%)	SPECIFICITY % C I (%)	POSITIVE PREDICTIVE VALUE % C I (%)	NEGATIVE PREDICTIVE VALUE % C I (%)	DIAGNOSTIC ACCURACY %
Microscopy	4.3 (7-15.1)	100 (73.2-100)	100 (73.2-100)	27.8 (13.5-35.8)	30.1
Culture	30.4 (17.0-43.4)	100 (73.2-100)	100 (73.2-100)	34.6 (17-43.4)	49.2
ELISA	47.8 (36.5-65.3)	35.2 (3.7-35.8)	66.6 (48.5-79.8)	20.0 (2-22.3)	44.4
PCR	93.4 (87.7-99.8)	70.5 (48.8-94.2)	89.5 (82.7-98.4)	80 (59.7-99.5)	87.3

ACKNOWLEDGEMENT: Authors thank Dr. P.C .verma, principal scientist, Divison of biological products, Indian veterinary Research institute, Izatnagar, Bareilly, for providing technical assistance in performing PCR by USP methodology and for providing H37Rv control strain of Mycobacterium tuberculosis.

REFERENCES:

1. Forbes B A, Sahm D F, Weissfeld A. Bailey and Scott's Textbook of diagnostic microbiology. 12th ed. St Louis: MOSBY ELSEVIER; 2007.
2. Kulkarni SP, Jaleel MA, Kadival GV. Evaluation of an in-house-developed PCR for the diagnosis of tuberculous meningitis in Indian children. J Med Microbiol 2005 Apr; 54(Pt 4):369-73.
3. Wilkinson RJ, Haslov K, Rappuoli R, et al. Evaluation of the recombinant 38-kilodalton antigen of Mycobacterium tuberculosis as a potential immunodiagnostic reagent. J Clin Microbiol 1997; 35: 553-7.
4. Espitia C, Cervera I, Gonzalez R, Mancilla R. A 38-kD Mycobacterium tuberculosis antigen associated with infection. Its isolation and serologic evaluation. Clin Exp Immunol 1989; 77: 373-7.
5. Palomino JC, Leao SC, Ritacco V. Tuberculosis 2007 - from basic science to patient care. 1st ed. Brazil: BourcillierKamps.com; 2007.
6. Roth A, Schaberg T, Mauch H. Molecular diagnosis of tuberculosis: current clinical validity and future perspectives. Eur Respir J 1997 Aug;10(8):1877-91.
7. C.N. Paramasivan, Pratibha Narang , G. Dakshayani , V. Chandrasekaran and P. Venkataraman. Evaluation of Bacteriological Diagnosis of smear positive Pulmonary Tuberculosis under Programme conditions in three Districts in the context of DOTS Implementation in India. Indian J Tuberc 2006; 53 :196-200.
8. Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. J Clin Microbiol 2005 Sep; 43(9):4357-62.
9. Jain Amita, Tiwari Vandana, R.S Guleria, R.K. Verma. Qualitative Evaluation of Mycobacterial DNA Extraction Protocols for Polymerase Chain Reaction. Molecular Biology Today (2002) 3: 43 – 50.
10. Kavita Modi Parekh, Vikas Inamdar, Anagha Jog,Anita Kar. A comparative study of the diagnosis of Pulmonary Tuberculosis using conventional tools and PCR. Indian J Tuberc 2006; 53: 69-76.
11. Jose Manuel Querol ,Maria Ampara Farga, Damiana Granda ,Concepcion Gimeno and Juan Garcia-de Lomas .CHEST 1995 ;107;1631-1635.
12. B sekar, L sevaraj, A Alexis et al .The utility of IS6110 sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of Extrapulmonary tuberculosis. Indian journal of medical microbiology,(2008) 26(4):352-5.
13. Mandira Varma-Basil, Rakesh Pathak et al. Direct Early identification of Mycobacterium tuberculosis by PCR – Restriction Fragment length Polymorphism Analysis from clinical samples. Jpn. J.Infect.Dis.63, 55-57,2010.
14. Parameet Kumar , Manas K. sen , Devendra S.Chauhan ,Vishwa M. Katoch ,Sarman Singh ,Hanumanthappa K. Prasad et al. Assessment of the N-PCR Assay in Diagnosis of Pleural Fluid and sputum collected in Tandem. PLos ONE 5(4):e10220.doi:10.1371/journal.pone.0010220.

15. Das S, Paramasivan CN, Lewis DB,Prabhakar R,Naraynan PR .IS6110 restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, south India. Tuberc lung disease 1995;76:550-4
16. SujathaN,VijayalakshmiP,FathimaR,ChitraS,GomathyD,KumaraswamiV,et al.Comparative evaluation of PCR using IS 6110 and a new target in the detection of tuberculosis lymphadenitis. Curr Sci 2000; 78 : 1367-70.
17. Shankar P ,Manjunath N,Lakshmi R,Aditi B,Seth P,Shriniwas.Identification of Mycobacterium tuberculosis by Polymerase chain reaction. Lancet 1990; 335:423.
18. Kent L ,Mc Hugh TD , Billington O, Dale JW, Gillespie SH.Demonstration of homology between IS 6110 of Mycobacterium tuberculosis and DNA'S of other Mycobacterium sp. J Clin Microbiology 1995;33 : 2290-3.
19. Sujatha Chandrasekaran, M.M. Chauhan & N. Parimala .Serodiagnosis of Pulmonary Tuberculosis and Evaluation of Two ELISA Kits. Ind J.Tub,1996, 43, 159.
20. Renuka Raju ,Sujai Suneetha, Karuna Sagili et al. Indian Journal of Clinical Biochemistry,2005,20 (1) 123 -128.

ANATOMICAL VARIATIONS OF NODAL ARTERIES IN HUMAN HEARTS.

Anjali S Sabnis, Nazmeen N Silotry

1. Associate Professor, Department of Anatomy, K. J. Somaiya Medical College, Sion, Mumbai,
2. Associate Professor, Department of Anatomy, MGM Medical College,Kamothe,Navi Mumbai.

CORRESPONDING AUTHOR

Dr. Anjali S. Sabnis,
D/3, Madhuban Society,
Atur Park, Chembur, Mumbai-71
E-mail: dranjus2003@yahoo.com,
Ph: 0091 9820493036.

ABSTRACT: BACKGROUND: Initiation of each heart beat is at sino-atrial (S.A) node and then it is transmitted to atrio-ventricular (A.V) node. The nodal arteries usually arise from Right Coronary Artery (RCA). The present study would be of use to cardiologists and interventional radiologist during invasive & non-invasive procedures. **AIMS:** The aim of the study is to investigate the anatomical variance in nodal arteries in human hearts. **MATERIAL & METHODS:** Study included 110 human hearts of both sexes between the ages of 20 to70 years obtained from Maharashtra were dissected to study coronary arteries. The S.A nodal and A.V nodal arteries were studied in detail in terms of their origin, course, diameter & length. **RESULTS:** In the study we found that S.A.node was supplied by RCA in 99 hearts and from left coronary artery (LCA) in 11 hearts while A.V node was supplied RCA in 77 hearts and by LCA in 32 hearts. In one heart it was supplied by both the coronary arteries. **CONCLUSION:** The data extracted from this study will be helpful for cardiac surgeons during atrial surgical interventions, radiologists and anatomists.

KEY WORDS: nodal arteries, S.A nodal artery, A.V nodal artery.

INTRODUCTION: S.A node is spindle shaped structure present at the junction of superior vena cava and right atrium. It acts as a pacemaker which fires at regular interval. The impulses reach AV node which is beneath the right atrial endocardium anterior to ostium of coronary sinus and directly above the insertion of septal leaflet of tricuspid valve. The main function of AV node is modulation of atrial impulse transmission to the ventricles, there by coordinating atrial and ventricular contractions[1].

SA node and AV node are the important structures in the conductive system of the heart. The arterial supply to S.A node and A.V node is also considered to be very important. The origin of S.A nodal artery is variable, when it comes from RCA it usually comes from anterior stem, When comes from LCA it arises from circumflex branch[2]. During the surgical procedures used in the treatment of arrhythmia injury to nodal arteries remains important possibility. So during the dissection of coronary arteries nodal arteries were studied in detail in terms of origin, course, diameter & length. Such detailed information regarding nodal arteries will be helpful in the application of cardiac surgery.

MATERIALS AND METHODS: 110 human hearts of both sexes between the age of 20 to 70 years were collected from B.J. Medical college and K.J.Somaiya medical college in the period for two years. They were numbered serially & fixed in 10% formalin solution. All hearts were dissected after the removal of epicardium. The right coronary artery, left coronary artery and

their branches to SA and AV node were dissected from origin to termination. The point of origin, course, external diameter & length of nodal arteries was studied. The length of nodal arteries was measured with the help of measuring scale and external diameter of S.A nodal artery at the origin was measured with divider and measuring scale.

RESULTS

Table No 1. Shows origin of S. A & A.V nodal artery

Origin From	S.A nodal artery	A.V nodal artery
Right Coronary Artery	99 hearts ((90%)	77 hearts (70%)
Left Coronary Artery	11 hearts (10%)	32 hearts (29.8%)
Both Coronary arteries	-	01 heart (0.96%)
Total no of hearts	110	110

- Among110, in 99 hearts S.A nodal artery was originated from RCA except in one heart where the artery originated from right marginal artery, rests originated from circumflex branch of LCA.
- In 77 hearts S.A nodal artery was originated from RCA, in 32 hearts it is originated from circumflex branch of LCA & in one heart it was originated from both coronary arteries.
- Table No 2 shows mean diameter and mean length of nodal arteries

S A Nodal artery			**A V Nodal artery**		
Mean Diameter	Mean Length		Mean Diameter	Mean Length	
1.1 mm	From RCA	From LCA	1.2 mm	From RCA	From LCA
	35 mm	70 mm		2 mm	4 mm

DISCUSSION: S.A node was first described by Keith & Flack in 1907[3]. It is a pacemaker and considered very important structure in the heart. The blood supply of this important structure has great value due to its functional importance. It usually comes from first part of right coronary artery, runs back in the sulcus between right auricle and aorta and branched around superior vena caval base. A ramus traverses S.A node and supplies it[4]. Vascular descriptions of nodal arteries have been reported in several published articles in literature and we agree that in majority of hearts nodal arteries are given by right coronary artery.

Table no 3 shows results of various authors[5,6,7,8,9].

	Name of author	**SA nodal from RCA**	**SA nodal from LCA**	**SA nodal from RCA & LCA**	**AV nodal from RCA**	**AV nodal from LCA**	**AV nodal from RCA & LCA**
1	Shilpa Bhimani[5]	66.6 %	25 %	3.3 %	80 %	16 %	Absent in 3.3 %
2	Pejkovic B[6]	63 %	37 %	--	90 %	10 %	--
3	Hutchinson MC[7]	50 %	07 %				
4	Lakshmi Raman[8]	53 %	42 %	4.33 %	72.4 %	27.6 %	--
5	Futamic[9]	73 %	3 %	23 %	80 %	10 %	10 %
6	**Present study**	**90 %**	**10 %**	**--**	**70 %**	**29 %**	**0.9 %**

There is high risk for intra-operative damage to S.A node during the superior trans-septal approach to mitral valve[10]. The point of origin, length, diameter & course of nodal arteries provides important information prior to surgical cardiac procedures. So by keeping this importance in mind, the point of origin of S.A.nodal artery was studied by measuring distance between origin of right coronary artery and origin of S.A nodal artery. In all hearts it is taking origin at mean distance of 35 mm from origin of right coronary artery (photograph no 1) with average length of 35 mm & diameter of 1.1 mm. The mean length of S.A nodal artery is 23 mm[5], 12 mm[6], 40.1 to 134 mm[11] & diameter is 1 mm[5],1.7 mm[6], 1.1 mm[11] within the node. In one of the 40 autopsy cases S.A nodal artery arose from the terminal part of right coronary artery[7].We did not notice any such origin from terminal part of right coronary artery.

In one heart right marginal artery was arising from right coronary artery much proximally. S.A nodal artery took origin from right marginal artery instead of right coronary artery (photograph no 3). It traveled around lateral border of right auricle and encircled the whole auricle and reached the base of superior vena cava ramifying there and supplying S.A node. Such origin from right marginal artery is not yet documented.

In 11 hearts (10%) S.A nodal artery was arising from circumflex artery with mean length of 70 mm & diameter of 1mm (photograph no 2). In all hearts it traveled from posterior aspect of heart and reaches S.A node. None of the heart showed S.A nodal artery coming from trunk of left coronary artery.In12% of cases it is arising from trunk[12] & mean diameter is 2.2 mm[6].

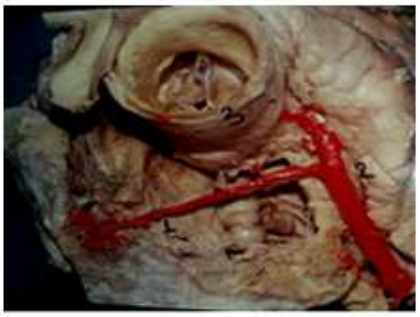
A.V node was first described by Tarawa in 1906[3]. It is a specialized tissue present in the posteroinferior region of the interatrial septum near the opening of coronary sinus. It electrically connects atrial and ventricular chambers[13]. The main function of A.V node is modulation of atrial impulse transmission to the ventricles, there by coordinating atrial and ventricular contractions. It delays the impulse received from S.A node and protect ventricles from atrial arrhythmia. The largest posterior septal artery from right coronary artery which is given at crux enters the interatrial septum and supplies A.V node. This artery is important vessel in the pathogenesis of heart block. Any variation in point of origin, course may provide information prior to catheter based interventional cardiac procedure[14].

With respect to that we have studied point of origin, course, length & diameter of A.V nodal artery. We found that A.V nodal artery is arising from RCA in 70% hearts with mean length of 2 mm, from circumflex artery in 29% hearts with mean length of 4 mm and from both the arteries in 0.9% heart. The mean external diameter is 1.2 mm. The origin of A.V nodal artery from right coronary artery is 90%[5], 80%[8], from left coronary artery is 10% [5,8] & from both arteries is 10% [8] with mean diameter of 2 mm[5].

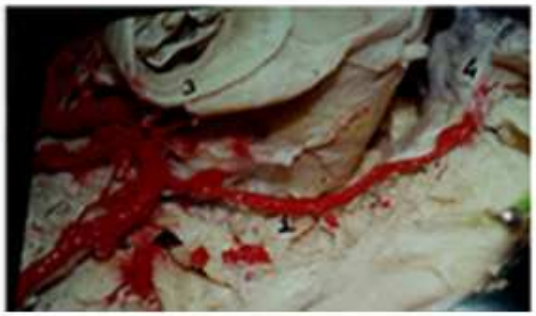
Awareness of anatomical variants of S.A nodal & A.V nodal arteries may guide the cardiac surgeons during cardiac procedures and reduces the risk of damaging them. The damage in the form of cut or occlusion will not give chance for repair & so special care should be taken to spare the artery during surgery.

Conclusion:

Detail knowledge of nodal arteries & awareness of variations is important from surgical point of view.

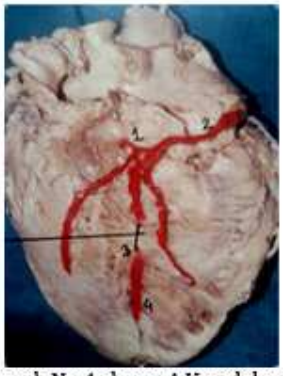


Photograph No 1 shows S.A nodal artery (1) is arising from 1st part of Right coronary artery (2)



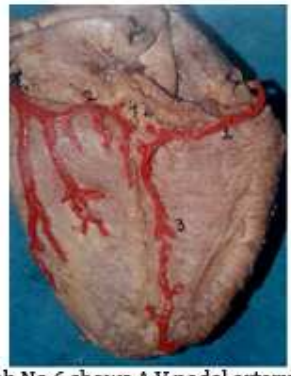
Photograph No 2 shows S.A nodal artery (1) is arising from circumflex artery (2)

Photograph No 3 shows S.A. nodal artery (4) is arising from right marginal artery (3)



Photograph No 4 shows A.V nodal artery (1) is arising from right coronary artery(2)

Photograph No 5 shows A.V nodal artery (1) is arising from circumflex artery (2)



Photograph No 6 shows A.V nodal artery (4) is arising from right coronary artery (1) & left coronary artery (2)

REFERENCES:

1. Lilly LS, Libby P, Bonow R, Mann D, Zips D. Braunwald's Heart Disease, 8th edition, Saunders, Philadelphia, 2008, p-727-762.
2. Susan Standring, Gray's Anatomy, 40thedition, Churchill Livingstone, Elsevier, London, 2008, pp-978-980
3. Hurst, Willis J. The Heart. 7th edition, Vol I, Part I, Mc-Graw Hill, New york, Information sevices company 1990, p-14-35.

4. Lawrence H. Bannister, Gray's Anatomy, 38th edition, Churchill Livingstone, London, 1995, pp-1451-1500,
5. Bhimalli S. A study of variations in coronary arterial system in cadaveric human heart. World J of science and technology, 2011,1(5):30-35
6. Pejkovic B. Anatomical aspects of the arterial blood supply to the sinoatrial and atrioventricular nodes of the human heart. J Int Med Res,2008;36(4):691-8
7. Hutchinson M C, A study of the artrial arteries in man J Anat:1978;125(1);39-54
8. Lakshmi R. Origin of the Sinoatrial and atrioventricular Nodal arteries in South Indians: an Angiographic Study, Arq Brs Cardiol, 2009:92(5)-319.
9. Futami C,The arterial blood supply of the conducting system in normal human hearts,Surg Radiol Anat,2003,25(1):42-9.
10. Berdajs D, The clinical anatomy of the sinus node artery.Ann Thorac Surg, 2003; 76:732-5.
11. Ortale JR. Anatomical Variations in human S.A nodal artery, Clinics 2006; 61(6)551-8.
12. Caetano A G. Critical analysis of the clinical and surgical importance of the variations in the origin of the sinoatrial node artery of the human heart.Rev Assoc Med Bras.1995:41(2):94-102.
13. Gray, Huon H, Keith D, Dawkins L, Simpson A & John M Morgan, Lecture notes on cardiology Boston ; Blackwell Science,2002, P-135,ISBN 978-0-86542-864-5
14. Duzgun Y, Anatomy & variations of arterial supply to sinoatrial node: Imaging with dual source cardiac multidetector CT angiography, Turk Gogus Kalp Damar Cer Derg 2010;18(4)290-292

DYSLIPIDEMIA IN PREECLAMPSIA – RISK FACTOR FOR FUTURE MATERNAL CARDIOVASCULAR DISEASES

Josephine Latha Pushparaj, Ganesan Subramanyam

1. Assistant Professor, Department of Biochemistry, K.A.P.V. Government Medical College, Tiruchirappalli, Tamil Nadu, Dr. M.G.R. Medical University.
2. Assistant Professor, Department of Biochemistry, K.A.P.V. Government Medical College, Tiruchirappalli, Tamil Nadu, Dr, M.G.R. Medical University.

CORRESPONDING AUTHOR
Dr. Josephine Latha Pushparaj
New no: 25 / old no: 6b,
Warners road, Cantonment,
Tiruchirappalli- 620 001, Tamilnadu.
E-mail: lathapasel@gmail.com, josephine.latha@yahoo.co.in ,
Ph: 0091 94863 27979.

ABSTRACT: Preeclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. It is associated with insulin resistance & increase in Free Fatty Acids and plasma Triglycerides well above that seen in normal pregnancy**.** The pattern of dyslipidemia seen in preeclampsia may predispose these women to future cardiovascular diseases. The aim of this study was to compare the alteration in lipid profile and also the calculated risk ratios of women who developed Preeclampsia with normotensive pregnant women. This cross sectional study was carried out in Mahatma Gandhi Memorial Government Hospital, Trichy, Tamilnadu.100 women with preeclampsia and 100 normotensive pregnant women as controls were included in the study after obtaining their informed consent. The diagnosis of Preeclampsia was done as per the norms of American college of Obstetrics and Gynecologists. Fasting blood samples were collected. Serum glucose, cholesterol, triglycerides, and HDL-C were estimated by standard methods using standard kits. LDL-C was calculated by Friedwald's equation. All the results were analyzed statistically using ANOVA technique. There was significant elevation of total cholesterol, triglycerides, LDL, VLDL and risk ratios TC/HDL and TGL/HDL and decrease in HDL in Preeclamptic group compared to normotensives ($p<0.0001$). The dyslipidemic pattern of elevated TGL and reduced HDL which was also reflected in highly elevated TGL/HDL seen in preeclamptic patients is definitely atherogenic and may lead to future maternal cardiovascular disease. Hence simple measurement of lipid profile at 20 weeks of gestation can help in preventing the complications of PIH and future cardio vascular risk of the mother. Further studies in larger population in various ethnic groups are required to predict maternal future cardiovascular risk and its prevention by lifestyle modifications and therapy.
KEY WORDS: Preeclampsia, dyslipidemia, atherogenesis, lipid profile, placental ischemia, cardiovascular disease.

INTRODUCTION: Preeclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. The incidence of preeclampsia ranges from 5-15% [(1)]. In India the incidence of preeclampsia is reported to be 8-10% of the pregnancies [(2)]. The incidence in primigravidae is about 10% and in multigravidae about 5% [(3)]. It causes IUGR leading to low birth weights. Low birth weight child is prone to suffer from diabetes, hypertension, and coronary vascular disorders in their later life[(1)]. Complications of Preeclampsia are HELLP syndrome [(4)], Renal

failure / Impairment, Pulmonary edema, Disseminated intravascular coagulation, Eclampsia and Placental abruption. According to the norms of American college of obstetrics and Gynecologist, the diagnostic criteria for Pregnancy Induced Hypertension (PIH) [5] is 1) Systolic Blood pressure ≥140 mm/Hg 2) Diastolic Blood pressure ≥90 mm/Hg Or 3) increase of ≥30 mm/Hg in Systolic pressure Or 4) increase of ≥15 mm/Hg in Diastolic pressure, in a previously normotensive woman. Preeclampsia usually starts after 20 weeks of pregnancy and is defined as a gestational blood pressure elevation (>140/90 mmHg), combined with proteinuria (>300 mg/24 h, or a positive dipstick 30 mg/dl).

The blood pressure is normally determined by Cardiac output and Peripheral vascular resistance [1]. In normal pregnancy, though cardiac output is increased [7] blood pressure is maintained because of the decrease in peripheral vascular resistance. But in PIH there is increased resistance due to the increased response to vasopressors[6], altered lipid synthesis leading to decrease in the ratio of PGI_2/TXA_2 and antioxidants/lipid peroxides[6] and changes in the local factors like NO, endothelins.

There is an accumulated evidence stating that abnormal placentation is one of the initial events leading to this disease. Impaired interstitial trophoblasts invasion and failure of vascular invasion leading to inadequate perfusion of feto-placental unit along with Impaired decidual remodeling,[7] Impaired function of uterine natural killer cells and maternal endothelial failure to express adhesion molecules and reduced placental L-arginine concentration due to excessive arginase II expression decrease the production of nitric oxide. This promotes abnormal placental perfusion and microvascular oxidative damage. Defective placental implantation causes placental ischemia leading to endothelial dysfunction [7]. This leads to Reduced perfusion of affected organs (Predominantly kidneys, liver & brain) that leads to the Clinical manifestation of Preeclampsia.

Normal pregnancy is hyperlipidemic (i.e.) 3 fold increase in TGL and fatty acids, 50% increase in LDL and HDL. One of the reasons for this increase may be due to the reduction in intestinal motility. Reduction of enterohepatic circulation with increased excretion of cholesterol in the bile also leads to alteration in lipid profile [8]. Normal pregnancy is also a state of hyperestrogenemia. Estrogen results in increase in HDL level and TGL level, decrease in LDL level.

Pre-eclampsia is associated with insulin resistance & increase in FFA (antedating clinical expression of the disorder) and plasma TGL well above that seen in normal pregnancy(Lorenzen et al 1995)[8] .Hypoestogenemia, predominance of smaller and denser serum LDL and significant concentration of soluble vascular cell adhesion molecule-1 are important contributors for endothelial dysfunction in Preeclampsia [6].

In lipid mediated endothelial dysfunction an essential step is oxidation of low-density lipoprotein [9-11]. The atherogenesis itself may be initiated by hypertriglyceridemia. Hypertriglyceridemia leads to elevated level of small dense LDL particles which is atherogenic. Since Triglyceride related vasculopathy may influence a future pregnancy as well as a woman's long term risk of cardiovascular disease, it is worthwhile to explore the metabolism and transport of various sub fractions of lipoproteins in pregnant women from 20 weeks of gestation who have developed increase in blood pressure. Early identification of women at risk for PIH may help in preventing the complications of the disease. This study aims at comparing the alteration in lipid profile and the cardiovascular risk ratios of women who developed Preeclampsia with normotensive pregnant women.

MATERIALS AND METHODS: This cross sectional study was carried out in Mahatma Gandhi Memorial Government Hospital, Trichy, Tamilnadu.100 women with preeclampsia and 100 normotensive pregnant women as controls were included in the study after obtaining their informed consent. The diagnosis of Preeclampsia was done as per the norms of American college of Obstetrics and Gynecologists. All the participants were inquired by a questionnaire containing their personal history, family history of PIH, Twins, Hypertension and Diabetes, Drug history and their symptoms.

The study included individuals of any gravida of age between 20 yrs and 45 yrs, after 20 wks of pregnancy with Blood Pressure ≥140/90 and proteinuria (positive dipstick) for preeclampsia and individuals with Blood Pressure ≤120/80 for control. Subjects with history of Diabetes, Hypertension, Hyperlipidemia before 20 wks and Edema, Proteinuria, oliguria, Hepatic disease and Involvement of other Organs were excluded. Clinical examination of participants were carried out to rule out Diabetes, Hypertension, Hyperlipidemia before 20 wks, edema, proteinuria, oliguria, Hepatic disease, involvement of other Organs..

Fasting blood samples were collected; the serum was separated and analyzed for the following parameters.

The automated analytical system applying routine methods was used for the following measurements: glucose (GOD-POD method), TC (cholesterol esterase method), and HDL-C after precipitation using phosphotungstate method (cholesterol esterase method), and triglyceride (lipase method). LDL-C was calculated by using the Friedwald equation [LDL-C = TC – (HDL-C + triglyceride/5)], where the triglyceride level was less than 400 mg/dL. The risk ratios were calculated from the estimated values. All the results were tabulated. 24 hrs urine collected and protein estimated by analysis using Roche URS-345 dipstick urinalysis stripe to diagnose preeclampsia.

The various results obtained were statistically analyzed using ANOVA technique. Correlations between the variables were estimated by Pearson's correlation coefficients. Significance was assumed if the *P* value was less than 0.05.

RESULTS: The values of the lipid fractions were tabulated as mean ± SD (Table 1). Total cholesterol, triglycerides, LDL-C, VLDL-C were significantly elevated and HDL-C was significantly decreased in Preeclampsia group compared to control ($p < 0.0001$).

Table 1

Test	Preeclampsia	Control	p value
TC	209.54 ± 32.46	175.16 ± 30.16	<0.0001
TGL	337.24 ± 77.29	197.48 ± 37.95	<0.0001
HDL	37.62 ± 7.71	47.50 ± 7.90	<0.0001
LDL	104.47 ± 34.62	88.164 ± 30.22	0.0006465
VLDL	67.45 ± 15.46	39.496 ± 7.60	<0.0001

(TC-Total cholesterol, TGL-Triglycerides, HDL-High density cholesterol, LDL-Low density cholesterol, VLDL-Very Low density cholesterol)

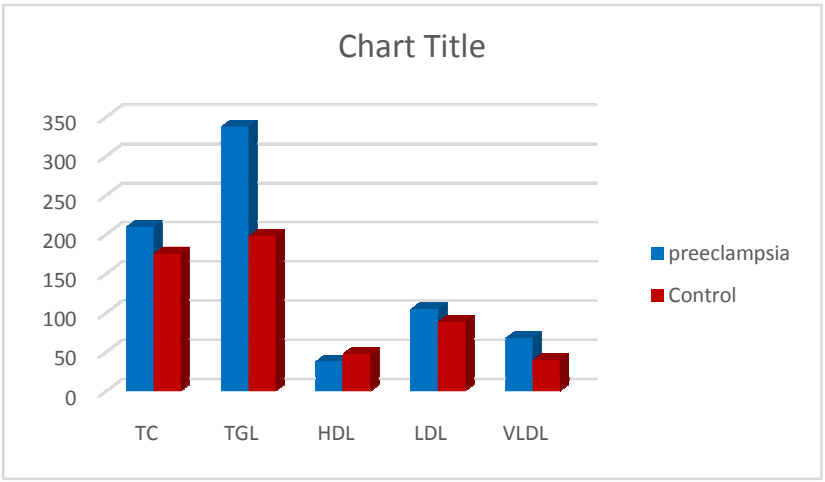
The risk ratios TC/HDL, TGL/HDL, and LDL/HDL were significantly elevated and HDL/VLDL significantly decreased in Preeclampsia group compared to control ($p < 0.0001$) (Table 2). The results of various lipid sub-fractions and the atherosclerotic risk ratios were

compared between the preeclampsia and control groups and shown in FIGURE 1 and FIGURE 2 respectively.

Table 2

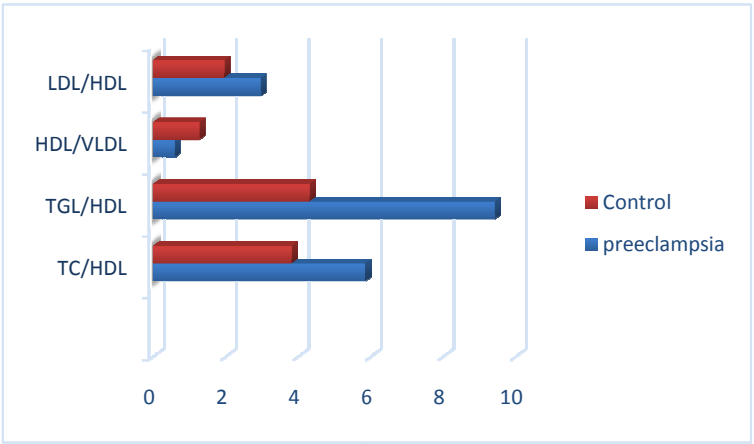
Test	Preeclampsia	Control	p value
TC/HDL	5.843 ± 1.74	3.81 ± 0.99	<0.0001
TGL/HDL	9.41 ± 3.27	4.30 ± 1.21	<0.0001
HDL/VLDL	0.58 ± 0.17	1.26 ± 0.37	<0.0001
LDL/HDL	2.96 ± 1.36	1.95 ± 0.85	<0.0001

Figure-1



Comparison Of Lipid Profile In Preeclampsia With That Of Control

Figure-2



Comparing Calculated Risk Ratios Between Preeclamptic and Control Groups

The risk ratio TGL/HDL had a significant positive correlation with triglycerides (r = 0.762) and a negative correlation with HDL-C (r = -0.674 in patients). The correlation was highly significant (P<0.001). From factor analysis by rotated component matrix, the 3 factors TGL/HDL, TGL or VLDL & TC accounted for 89.644 percent of the variance and hence contribute more to the preeclampsia. The significant elevation of Triglycerides and the ratio TGL/HDL in preeclamptic women compared to the normotensive pregnant women can be seen in the following charts.

Figure-3: Triglyceride values in Preeclampsia and Control Group

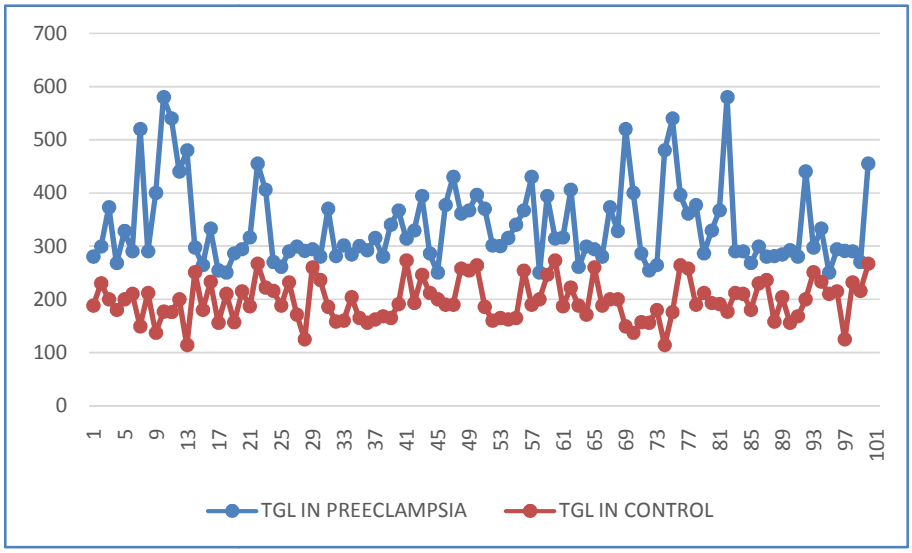
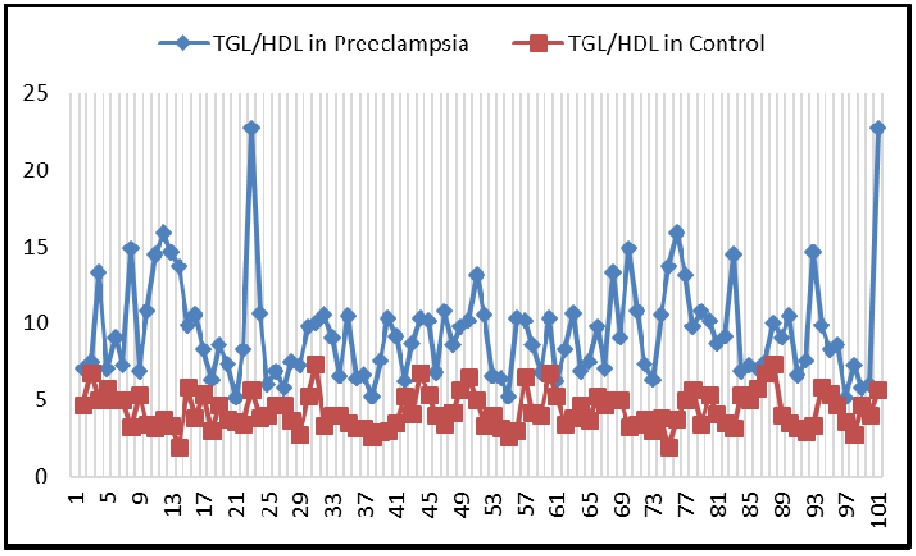


Figure-4: Atherogenic Index(TGL/HDL) In Preeclampsia And Control Group



DISCUSSION: There was dramatic alteration in lipid profile which has been elevated since 20 weeks of gestation. In our study all the lipid sub fractions were significantly elevated, more in the case of triglycerides (figure 1) and HDL was significantly decreased. The significant rise in serum triglyceride concentration in pre-eclampsia in our study was established in the studies of many workers [12, 13]. The exaggerated TGL rise leads to elevated atherogenic small dense low density lipoprotein concentrations [7, 14-16]. Hepatic lipase activity has been shown to be elevated in PE and could contribute to increased small dense low density lipoprotein concentration [7], via increased TGL exchange into LDL, followed by hepatic lipase induced lipolysis of the particle [17]. HDL cholesterol levels are reduced, probably as a consequence of the increased TGL levels and elevated hepatic lipase activity [18].

One of the reasons for this dyslipidemia is due to hormonal imbalance in preeclampsia. Preeclampsia is a state of hypoestrogenemia [6]. Decreased utero-placental blood flow which is the main pathophysiological event in preeclampsia leads to impairment in the formation of Dehydroepiandrosterone sulphate (DHEA) by fetal adrenal glands by interfering with the uptake of lipids by the fetus. DHEA is the important source of estrogen in pregnancy, (i.e.) 90 % of estrogen in maternal circulation is from fetal DHEA which on desulphuration and aromatization by the enzymes sulfatase and aromatase in the placenta forms estriol [19]. Because of the impairment in the formation of DHEA estrogen levels decrease [6]. Moreover decrease in utero-placental blood flow leads to relative stasis of maternal blood in the intervillous space resulting in redistribution of steroids formed in syncytium back to the fetus instead of entering into maternal circulation which also results in a state of hypoestrogenemia[20].

The state of hypoestrogenemia leads to decreased expression of VLDL / apo E receptors in the placenta that are essential for the lipid transport to the fetus. This results in reduced transport of VLDL to fetal compartment, which may be the reason for maternal hypertriglyceridemia. Further LDL taken up by the fetus for the synthesis of DHEA is decreased due to reduced fetoplacental perfusion leading to increased LDL. The decreased catabolism of TG rich lipoproteins by reduced placental uptake and the simultaneous decrease of lipoprotein lipolysis results in the accumulation of TG rich remnant lipoproteins in the maternal circulation [21, 22].

The changes in lipoprotein sub fraction seen in our study were compatible with changes seen in coronary artery disease (Kaaja et al 1995, sattar et al 1997). The dramatic increase in TGL(as shown in figure 3) causes increase in VLDL, small changes in LDL and decrease in HDL which finally leads to Vascular damage and dysfunction directly & increased oxidative stress potentiating insulin resistance – Vascular dysfunction indirectly (Hayman et al 1999& saltar & Green 1999) [23].

In the late phase of normal pregnancy, estrogen enhances the production of VLDLs and decreases maternal lipolytic activity [24]. Together with the increased expression of the VLDL/apoE receptors in the placenta [25], this may result in a coordinated rerouting of TG-rich lipoproteins from the mother toward the fetoplacental unit to meet the nutritional demands of the growing fetus[26]. But in pre-eclampsia, the reduced maternal lipolysis and the hampered uptake of TG-rich lipoproteins by the fetoplacental unit lead to the accumulation of TG-rich remnant lipoproteins in the maternal circulation. Renal protein excretion and hypertension may both reflect endothelial dysfunction. Arbogast et al. [27] hypothesized that TG-rich lipoproteins from preeclamptic women damage the endothelium.

Some studies show that mutation in LPL gene in pre-eclampsia may lead to lowered activity of LPL resulting in decreased hydrolysis of Triglycerides and predispose to dyslipidemia

and cardiovascular disease [28]. There is also evidence that there is active role of placental LPL and Apo E in the metabolism of maternal lipoproteins and so fetal genes may modulate the risk for problems related to maternal dyslipidemia. [29]

The recently published results of the ongoing Copenhagen Male Study, which studied the effect, the TGL/HDL ratio has on the long-term development of heart disease showed that TGL/HDL ratio > 6 had a much higher risk of developing heart disease[29][30] . This was observed in our study also, (the TGL/HDL ratio in preeclamptic population was 9.41 (high risk) against 4.30 in normal pregnancy). The significant increase of TGL/HDL ratio in preeclamptic population is shown in figure 4. The dyslipidemia of elevated triglycerides and lowered HDL in our study was similar to that of previous studies [31- 33].

CONCLUSION: The pattern of dyslipidemia seen in Preeclampsia is similar to the pattern in coronary heart disease. Atherogenic dyslipidemia (elevated triglycerides and low HDL-C) appears to be an important independent risk factor for CVD, confirmed by principal component analysis of lipoprotein subfractions in a large prospective cohort study [34]. Elevation in the ratio of TGL to HDL-C was shown to be the single most powerful predictor of extensive coronary heart disease among all the lipid variables [35]. From all these facts it is clear that dyslipidemia in pre-eclampsia is definitely predisposing to future maternal cardiovascular disease. Hence simple measurement of lipid profile at 20 weeks of gestation can go a long way in prevention of the complications of PIH like, eclampsia, intrauterine growth retardation, HELLP syndrome future cardio vascular risk of the mother, future development of Hypertension and stroke. Further prospective cohort studies in larger population in various ethnic groups are required to predict maternal future cardiovascular risk and its prevention by lifestyle modifications and therapy in preeclampsia. Women with hypertensive disorders in pregnancy should therefore be monitored more closely for the future development of hypertension and the presence of other cardiac risk factors. Furthermore, the obstetric history may be an important additive tool in the cardiovascular risk evaluation in women with hypertension and/or chest pain syndromes.

REFERENCES:

1. Carno TA, Nitrini SM. Drug Prescription for Pregnant Women: A pharmacoepidemiological study. Cad Saud Publica 2004; 20: 1004-13.
2. Krishna Menon MK, Palaniappan B. Hypertensive disorders of pregnancy. In: Mudaliar Menon editor. Clinical Obstetrics 9th ed. Madras: Orient Longman; 1994. 133-54.
3. Dutta DC. In: Text Book of Obstetrics. Hiralal K. editor. 6th ed. Calcutta: New central book agency ; 2006:46, 52 .
4. D.J. Williams M. de swiet, Review Article: The Patho physiology of Pre-eclampsia. Intensive Care Med :1997: 23:620-29.
5. F.Gary Cummingham, Norman FG,Kenneth et al. Hypertensive disorders in pregnancy, Williams Obstetrics, 22nd Edition, Mc. Graw Hill 2005:761-64.
6. Jayanta De, Anandhakumar Mukhopadhyay and Pradip Kumar Saha Study of Serum Lipid Profile in Pregnancy Induced Hypertension. Indian Journal of Clinical Biochemistry 2006/21 (2) 165-68.
7. Marina Noris, Norberto Perico and Giuseppe Remuzzi Mechanisms of Disease: Pre-eclampsia Nature Clinical practice Nephrology 2005:1(2) 98-114.
8. Turnbull's Obstetrics. Geoffrey Chamberlain, Philip J.Steer 3rd Edition. Churchill Livingstone 2001.

9. Ray J, Diamond P, Singh G, Bell C. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. BJOG 2006; 113:379–86.
10. Gratacos E, Casals E, Gomez O, Liurba E, Mercader I, Cararach V, et al Increased susceptibility to low density lipoprotein oxidation in women with a history of Preeclampsia.BJOG 2003:110:400-04.
11. Staff AC, Ranleim T, Khoury J, Henriksen T Increased contents of phospholipids, cholesterol and lipid peroxides in Decidua Basalis in women with Preeclampsia Am J Obstet Gynecol 1999:180:587-92
12. Enquobahrie, DA, Williams MA, Butler CL, Frederick IO, Miller RS and Luthy DA. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J Hypertens.2004 July;17(7):574-81
13. Cekmen MB, Erbagci AB, Balat A, Duman C, Maral H, Ergen K, et al. Plasma lipid and lipoprotein concentrations in pregnancy induced hypertension. Clin. Biochem. 2003; 36(7):575-78.
14. Sattar N, Bendomir A, Berry C, et al. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. Obstet Gynecol 1997;89: 403-08.
15. Ogura K, Miyatake T, Fukui O, et al. Low-density lipoprotein particle diameter in normal pregnancy and preeclampsia. J Atheroscler Thromb 2002;9: 42-7.
16. Lorentzen B, Henriksen T. Plasma lipids and vascular dysfunction in preeclampsia. Semin Reprod Endocrinol 1998;16:33-9.
17. Belo L, Caslake M, Gaffney D, et al. Changes in LDL size and HDL concentration in normal and preeclamptic pregnancies. Atherosclerosis 2002; 162:425-32.
18. Tan CE, Foster L, Caslake MJ, et al. Relations between plasma lipids and post heparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women. Arterioscler Thromb Vasc Biol 1995;15: 1839-48.
19. Williams Obstetrics. F.Gary Cummingham et al 23rd edition. Mc. Graw Hill 2010:65-70.
20. Frank Hytten, Geoffrey Chamberlain's Clinical physiology in Obstetrics. 2nd edition, Boston: Blackwell Scientific; 1991.
21. Rubina A, Tabassum M. Pre-eclampsia and lipid profile. Pakistan Journal of Medical Sciences. 2007 Oct-Dec (Part-I) ; 23(5): 751-54.
22. Karl W, Birgit W, Michael M. H et al.Triglyceride Rich Lipoproteins Are Associated with Hypertension in Preeclampsia. The Journal of Clinical Endocrinology & Metabolism 2003;88(3):1162-66.
23. Kaaja R, Tikkanen MJ, Viinikka L et al. Serum lipoprotein, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. Obstet Gynecol 1995;85:353-56.
24. Kinnunen PK, Unnerus HA, Ranta T, Ehnholm C, Nikkila EA, Seppala M 1980. Activities of postheparin plasma lipoprotein lipase and hepatic lipase during pregnancy and lactation. Eur J Clin Invest 10:469 –74.
25. Wittmaack FM, Gafvels ME, Bronner M, Matsuo H, McCrae KR, Tomaszewski JE, Robinson SL, Strickland DK, Strauss III JF 195. Localization and regulation of the human very low density lipoprotein/apolipoprotein-E receptor: trophoblast expression predicts a role for the receptor in placental lipid transport. Endocrinology 136: 340–48.
26. Winkler K, Wetzka B, Hoffmann MM, Friedrich I, Kinner M, Baumstark MW, Wieland H, Marz W 2000 Low Density Lipoprotein(L DL) subfractions during pregnancy:

accumulation of buoyant LDL with advancing gestation. J Clin Endocrinal Metab 85: 4543-50.

27. Arbogast BW, Leeper SC, Merrick RD, Olive KE, Taylor RN 1994 Which plasma factors bring about disturbance of endothelial function in pre-eclampsia. Lancet 343:340-41.
28. Williams RR, Hunt SC, Hopkins PN, Hasstedt SJ, Wu LL, Lalouel JM. Tabulations and Expectations regarding the genetics of human hypertension. Kidney Int. 1994; 45 : 57 – 64.
29. Descamps,O. S., M. Bruniaux, P-F. Guilmot, R. Tonglet, and F. R.Heller.Lipoprotein metabolism of pregnant women is associated with both their genetic polymorphisms and those of their newborn children. J. Lipid Res.2005.46:2405–14.
30. Jørgen Jeppesen,Hans Ole Hein, Poul Suadicani, Finn Gyntelberg Relation of High TG–Low HDL Cholesterol and LDL Cholesterol to the Incidence of Ischemic Heart Disease An 8-Year Follow-up in the Copenhagen Male Study. Arteriosclerosis, Thrombosis and Vascular Biology: 1997; 17: 1114-20
31. Vidyabati RK , Hijam Davina , Singh NK , Singh W Gyaneshwar Serum βhCG and lipid profile in early second trimester as predictors of pregnancy induced hypertension J Obstet Gynecol India 2010 February : 60(1) 44-50
32. Adiga Usha and Adiga Sachidananda Dyslipidemia In Pregnancy Induced Hypertension Journal of Global Pharma Technology ; 2010:2(5): 69-72
33. Shalini Maksane, Rashmi Ranka, Nitin Maksane and Anjali Sharma Study of Serum Lipid Profile and Magnesium in Normal Pregnancy and in Pre-Eclampsia: A Case control Study Asian Journal of Biochemistry 2011:6 (3): 228-39.
34. Kiran Musunuru Atherogenic Dyslipidemia: Cardiovascular Risk and Dietary Intervention Lipids. 2010 October; 45(10): 907–14.
35. Protasio Lemos da Luz, Desiderio Favarato, Jose Rocha Faria-Neto Junior, Pedro Lemos, and Antonio Carlos Palandri Chagas, High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease Clinics. 2008 August; 63(4): 427–32.

COMPARATIVE EVALUATION OF EFFICACY OF CAUDAL BUPIVACAINE ALONE OR IN COMBINATION WITH BUTORPHANOL OR CLONIDINE FOR POSTOPERATIVE ANALGESIA IN CHILDREN

Veena Chatrath, Sarabjit Kaur, Joginder Pal Attri, Ranjana Khetarpal, Radhe Sharan, Gurmeet Kaur

1. Professor and Head, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.
2. Professor, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.
3. Associate Professor, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.
4. Assistant Professor, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.
5. Assistant Professor, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.
6. Senior Resident, Department of Anaesthesia, Government Medical College, Amritsar, Punjab.

CORRESPONDING AUTHOR

Dr Veena Chatrath, Prof. & Head,
Dept. of Anaesthesia,
Govt. Medical College,
Amritsar (Punjab)-143001,
E-mail: drveenachatrath@yahoo.com,
Ph: 0091 9814112355.

ABSTRACT: BACKGROUND: Addition of adjuvant in caudal block along with bupivacaine prolongs the duration of postoperative analgesia. **AIMS:** The aim of this study was to evaluate the efficacy of caudal bupivacaine alone or in combination with butorphanol or clonidine for postoperative analgesia in children undergoing infra-umbilical surgeries. **MATERIAL AND METHODS:** This study was a prospective, randomized, double blind study and seventy five children of ASA grade I and II of either sex aged 3-8yr were randomized to one of the three groups. Group A received 1 ml/kg of 0.25% bupivacaine; Group B received 20 µg/kg of butorphanol in combination with 1 ml/kg of 0.25% bupivacaine; and Group C received 2 µg/kg of clonidine in combination with 1 ml/kg of 0.25% bupivacaine caudally after general anaesthesia was induced. Hemodynamic variables (HR, SpO2, RR and NIBP) were monitored in all patients. Sedation score, mean duration of analgesia, modified objective pain score and requirement of rescue analgesia were recorded at preset time intervals along with various complications like nausea, vomiting, hypotension, bradycardia, respiratory depression, retention of urine, sweating, pruritis, hallucinations. **STATISTYICAL ANALYSIS:** ANOVA test (Analysis of variance) for intergroup comparison with parametric data, Student's paired t test for intragroup comparison, chi square test for non parametric data and complications and coefficient of variation for variation of parameters from the baseline. **RESULTS:** Mean duration of analgesia was maximum in group B (822.0±217.41 min) than in group A (383.2±81.04 min) and group C (745.4± 216.69 min) and it was statistically significant. The longer duration of sedation observed in group C was also statistically significant. No significant difference was observed in incidence of hemodynamic changes or side effects. **CONCLUSION:** Our results concluded that the addition of butorphanol and clonidine to bupivacaine resulted in superior analgesia with a longer duration of analgesia as compared to caudal bupivacaine alone. Moreover, addition of clonidine to bupivacaine resulted in a higher sedation score which is desirable in children.

KEYWORDS: Paediatric surgery, caudal analgesia, butorphanol, clonidine, bupivacaine.

INTRODUCTION: Caudal epidural block is one of the most popular, reliable, and safe techniques in paediatric analgesia that can provide intra-operative and post-operative analgesia for a variety of infra- and supraumbilical surgical procedures. The main disadvantage of caudal analgesia is the short duration of action after a single injection.[1] Prolongation of caudal analgesia using a 'single-shot' technique has been achieved by the addition of various adjuvants, such as epinephrine, opioids, ketamine, and α_2 agonists.[2]

Butorphanol is a totally synthetic compound of nalorphine cyclazocine series. It is a mixed agonist – antagonist opioid having intrinsic activity at 'μ' type of opioid receptors and agonist at 'κ' receptors. Its interaction with these receptors in the central nervous system apparently mediate most of its pharmacological effects, including analgesia. It has an analgesic action similar to that of morphine but with less respiratory depression and may produce less nausea and vomiting. Its high lipid solubility and high affinity for opioid receptors are additional factors that contribute to the paucity of side effects with its use.[3]

Clonidine is an imidazoline derivative with α_2 agonist activity. After it's administration into the subarachnoid or epidural space, clonidine provides a substantial antinociceptive effect by acting on the α_2 receptors in the dorsal horn of spinal cord and brain stem nuclei implicated in pain. Clonidine has sedative property and it is often a desirable feature as it reduces the requirement of hypnotics.[4]

Krane conducted a study in 1987 in which caudal morphine was used with bupivacaine for caudal analgesia and it was found to prolong the duration of analgesia but with significant side effects like nausea, vomiting, pruritis, urinary retention and delayed respiratory depression.[5,6] Another study compared caudal bupivacaine-clonidine mixture with plain bupivacaine and concluded that addition of clonidine to bupivacaine improve the efficacy of caudal anaesthesia along with prolonged sedation in children[7]. Lawhorn C D et al in 1997 added butorphanol to bupivacaine for caudal analgesia and found an increase in the duration of analgesia[8].

We planned this study to compare the post-operative analgesic effects and side-effects of caudal bupivacaine alone or in combination with butorphanol or clonidine for caudal analgesia in children undergoing infra-umbilical surgeries.

MATERIAL AND METHODS: After obtaining approval from hospital ethical committee, and taking written informed consent from parents, we included 75 patients of ASA I and II aged 3-8 years in this prospective randomized double blind study. The children were scheduled for elective infraumbilical surgeries under caudal block. The study were conducted in a duration of 2 years.

Exclusion criteria included refusal by the parents, presence of a significant sacral deformity, infection of the skin or subcutaneous tissue in the puncture area, coagulation defects, demyelinating disease of the CNS, hypersensitivity to local anaesthetics, severe hypovolemia, mental retardation and history of convulsions. Patients were randomly assigned to one of three groups by computerised random number by an investigator with no clinical involvement in the trial. Blinding was used to avoid patient selection bias. The anaesthesiologist giving the caudal block as well as the observer who monitored the post-operative analgesia were both blinded to the study drug. Using computer generated random numbers patients were allocated into three groups of 25 each. Group A patients received 1 ml/kg of 0.25% bupivacaine with 0.1ml/kg normal saline as control. Group B patients received 1 ml/kg of 0.25% bupivacaine and butorphanol 20 μg/kg. Group C patients received 1 ml/kg 0.25% bupivacaine and clonidine 2

μg/kg. Drug solutions were prepared by an anaesthesiologist who was blinded to the nature of the study. For each patient two different syringes were prepared. One syringe contained bupivacaine and the other contained either 0.1ml/kg of normal saline or clonidine or butorphanol. Normal saline was added to clonidine or butorphanol to achieve a total volume of 0.1ml/kg. Volume of the drug solutions was kept constant in all the three groups.

Detailed preanaesthetic check up of the children was done a day prior to surgery and the children were kept fasting for 4-6 hrs. On the day of surgery, the children were reassessed in the preanaesthetic room. Premedication was given orally with syrup promethazine 1 mg/kg 45 minutes before surgery.

In the operating room, precordial stethoscope and monitor to check for heart rate, respiratory rate, non invasive blood pressure and SpO2 were attached and the baseline parameters were recorded. The children were induced with oxygen, nitrous oxide (50:50) and halothane with appropriate size of face mask and Jackson-Ree's circuit. Intravenous line was started and intravenous fluids were given according to the body weight of the child. Operative procedures performed were circumcision, herniotomy, hypospadias, orchidopexy etc.

After adequate level of anaesthesia caudal block procedure was performed with short bevelled 22G needle. Identification of the space was done with whoosh test. A mechanical stimulus was applied at the surgical dermatome or at the immediate superior dermatome with a modified Alis clamp for the evaluation of block onset. Surgery was started 15 minutes after the caudal block when there was no limb movement to the mechanical stimulus. Surgery was allowed only after an adequate effect was achieved. Caudal effectiveness score was assessed

Patients were monitored intraoperatively for heart rate, respiratory rate, blood pressure, oxygen saturation after every 5 min interval during the surgery. Postoperatively in the recovery room these parameters were monitored for 2 hrs at the interval of 15 min and in the ward 2 hourly for six hours and then at 12 and 24 hrs.

Postoperatively sedation and severity of pain was assessed using sedation score[9] (table 2) and modified objective pain score[10] (OPS) (table 3) respectively.

Side effects including nausea, vomiting, hypotension, bradycardia, respiratory depression, retention of urine, sweating, flushing, pruritis, hallucination or any other side effect was noted.

Rescue analgesia was given with oral paracetamol 20 mg/kg to the children when the modified objective pain score was ≥4. The number of rescue analgesic doses and total requirement of analgesic doses was noted in the postoperative period.

STATISTICAL ANALYSIS: At the end of this study, decoding of the drug solutions was done and the results were analysed statistically using ANOVA test for intergroup comparison and student's T test for intragroup comparisons. Chi-square test was performed to analyse non-parametric data.

RESULTS: The age, sex, weight, ASA status and duration of surgery of the patients were comparable in the three groups and there was no statistical difference in between them (Table-4). Caudal effectiveness score was assessed in all the patients and no significant difference was found in between the three groups.

In the postoperative period urinary retention was noted in all the groups and nausea was seen in group A & B but the difference was not statistically significant. No patient had

respiratory depression, pruritis, hallucinations, sweating/ flushing, laryngospasm, hypotension and cardiac event.

The sedation score was maximum at '0' minute in the recovery room. i.e. 1.48±0.71, 2.08±0.70 and 2.36±0.70 in Group A, B and C respectively and after that it gradually diminished so that at 90 min., it was 0.40±0.50, 0.84±0.55 and 1.32±0.55 in the three groups respectively. After 90 min the patients in Group A were fully conscious. The sedation score of the patients in groups B was 0.52±0.51 up to 4 hrs in the ward and in group C the sedation score was 0.52±0.43 up to 6 hrs in the ward. The comparison of sedation score between Group A, B and C was statistically significant in the recovery room and in the ward (p value <0.05, paired t test. (Table 5).

The mean duration of analgesia was statistically longer (p value < 0.01) in the group B (822.0± 217.41 min) and group C (745.4± 216.69 min) than group A (383.2±81.04 min). The total number of 'rescue' analgesic doses required in the first 24 hrs was lesser in group B (0.80 ± 0.41) than both groups A (1.96 ± 0.61) and C (0.96 ± 0.45) (Table 5).

The objective pain score was less in group B and C compared to group A. The OPS was again statistically significant in comparing group A & B and A & C throughout the postoperative period (p value<0.05, paired T test). But OPS score was not statistically significant between Group B and C.(Table 5).

DISCUSSION: Surgical procedures in children are followed by pain which may give rise to restlessness, tachycardia, fear, crying, anxiety and agitation in children. To negate this physiological and psychological effects of pain and to improve the quality of analgesia, caudal epidural block is a well known method.[11]

Caudal block is easy to perform and has been found to be very effective in children. Caudal anaesthesia is the most frequently used regional technique in children, accounting for almost 50% of all regional techniques. When caudal block is used as a supplement to general anaesthesia in children, it decreases the intra-operative requirement for general anaesthesia, with lesser use of parenteral opioids thereby limiting the incidence of respiratory depression and it also limits the stress hormone response.[11] Many anaesthetic agents have been used for caudal analgesia in paediatric patients, with bupivacaine and lignocaine being most common.

Bupivacaine when used alone through caudal route has the limitation of short duration of action. Different additives have been used to prolong the duration of analgesia even with longer acting local anaesthetics. To prolong the duration of single shot caudal analgesia and to decrease the individual dose of the drugs, many adjuvants have been administered along with bupivacaine eg. Morphine, clonidine, tramadol, ketamine, neostigmine and butorphanol.[12,13]

Butorphanol is a mixed agonist – antagonist with intrinsic activity at receptors of the mu opioid type (morphine like). It is also an agonist at kappa opioid receptors. Its interactions with these receptors in the CNS apparently mediate most of its pharmacological effects, including analgesia. Butorphanol has an analgesic action similar to that of morphine, with less respiratory depression, less nausea and vomiting, no undesirable psychomimetic effects.[14]

Clonidine an α_2 agonist has also been used as additive to local anaesthetics. It's addition increases duration and improves quality of analgesia provided by single shot caudal anaesthesia. Clonidine when used extradurally provides analgesia by nonopioid spinal effects.

The present study was conducted to compare the analgesic efficacy of caudal bupivacaine alone or in combination with butorphanol or clonidine for post-operative analgesia. The demographic profile of our patients was comparable with respect of age, sex, body weight,

ASA status and duration of surgery. The results of our study have shown that caudal administration of bupivacaine with the addition of 20μg/kg butorphanol and 2μg/kg clonidine resulted in significant increase in analgesia time than bupivacaine alone. The mean duration of post-operative analgesia was 383.2±81.04 min in bupivacaine group, 822.0±217.41min in butorphanol group and 745±216.69min in clonidine group. The prolongation of duration of analgesia was significant in both butorphanol and clonidine group when compared to bupivacaine alone group. The findings of our study were consistent with the studies conducted by others.[7,8,9].

Sedation may be beneficial especially in paediatric population. Children who sleep for many hours after surgery are perceived more comfortable by the attending nurses and families. Also it offers advantages in children who would benefit from sedation in order to tolerate any necessary monitors, lines or drains postoperatively. In a previous study by Bailey et al, the incidence of sedation was significantly higher in paediatric patients receiving caudal butorphanol.[15]

In our study also we found that sedation score was significantly higher in Group C (clonidine 2 μg/ kg with 0.25% bupivacaine 1 ml/kg) compared to Group A and Group B. The findings of our study are consistent with the findings of Lee JJ et al who found significant sedation with clonidine in the doses of 2 μg/ kg.[9]

In our study Modified Objective Pain Score (OPS) was used to evaluate the pain in children and gives an objective evaluation of pain. None of the patients had pain in the recovery room i.e. for up to 2 hours postoperatively and hence none of the patients required any rescue analgesia during this period. In the ward OPS started increasing at 4 hrs in Group A and at 6 hrs in Group B and C. After that OPS started falling till the end of observation period. This can be contributed to the fact that most of the patients received the rescue analgesia during this period. Singh V et al concluded that need for rescue analgesia in PACU and total numbers of doses of morphine administered were significantly less in patients in whom butorphanol was added to bupivacaine in caudal epidural analgesia.[8]

In our study, the variation in the heart rate, blood pressure, respiratory rate and SpO_2 was comparable in all the three groups in both the intraoperative and the postoperative periods and this was not statistically significant. This implies that addition of butorphanol/clonidine to bupivacaine did not produce any significant effect on haemodynamics.[8,9]

Martin et al carried out a study and found that with caudal anaesthesia the most common side effect is urinary retention.[15]In our study 4% patients in Group A, 8% patients in Group B and 4% patients in Group C had urinary retention. In a previous study by Aggarwal and Taylor, no patient had pruritis and respiratory depression following caudal butorphanol.[16]Similarly no patient in our study had pruritis and respiratory depression. The side effect profile of bupivacaine, butorphanol and clonidine were favourable and non-significant on statistical comparison among the three groups.

CONCLUSION: From the present study it is concluded that caudal administration of bupivacaine with addition of butorphanol or clonidine, is a reasonably safe and effective means for paediatric caudal analgesia in children undergoing infraumbilical surgeries to increase the duration and quality of analgesia without any haemodynamic instability and increase in side effects. Furthermore, the addition of clonidine also increased the duration of sedation, which is a desirable feature in children.

Table – 1 Caudal effectiveness score

Score	Definition
0.	Impossible to reduce halothane concentration at any time during surgery.
1.	Halothane concentration increased after initial reduction.
2.	Halothane concentration reduced; heart rate or blood pressure increase ≥ 20% of the baseline.
3.	Halothane concentration reduced; heart rate and blood pressure increase < 20% of baseline.

Table 2- Sedation score

Grade	Definition
0	Fully awake
1	Slightly drowsy
2	Asleep but easily arousable
3	Fully asleep but arousable
4	Fully asleep but not arousable

Table 3- Modified Objective pain score

VARIABLE	SCORE 0	SCORE 1	SCORE 2
Crying	None	Consolable	Not consolable
Movement	None	Restless	Thrashing
Agitation	Asleep/Calm	Mild	Hysterical
Posture	Normal	Flexed	Holds injury site
Verbal	Asleep/No complaint	Complains but cannot localize	Complains and can localize

Table 4- Showing demographic Data and duration of surgery among various groups

Group	A	B	C	P value
Age (yr)	4.56±1.82	4.6±1.75	5.08±1.55	>0.05
Sex (Male : Female)	20 : 5	22 : 3	21 : 4	>0.05
Weight (kg)	15.8 ± 3.4	16.12 ±3.95	16.88 ±2.71	>0.05
Duration of Surgery (min)	30.40±12.82	31.00 ±11.99	38.60 ±18.51	>0.05

Table 5- Showing Sedation score, Mean duration of analgesia,

Mean dose of rescue analgesia & OPS

Group	A	B	C
Sedation score	1.48±0.71	2.08±0.70*	2.36±0.70*
Mean duration of analgesia (min)	383.2±81.04	822.0± 217.41*	745.4± 216.69*
Mean dose of rescue analgesia	1.96 ± 0.61	0.80 ± 0.41*	0.96 ± 0.45*
Objective Pain Score (OPS)	3.44 ± 0.91	2.52 ± 0.65*	2.28 ± 0.84*

*Statistically significant ($p < 0.05$

Figure 1: Showing mean sedation score in the three groups at different time intervals in recovery room

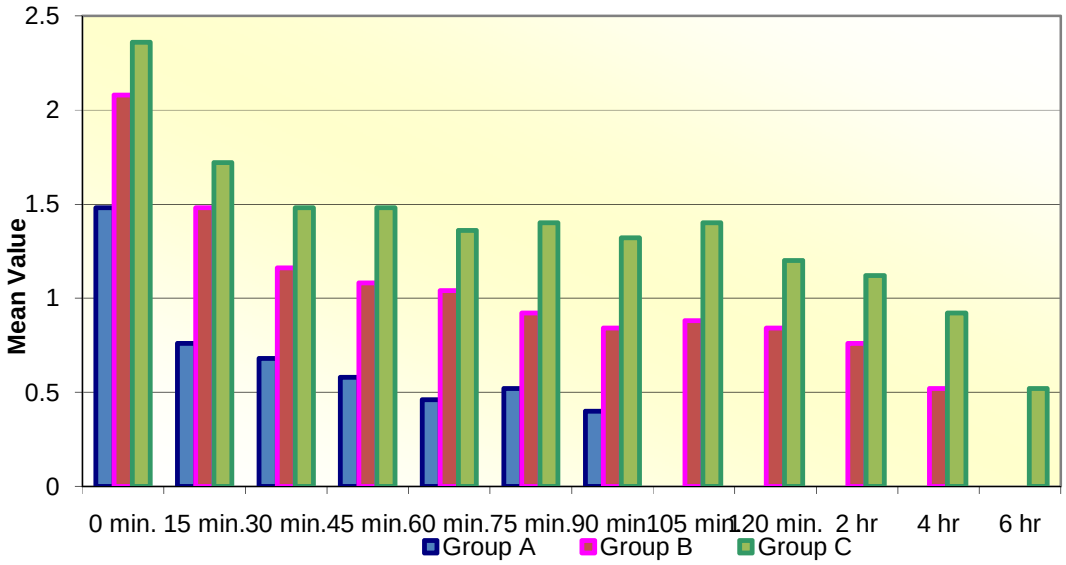
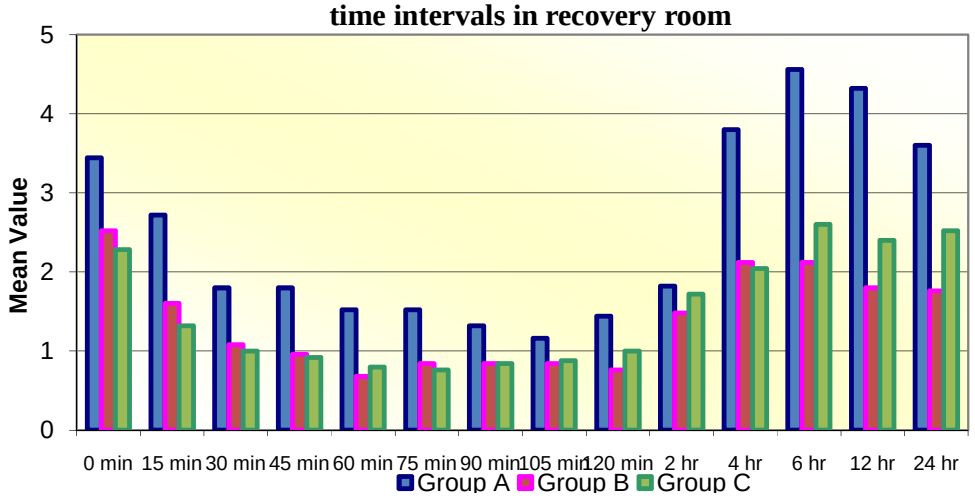


Figure 2: Showing mean ops in the three groups at different time intervals in recovery room



REFERENCES:

1. Lloyd-Thomas AR. Pain management in paediatric patients. Br J Anaesth 1990;64:85-104.
2. Vetter TR, Carvallo D, Johnson JL, Mazurek MS, Presson RG. A comparison of single-dose caudal clonidine, morphine, or hydromorphone combined with ropivacaine in pediatric patients undergoing ureteral reimplantation. Anesth Analg 2007; 104:1356-63.
3. Sung YF, Weinstein MS, Ghani GA. Balanced anesthesia: a comparision of butorphanol and morphine. Southern Medical Journal 1984; 77:180-182.
4. Eisenach J, Detweiter D, Hood D. Haemodynamic and analgesic action of epidurally administered clonidine. Anaesthesiology 1993; 78:277-87.
5. Krane EJ. Tyler DC. Jacobson LE. The dose response of caudal morphine in children. Anaesth 1989; 71:48-52.
6. Krane EJ. Delayed respiratory depression in a child after caudal epidural morphine. Anaesth analg 1988; 67:79-82.
7. Klimscha W, Chiairi A. The efficacy and safety of clonidine/bupivacaine combination in caudal blockade for paediatric hernia repair. Anesth Analg 1998;86:54-61.
8. Lawhorn CD, Stoner JM, Schimtz ML, Brown RE, Jr. Stewart FW, Volpe P et al. Caudal epidural butorphanol plus bupivacaine versus bupivacaine in pediatric outpatient genitourinary procedures. J Clin Anesth 1997; 9:103-8.
9. Lee JJ, Rubin AP. Comparison of a bupivacaine-clonidine mixture with plain bupivacaine for caudal anaelgesia in children. Br J Anaesth 1994; 72:258-62.
10. Nordon J, Hannallah R, Geston P. Reliability of objective pain scale in children. Anesth Analg 1991; 72:99.
11. Yaster M, Maxwell LG. Pediatric regional anesthesia. Anesth 1989; 70:324-38.
12. Armitage EN, Caudal block in children. Anaesth 1979; 34:396.
13. Armitage EN, Lunn JN. Analgesia after circumcision. Anaesth 1980; 35:77-78.
14. Singh V, Kanaujia A, Singh GP. Efficacy of caudal butorphanol. In J Pediatr 2006; 73:147-50.
15. Martin L M. Penile block associated with less urinary retention than caudal anaesthesia in distal hypospadias repair in children. World J of Urology 2010;28:87-91.
16. Aggarwal R, Taylor BD. Caudal epidural butorphanol in children. Anesth Analg 1993; 76:3.

CARDIAC SARCOIDOSIS- A RARE CASE REPORT IN AUTOPSY SPECIMEN WITH REVIEW OF LITERATURE

Shaista Choudhary, Y. A. Manjunatha, Priyadarshini M. M.

1. Associate Professor, Department of Pathology, Dr. B. R Ambedkar Medical College, Bangalore.
2. Professor & HOD, Department of Pathology, Dr. B. R Ambedkar Medical College, Bangalore.
3. Post Graduate, Department of Pathology, Dr. B. R Ambedkar Medical College, Bangalore.

CORRESPONDING AUTHOR

Dr Shaista Choudhary,
No60, 10thA Cross,
West of Chord road,
2nd stage, Bangalore-80,
E-mail: drshaista5@rediffmail.com,
Ph: 0091 9901474389.

ABSTRACT: Sarcoidosis is an uncommon disease in India and Cardiac Sarcoidosis is a known but uncommon manifestation of Sarcoidosis. The clinical features and presentations of cardiac sarcoidosis are protean and vary from benign ectopic to life threatening ventricular tachycardia. The outcome of most of the cases that are not diagnosed in time is sudden death. [1]

KEY WORDS: Cardiac sarcoidosis, interventricular septum, sudden death.

INTRODUCTION: Cardiac sarcoidosis occurs with more frequency in young adults[2]Bernstein et al were the first to recognise this entity. Gozo and his associates were the first to describe its clinical and pathologic features. Hagemann and Wurms estimated that the heart was affected in about 5% of patients with chronic sarcoidosis.[3]But the actual incidence of cardiac sarcoidosis is much higher than the number of cases reported. This could be due to the lack of awareness of the disease with increased chances of misdiagnosis clinically. The incidence of sarcoidosis in developing countries like India has been underestimated, due to overwhelming incidence of tuberculosis which mimics this condition closely. Most of the affected patients are young adults and survival is limited to 2-3 years after cardiac involvement. The diagnosis based on clinical features is really difficult because of its varied presentation, ranging from benign arrhythmias to fatal ventricular fibrillations.[3]Therefore one has to be very careful while dealing with cases suspected to have any cardiac symptom .The same holds good even for cases with abnormal cardiac or pulmonary investigations.

CASE REPORT: We describe a case of myocardial involvement by sarcoidosis that lead to sudden death of a 38 years old man. He was found dead and autopsy was conducted to know the cause of death. Viscera were sent for histopathological examination. Grossly there was hypertrophy of heart (figure1).Cut surface of heart showed grey white patchy areas over the interventricular septum and extending towards right and left ventricular wall (figure2).Multiple sections were processed from the affected area. Microscopy revealed the presence of non caseating granulomas with numerous giant cells in the background (figure3).Gross appearance of lungs was unremarkable. The microscopy showed non caseating granulomas with giant cells in the lungs too (figure4). The autopsy report was finalised as cardiac sarcoidosis to be the cause of sudden death after ruling out other causes of granulomatous lesions affecting the heart. Since it was a post-mortem case, investigation reports were not available.

DISCUSSION: Cardiac sarcoidosis is a potentially fatal condition and not frequently diagnosed during clinical examination.[4]It is an incidental finding in autopsy cases. In necropsy studies of sarcoidosis it was found that the proportion of cases with cardiac involvement by granulomas was higher than the proportion of cases with cardiac sarcoidosis during life. There are various other causes for granulomas in the heart such as giant cell myocarditis, idiopathic granulomatous myocarditis, tuberculous myocarditis, fungal myocarditis, Whipple disease, S.L.E, rheumatoid nodule and Wegener's granulomatosis.

Idiopathic granulomatous myocarditis and giant cell myocarditis stand as two most important differential diagnosis for cardiac sarcoidosis. In case of idiopathic granulomatous myocarditis there are non caseating granulomas in the heart without granulomatous involvement of the other organs. In giant cell myocarditis on the other hand is characterized by an infiltrate of eosinophils, lymphocytes, macrophages and giant cells associated with myocyte necrosis. The macrophage does not aggregate to form granulomas. This can be appreciated in routine Hematoxylin-Eosin (H & E) stained sections. Infective granulomatous myocarditis (tuberculous myocarditis) is quite rare and granulomas are of caseating type and have larger lymphocytes as compared to granulomas of sarcoidosis. Fungal infection of the heart can also give rise to granulomas but the fungal elements can be identified under routine stain because of the presence of plenty of inflammatory cells predominantly eosinophils and plasma cells.

In case of Cardiac Sarcoidosis there are non caseating granulomas in the cardiac tissue along with same features in other organs. Thus, the other causes of granulomatous inflammation of the heart can be ruled out. A.F.B (Acid fast Bacilli) stain on the representative tissue which was also negative. However the microscopic features on H and E itself were quite characteristic of the lesion[5].Clinical evidence of myocardial involvement is present in approximately five percent of patients with sarcoidosis, although autopsy studies indicate that subclinical cardiac involvement is present in 20-30% of cases. R. Guleria et.al in their article have discussed the varied presentations of cardiac sarcoidosis.[1]The clinical features may vary between body ache, dyspnoea, palpitations, fatigue etc .It is advisable to start with preliminary investigations like chest X-ray, pulmonary function tests and ECG. Transbronchial lung biopsy can be done in patients with hilar lymphadenopathy. Cardiac biopsy can be performed in few cases which show e.g. any abnormality. Investigators have recommended that all patients of sarcoidosis must be investigated for cardiac involvement with halter and thallium scan.

Cardiac sarcoidosis is a disease of remission and relapse. Cardiologist and pulmonologist have to work up the case together in order to reach an early diagnosis.[1]If the patient presents with cardiac and respiratory symptoms and pulmonary investigations point towards sarcoidosis of lung, then it is mandatory to have high suspicion towards cardiac sarcoidosis, provided the patient also has cardiac symptoms.

Cardiac biopsy is very specific but on rare occasions diagnosis can still be missed because of the patchy distribution of the disease. Increased numbers of cases of cardiac sarcoidosis are being incidentally reported at autopsy [6], so one has to be careful in diagnosing subclinical cases. This case is being presented here for its academic interest and also to emphasize on subclinical cases which go unnoticed because of lack of awareness.

Figure-1: Gross appearance of autopsy specimen showing hypertrophy of heart.

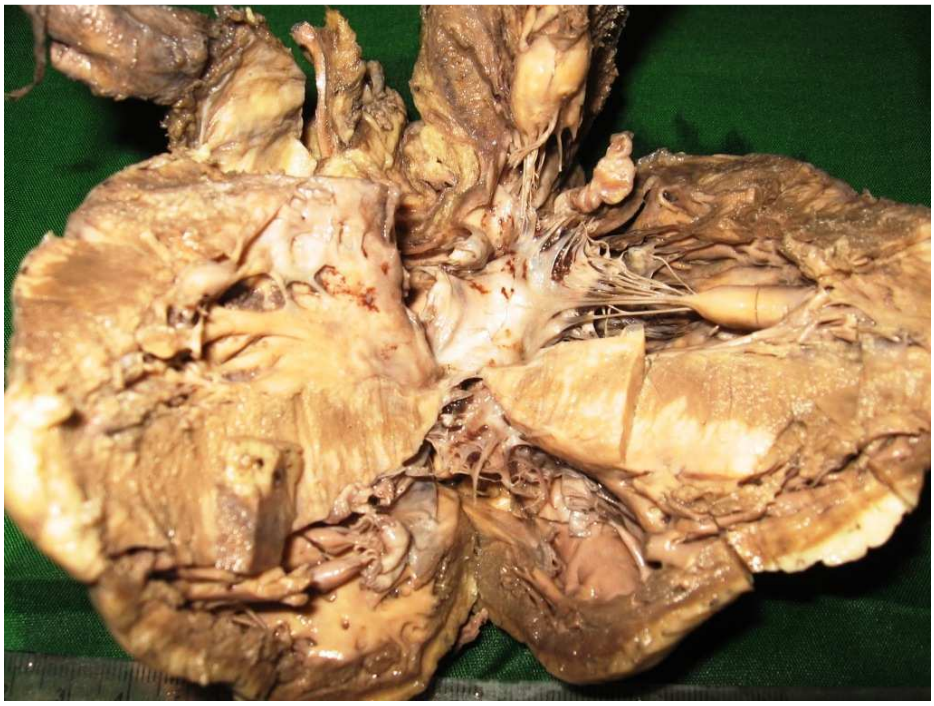


Figure-2: Cut surface show grey-white areas with thickening of interventricular septum.

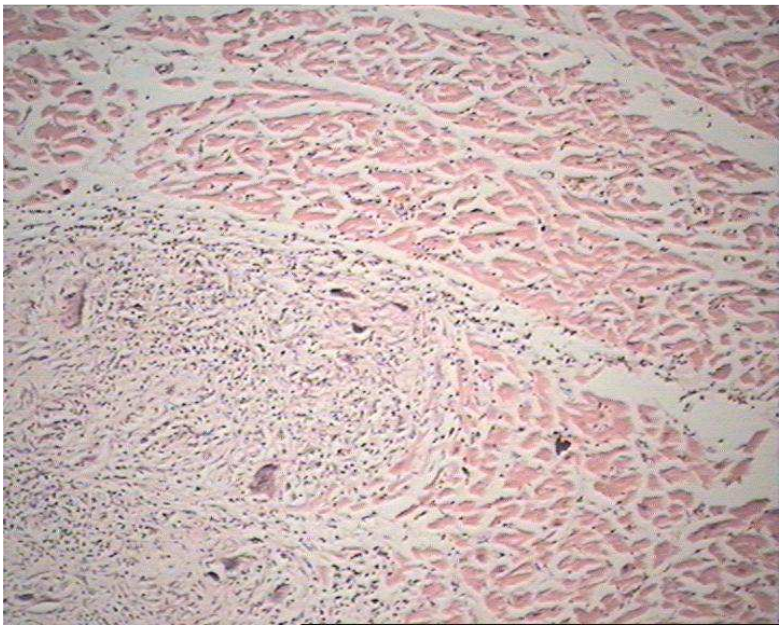


Figure-3: Show non-caseating granulomas in the cardiac muscle.

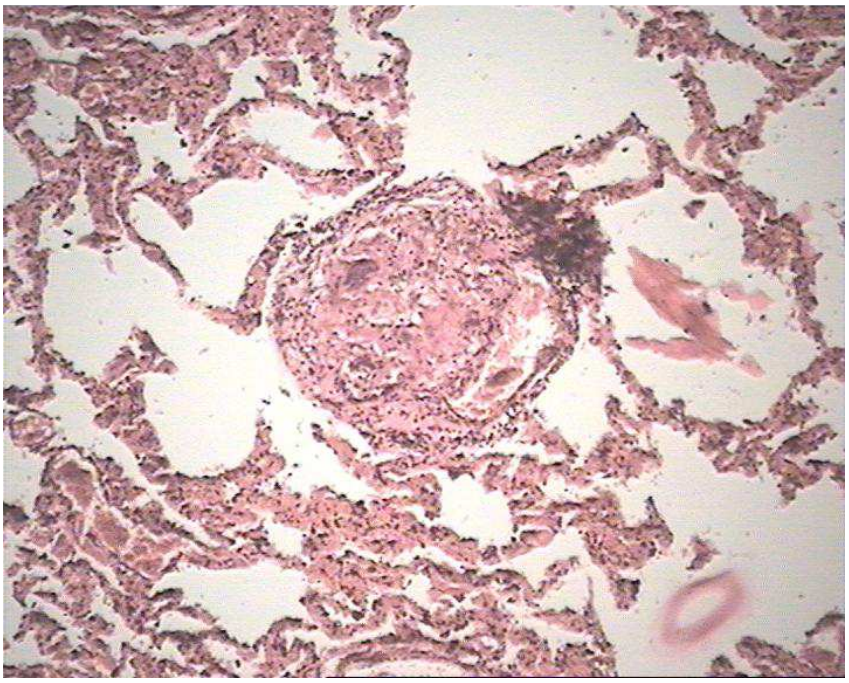


Figure-4: Show granuloma with giant cells in the lung parenchyma.

REFERENCES:

1. Guleria R, Sharma R, Mohan A and Das C. Cardiac Sarcoidosis. Ind journal of Chest disease allied sci 2006; 48:133-37.
2. Wan Muhaizan WM, Swaminathan M, David M S. Cardiac Sarcoidosis-two cases with autopsy findings. Malays journal of pathol 2004; 26(1):59-63.
3. Sharma Om P. Diagnosis of cardiac sarcoidosis-An imperfect science: A hesitant art. CHEST 2003; 123(1) :18-19.
4. Kim Jessica S, Judson Mark A, Domino Robert, Gold Michael, Cooper Leslie T. Prystowsky Eric N et.al. Cardiac Sarcoidosis. American heart journal 2009; 157(1):9-21.
5. Lagna S M, Parvani A V , Nichols L C. Cardiac Sarcoidosis: a pathology focussed review. Arch Pathol Lab Med.2010; 134(7):1039-46.
6. Iwai Kazuro, Tachibana Jeruo, Takemura Tamiko, Matsui Yasuo, Kitalchi Masanori, Kawabata Yoshenori. Pathological studies on Sarcoidosis autopsy-epidemiological features of 320 cases in Japan. Pathology international. ; 43(7-8):372-76.

MICROBIOLOGICAL PROFILE OF VAGINAL SWABS.

Sevitha Bhat, Nilica Devi, Shalini Shenoy

1. Assistant Professor, Department of Microbiology, Kasturba Medical College, Mangalore.
2. MSc Student, Department of Microbiology, Kasturba Medical College, Mangalore.
3. Professor, Department of Microbiology, Kasturba Medical College, Mangalore.

CORRESPONDING AUTHOR
Dr Sevitha Bhat,
Flat 102, Meadows,
Mannagudda Road, Ballalbagh,
Mangalore, Karnataka.
E-mail: sevitha@rediffmail.com,
Ph: 0091 9449831631.

ABSTRACT: BACKGROUND/OBJECTIVES: Bacterial vaginosis (BV), an alteration of vaginal flora involving a decrease in Lactobacilli and predominance of anaerobic bacteria, is the most common cause of vaginal complaints for women of childbearing age .Causative agents include Gardnerella vaginalis, facultative lactobacilli, Mycoplasma hominis, Viridans streptococci & anaerobic bacteria. The most frequent manifestations of genitourinary candidiasis is vulvovaginal candidiasis & is diagnosed in 40 % of women with vaginal complaints. The present study was undertaken to study the occurrence of Bacterial vaginosis & Vulvovaginal candidiasis in women with vaginal complaints. **MATERIALS AND METHODS:** The study included 100 High vaginal swabs with H/O white discharge per vaginum for a period of 6 months from June 2011.Swabs were stained by Grams method & were cultured. **RESULTS:** Out of 100 samples, in 35(35%)Gram smear findings were normal & no growth in culture. 26(26%)samples had polymorphs with Gardnerella morphotype, gram negative curved rods, fusiforms & Lactobacilli were absent/present in low numbers(1 to 2+), the smear was interpreted as consistent with BV. 15 samples (15%) had the growth of Candida spp.

In the remaining 24 samples, the Gram stain findings had only vaginal epithelial cells with Lactobacilli, but the culture had the growth of Group B Streptococcus 10(10%), E. coli & coliforms 10(10%),Enterococcus spp 14(14%)which could just be colonisers.

CONCLUSION: Bacterial vaginosis is the predominant cause of leucorrhoea (26%) followed by vulvovaginal candidiasis accounting for 15 % of the cases. Detection of intrapartum vaginal colonisation of Group B Streptococci, E. coli & coliforms & Enterococcus spp. in pregnant women is of significance as it can get transmitted to the neonate & cause sepsis.

KEY WORDS : Bacterial vaginosis , Vaginal swabs, G. vaginalis

INTRODUCTION: Bacterial vaginosis (BV), an alteration of vaginal flora involving a decrease in Lactobacilli and predominance of anaerobic bacteria, is among the most common cause of vaginal complaints for women of childbearing age. It is well known that BV has an influence in acquisition of certain genital infections. This infection is characterized by a loss of indigenous (hydrogen peroxide-producing) Lactobacillus-predominant vaginal microflora, and a concurrent massive overgrowth of anaerobic bacteria. The most common include Gardnerella vaginalis, Mobiluncus species, Prevotella species (anaerobic bacteria), Mycoplasma hominis, and Atopobium vaginae[1]. At least 50% of patients have no symptoms. In the other half, it most often manifests clinically as a thin homogenous vaginal discharge, a vaginal pH > 4.5, presence

of 'clue cells', and an amine odour after addition of 10% of potassium hydroxide. BV has been shown to increase the risk of obstetric and gynaecologic complications such as preterm labour and delivery, chorioamnionitis, post-caesarean endometritis, post-abortion pelvic inflammatory disease, and cervicitis [1,3]. Moreover, BV has been associated with many sexually transmitted infections (STIs), including infection with Chlamydia trachomatis, Neisseria gonorrhoeae, HSV-1 and 2, and an increased risk of HIV acquisition . Diagnostic criteria for BV included Nugent's method and culture.

The most frequent manifestations of genitourinary candidiasis include vulvovaginal candidiasis (VVC) in women, balanitis and balanoposthitis in men, and candiduria in both sexes. These diseases are remarkably common but occur in different populations, immunocompetent as well as immunocompromised. While VVC affects mostly healthy women, candiduria is commonly diagnosed in immunocompromised patients or neonates. In the majority of women, a diagnosis of VVC is made at least once during their childbearing years. Among the many causes of vaginitis, VVC is the second most common after bacterial vaginosis and is diagnosed in up to 40% of women with vaginal complaints in the primary care setting. A 10% potassium hydroxide (KOH) preparation is more sensitive than a saline preparation in identifying yeast cells or hyphae [2]. Vaginal culture is the most accurate method for the diagnosis of VVC and is indicated if microscopy is negative [2].

In pregnant women, Group B Streptococci (GBS) is a cause of cystitis, amnionitis, endometritis, and stillbirth. Occasionally, GBS has been a cause of endocarditis and meningitis, while in postpartum women; it has been identified as a cause of urinary tract infections (UTIs) and pelvic abscesses4. Prenatal screening for this condition is now established. Intrapartum colonisation of the mother's vagina with coliforms, enterococci is also associated with neonatal sepsis and meningitis in the newborn.

The present study was undertaken to study the occurrence of BV & Vulvovaginal candidiasis in women with vaginal complaints.

MATERIALS AND METHODS: A retrospective study was undertaken . The study included 100 High vaginal swabs received in the laboratory from women in the age group 21-35 years who attended the hospital with H/O white discharge per vaginum, abdominal pain and any other complaints formed the study population .The study was carried out in the Department of Microbiology, Kasturba Medical College, Mangalore for a period of 6 months from June 2011 .

The swabs were stained by Grams method & were cultured onto Thioglycollate broth, Mac Conkey Agar, Chocolate agar, Blood agar. The culture was incubated aerobically & was examined after 18 to 24 hours[5].

Gram smear examination: Gram smear was evaluated for the presence of pus cells, vaginal epithelial cells, various bacterial morphotypes , clue cells & yeasts .

Each microbial morphotype was quantitated under an oil immersion objective (l000 x) by using the following scheme: 1+(<1 per field); 2+ (1 to 5 per field); 3+(6 to 30 per field); 4+(>30 per field). Large gram-positive rods were taken as lactobacillus morphotypes; smaller gram-negative to gram-variable rods were considered as G. vaginalis and Bacteroidesspp[5]. As per table 1.

When the Lactobacillus morphotype was present alone or in combination only with the Gardnerella morphotype, the smear was interpreted as normal. When mixed flora, including not only the Gardnerella morphotype but also other gram-negative and gram-positive bacteria such as curved rods, gram-negative rods, fusiforms, and gram-positive cocci was present and when

the Lactobacillus morphotype was absent or present only in low numbers (1 to 2+), the smear was interpreted as consistent with BV. The presence of morphotypes other than the Lactobacillus or Gardnerella morphotype was considered abnormal, regardless of quantity[5].

The growth obtained on the culture plates was identified using conventional biochemical tests [6]. Group B Streptococcus was identified by Strep Pro grouping kit. &Candida spp. were further identified by Germ tube test and CHROM agar.

RESULTS: Out of 100 samples, in 35(35%) Gram smear findings were normal & no growth in culture. 26(26%) samples had polymorphs with Gardnerella morphotype , gram negative curved rods, fusiforms& Lactobacilli were absent/present in low numbers(1 to 2+), the smear was interpreted as consistent with BV.

15 samples (15%) had the growth of Candida spp.

In the remaining 24 samples, the Gram stain findings had only vaginal epithelial cells with Lactobacilli, but the culture had the growth of Group B Streptococcus 10(10%), E.coli & coliforms 10(10%),Enterococcus spp 4(4%) which could just be colonisers.

DISCUSSION: Bacterial vaginosis is characterized by a reduction in the prevalence and the numbers of hydrogen peroxide-producing lactobacilli and an increase in the concentration of Gardnerella vaginalis and resident anaerobic bacteria. Causative agents of BV thus include Gardnerella vaginalis, facultative lactobacilli, Mycoplasma hominis, Viridans streptococci & anaerobic bacteria[7].

It is associated with adverse outcomes related to upper genital tract and increased risk of HIV acquisition. An interesting observation is that new genital HPV infection in young women is associated with increased subsequent risk of developing BV. Other risk factors include multiple sexual partners and recent intercourse with a new partner, but metronidazole treatment of male partners has not reduced the rate of recurrence among affected women[8].

The delicate balance of the vaginal ecosystem is challenged constantly by several factors such as hormonal changes, medications, intercourse, stress, infection, douching, and hygiene.

The unhealthy vaginal environment can be described as an imbalance in the vaginal bacterial ecosystem[8].

At least 50% of patients were asymptomatic. In the other half, it most often manifests clinically as a thin homogenous vaginal discharge and presence of 'clue cells' [1,2] .

Although there are no universal standards, as a point of reference, the PHLS SOP recommends an High vaginal swabs from patients be Gram stained for BV and cultured for Trichomonas and yeasts.

We conducted a study on 100 cases of BV which were diagnosed by Gram stain and culture. It classifies gram stained vaginal smears into normal, intermediate and bacterial vaginosis based on the gram stain scoring system.

In our study , out of the 100 smears examined , 26 smears(26%) were interpreted as consistent with BV. 15 samples (15%) had the growth of Candida spp and is comparable to previous studies where bacterial vaginosis is common compared to VVC in women with vaginal complaints in the primary care setting [2].

Culture is not reliable for diagnosis of most of the bacterial diseases; as the organisms which are involved in BV cannot be isolated in the laboratory easily and as normal women also have this flora in their vagina in small numbers .

Culture was used to detect the vaginal colonisation with coliforms, Group B Streptococcus, Enterococcus species which is of importance in pregnant women .

CONCLUSION: BV is the predominant cause of leucorrhoea (26%) followed by vulvovaginal candidiasis accounting for 15 % of the cases. Detection of intrapartum vaginal colonisation of Group B Streptococci, E. coli & coliforms & Enterococcus spp. in pregnant women is of significance as it can get transmitted to the neonate & cause sepsis.

ACKNOWLEDGEMENT: The authors are grateful to Manipal University for providing the facilities to perform the study.

Table 1: Nugent's method of diagnosis of bacterial vaginosis

*Score	Lactobacillus Morphotypes	G. vaginalis and Bacteroides spp. Morphotypes	Curved gram variable rods
0	4+	0	0
1	3+	1+	1+ to 2+
2	2+	2+	3+ to 4+
3	1+	3+	
4	0	4+	

Table 2: Gram smear and culture findings of 100 High vaginal swabs

	Number and percentage
Normal(Gram smear findings were normal & no growth in culture)	35 (35%)
BV{ Gardnerella morphotype , gram negative curved rods, fusiforms& Lactobacilli were absent/present in low numbers(1 to 2+)}	26 (26%)
Candida	15 (15%)
Colonisers (Gram stain findings had only vaginal epithelial cells with Lactobacilli, but the culture had the growth of Group B Streptococcus, E. coli & coliforms , Enterococcus spp.)	24 (24%) Group B Streptococcus 10(10%) E. coli& coliforms 10(10%) Enterococcus spp 14(14%)

REFERENCES:

1. Morris M, Nicoll A, Simms I, et al.: Bacterial vaginosis: a public health review. BJOG 2001, 108:439-450.
2. Jacqueline M. Achkar and Bettina C. Fries .Candida Infections of the Genitourinary Tract. Clin. Microbiol. Rev 2010; 23 (2): 253-273.
3. Rao PS, Devi S, Shriyan A, Rajaram M, Jagdishchandra K. Diagnosis of bacterial vaginosis in a rural set up: comparison of clinical algorithm, smear scoring and culture by semiquantitative technique. Indian J Med Microbiol2004; 22(1):47-50.
4. Ugwumadu AH: Bacterial vaginosis in pregnancy**.** CurrOpinObstetGynecol 2002, 14:115-118
5. Udayalaxmi, GopalkrishnaBhat, SubbannayyaKotigadde, ShaliniShenoy Comparison of the Methods of Diagnosis of Bacterial Vaginosis . Journal of Clinical and Diagnostic Research. 2011 June, Vol-5(3): 498-501.
6. MacFaddin, J.F. Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Baltimore: Williams and Wilkins, 1980.
7. Mohanty S, Sood S, Kapil A, Mittal S. Interobserver variation in the interpretation of Nugent scoring method for diagnosis of bacterial vaginosis. Indian J Med Res 2010;131:88-91.
8. Verstraelen H, Verhelst R: Bacterial vaginosis: an update on diagnosis and treatment.Expert Rev Anti Infect Ther 2009, 7:1109- 1124.

MODULATION OF ANTI-INFLAMMATORY ACTIVITY OF NSAIDS IN NORMAL RATS TREATED WITH ANTIHISTAMINICS.

Nilam Nigam Sanjay Kumar Nigam, Anand Mishra.

1. Associate Professor, Department of Pharmacology, Rama Medical College Hospital & Research Centre, Mandhana, Kanpur, Uttar Pradesh.
2. Associate Professor, Department of Pathology, Rama Medical College Hospital & Research Centre,Mandhana, Kanpur, Uttar Pradesh.
3. Associate Professor, Department of Biochemistry, Rama Medical College Hospital & Research Centre,Mandhana, Kanpur, Uttar Pradesh.

CORRESPONDING AUTHOR
Dr. Nilam Nigam,
House no. 205,
Rama Medical College, Mandhana,
Kanpur, U.P -209217.
E-mail: drnilamnigam@yahoo.com,
Ph: 0091 8604560152.

ABSTRACT: NSAIDs are frequently used for relief of inflammation and Antihistaminics are indicated for simultaneous administration for allergic manifestations. Opioids analgesics have been reported to interact with Antihistaminics. To explore the interacting potentiality, in the present study the effects of combined treatment with NSAIDs and antihistaminics were examined in rats. Anti-inflammatory effect was evaluated by Carrageenan induced hind paw oedema in rats. NSAIDs like aspirin, ibuprofen and piroxicam were selected for study on per se and on concurrent administration with Antihistaminics such as Promethazine, Cetrizine and Astemizole. All NSAIDs protected animals show anti-inflammatory activity. Aspirin shows highly significant potentiation of anti-inflammatory effect at all dose level, however ibuprofen and piroxicam show highly significant anti-inflammatory effect at higher doses only and on concurrent administration of antihistaminics, aspirin, piroxicam and ibuprofen with all antihistaminics produced highly significant potentiation except ibuprofen with Cetrizine produced significant potentiation of anti-inflammatory response at higher doses only.
KEY WORDS: antihistaminics, inflammation, NSAIDs drug interaction

INTRODUCTION: Plethora of drugs available to the physicians and frequent need of several drugs for concurrent use has resulted in increased frequency of drug interaction. Use of multiple drugs concurrently, in practice of medicine, and excess of drugs available to the physicians, has resulted in increased frequency of drug interactions.

It is presumed that drug interactions comprise about 7.0% of all adverse reactions and that among the few patients who succumb to adverse drug reactions (about 4.0% of all deaths) about a third are due to interactions (1).

Genetic or environmental factors, intercurrent illness or polypharmacy might affect the response of concurrently administered drug combinations.

All NSAIDs protected animals show anti-inflammatory activity. Aspirin shows highly significant potentiation of anti-inflammatory effect at all dose level, however ibuprofen and piroxicam show highly significant anti-inflammatory effect at higher doses only and on concurrent administration of aspirin, piroxicam and ibuprofen with all antihistaminics

produced highly significant potentiation except ibuprofen with Cetrizine produced significant potentiation of anti-inflammatory response at higher doses only.

AIMS AND OBJECTIVES:

1. To explore anti-inflammatory effects of NSAIDs per se.
2. To explore the drug interacting potentiality on concurrent administration of NSAIDs with antihistaminics.

MATERIAL AND METHODS: Anti-inflammatory effect was evaluated by Carrageenan induced hind paw oedema in rats (2). This study was conducted in Albino rats of either sex with ten rats in each group weighing (100-200 gms). Rats were maintained under standard laboratory conditions in animal house of M.L.B. medical college Jhansi, U.P. India. The rodents had access to food and water. The study was permitted by institutional animal ethical committee. One group was treated with 2ml/kg distilled water served as control while other groups were treated with drugs with three dose level of each drug. All the drugs used in this study were given in suspension of 2% gum acacia orally except promethazine which were administered intramuscularly. The inflammation was induced by subcutaneous injection 0.1ml 1.0% Carrageenan suspension in the planter aponeurosis of right hind paw. The oedema resulting after 3 hours of Carrageenan injection was measured by plethysmometer (3). The hind paw oedema was noted as paw volume. The difference between the paw volume before and 3 hours after Carrageenan injection was recorded as volume of paw oedema. The drugs administered orally as suspension in 2.0 % gum acacia except prometazine one hour before Carrageenan injection. The percentage inhibition of oedema volume (anti-inflammatory effect) was calculated by the following formula:-

$(1- V_t/V_c \times 100)$

Where V_t= volume of paw oedema in treated rats

V_C = volume of paw oedema in control rats.

Statistical analysis is done by using paired t- test.

RESULTS:

Table- 1 Anti-inflammatory effect of non – opioid analgesics on Carrageenan induced oedema

S.No.	Group	No. Of animals	Dose (mg/kg)	Carrageenan induced oedema volume (ml) (Mean ± S.E.)	(% inhibition)
1	Control (distilled water)	10	2 ml	.64±.01	-
2	Aspirin	10	50	.54±.003***	16
			100	.44±.01***	32
			200	.34±.01***	47
3	ibuprofen	10	50	.60±.005*	7
			100	.56±.018**	13
			200	.48±.01***	25
4	piroxicam	10	0.29	.60±.003*	7
			0.58	.55±.09***	15
			1.16	.49±.01***	24

*P < .05 **P < .01 ***P <.001

Table-2. Anti-Inflammatory effect of Aspirin with Antihistaminics on Carrageenan induced oedema –

S.No.	Group	No. Of animals	Dose (mg/kg)	Carrageenan induced oedema volume (ml) (Mean ± S.E.)	(% inhibition)
1	Control (distilled water)	10	2 ml	0.64±0.009	-
2	Aspirin+ Cetrizine	10	50+1.60	0.57±0.008***	11
			100+3.20	0.48±0.01***	25
			200+6.40	0.36±0.01***	44
3	Aspirin+Astemizole	10	50+1	0.50±0.005***	22
			100+2	0.46±0.01***	29
			200+4	0.32±0.007***	50
4	Aspirin+Promethazine	10	50+1	0.55±0.01***	15
			100+2	0.49±0.01***	24
			200+4	0.42±0.007***	35

*P<0.05 **P<0.01 ***P<0.001

Table-3. Anti-inflammatory effect of ibuprofen with Antihistaminics on Carrageenan induced oedema –

S.No.	Group	No. Of animals	Dose (mg/kg)	Carrageenan induced oedema volume (ml) (Mean ± S.E.)	(% inhibition)
1	Control (distilled water)	10	2 ml	0.88±0.01	-
2	Ibuprofen+Cetrizine	10	50+1.60	0.74±0.01	16
			100+3.20	0.66±0.01***	25
			200+6.40	0.52±0.01***	41
3	Ibuprofen+Astemizole	10	50+1	0.72±0.006***	19
			100+2	0.55±0.01***	38
			200+4	0.45±0.01***	49
4	Ibuprofen+Promethazine	10	50+1	0.71±0.003***	20
			100+2	0.61±0.006***	31
			200+4	0.54±0.008***	39

*P<0.05 **P<0.01 ***P<0.001

Table- 4. Anti-inflammatory effect of piroxicam with on Carrageenan induced oedema –

S.No.	Group	No. Of animals	Dose (mg/kg)	Carrageenan induced oedema volume (ml) (Mean ± S.E.)	(% inhibition)
1	Control (distilled water)	10	2 ml	0.67±0.009	-
2	Piroxicam+Cetrizine	10	0.29+1.60	0.57±0.003***	15
			0.58+3.20	0.51±0.01***	24
			1.16+6.40	0.40±0.008***	41
3	Piroxicam+Astemizole	10	0.29+1	0.53±0.009***	21
			0.58+2	0.49±0.006***	27
			1.16+4	0.42±0.008***	38
4	Piroxicam+Promethazine	10	0.29+1	0.54±0.01***	20
			0.58+2	0.51±0.008***	24
			1.16+4	0.42±0.008***	38

*P<0.05 **P<0.01 ***P<0.001

RESULTS: Anti-inflammatory effect (% inhibition) of oedema volume was evaluated by Carrageenan induced oedema volume in (ml) (mean ± SE) in the control group (0.64±0.01) no % inhibition on per se administration of NSAIDs used in this study, aspirin showed highly significant % inhibition in oedema and ibuprofen and piroxicam showed significant inhibition of oedema at higher doses only (Table1.)

On concurrent administration of all NSAIDs with Cetrizine, Astemizole and Promethazine produced highly significant ($P < 0.001$) % inhibition of oedema volume except Ibuprofen with Cetrizine at low dose does not produce 0.74 ± 0.01 significant % inhibition of oedema volume. (Table-3)

Concurrent administration of aspirin with all antihistaminics produced highly significant % inhibition of oedema ($p < 0.001$) (Table-2) and concurrent administration of ibuprofen with Astemizole and promethazine produced highly significant activity ($p <0.001$) except ibuprofen with Cetrizine at low dose does not produce % inhibition of oedema (0.74 ± 0.01) (table-3). Piroxicam with Cetrizine, Astemizole and promethazine produced highly significant effect ($p <0.001$). Interaction of piroxicam with Cetrizine, Astemizole and promethazine used in this study was evaluated by % inhibition of oedema volume at 3 dose levels as compared to control in table-4.

DISCUSSION: Enhanced toxicity or decreased therapeutic effects resulting from administration of drug combinations have recently gained tremendous importance in therapeutic practice. Drug interaction between opioid analgesics and phenothiazine, monoamine oxidase inhibitors and tricyclic antidepressants is well established (4).

Superior analgesic and comparable anti-inflammatory property with gastrointestinal sparing effect with zinc –naproxen complex when impaired with naproxen alone (5).

Both Phenylybutazone and Aspirin enhanced Glibenclamide induced hypoglycaemia (6). (Diwan et al 1992) showed potentiation of hypoglycaemic response of Glibenclamide by Piroxicam (a newer NSAIDs) in rats, human volunteers and diabetics (7).

Potent anti-inflammatory activity of μ and kappa agonists and identification of selectin as important molecules governing the homing of opioid cells to injured tissue (8). Some opioid analgesics have anti-inflammatory activity but little information is availed about NSAIDs and antihistaminic interaction.

In this study per se administration of NSAIDs aspirin shows highly significant % inhibition of oedema. Ibuprofen and Piroxicam showed significant inhibition of oedema at higher doses only. On concurrent administration of all NSAIDs with H1 blockers produced highly significant ($P < 0.001$) % inhibition of oedema except ibuprofen with Cetrizine which produced significant effect at higher doses only.

All NSIADs have protected animals effectively although aspirin was most potent which confirm the possessions of NSAIDs anti-inflammatory activity by these drugs. On per se administration of NSAIDs in which aspirin produced highly significant anti-inflammatory activity and ibuprofen and piroxicam produce significant anti-inflammatory activity only at higher dose level and with concurrent administration of antihistaminics, all NSAIDs produce highly significant anti-inflammatory activity. However ibuprofen with Cetrizine produces highly significant anti-inflammatory activity at higher doses only.

In view of these observations it can be safely concluded that antihistaminics possess interaction potentiality with NSAIDs. So caution should be taken in use of these drugs on concurrent administration in clinical practice.

BIBLIOGRAPHY:

1. Graham-smith, D.G. and Aronson, J.K.: Drug interaction. In clinical textbook of clinical pharmacology and drug therapy: Oxford University press, oxford, 1984, 638.
2. Winter, C.A.; Risley, E.A. and Nuss, G.W.: Carrageenane induced oedema in hind paw of rat as an assay for anti inflammatory drugs. Proc. Soc. Exp. Biol. Med. 1962, 111, 544-547.
3. BUTTLE GA, D'ARCY PF, HOWARD EM, KELLETT DN. Plethysmometric measurement of swelling in the feet of small laboratory animals. Nature. 1957 Mar 23;179(4560):629.
4. Reisine, T. And Pasternak, G. : opioid analgesics and antagonists: in Goodman and Gilman's the pharmacolocal basis of therapeutics, Ed. P.B. Molinoff, R.W. Ruddon and A.G. Gillman, Mc Graw – Hill, Newyork, 1996, 521-555,.
5. Jain N.K., Singh, A. And Kulkarni, S.k.: comparative analgesic, anti-inflammatory and ulcerogenic study of Zinc Naproxone Vs Naproxone. Ind.J.Pharamacol.2000 32 (1) :53.
6. Sharma, V.V.; Srivastava V.K.; Kulshestha, V.K,; and Prasad, D.N. : interaction of anti-inflammatory agents with glebenclamide in rabbits. Ind.J.Pharmac.1981, 13(2)207-210.
7. Diwan PV, Sastry MS, Satyanarayana NV Potentiation of hypoglycemic response of glibenclamide by piroxicam in rats and humans Indian J Exp Biol. 1992 Apr;30(4):317-9.
8. Stein C, Machelska H, Schäfer M Peripheral analgesic and antiinflammatory effects of opioids. Z Rheumatol. 2001 Dec;60(6):416-24.

ASSESSMENT OF RISK FACTORS OF HYPERTENSION: A CROSS-SECTIONAL STUDY

Makwana Naresh, Shah Viral, Khambhati Sudham, Choudhary Mahesh, Goswami Kalpesh, Yadav Sudha

1. Associate Professor, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat
2. Assistant Professor, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat
3. Medical Officer, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat
4. Resident, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat
5. Resident, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat
6. Professor & Head, Department of Community Medicine, Shri M P Shah Medical College, Jamnagar, Gujarat

CORRESPONDING AUTHOR

Dr. Mahesh Choudhary, Resident,
Department of Community Medicine,
Shri M P Shah Medical College, Jamnagar,
E-mail: drmahesh2711@gmail.com,
Ph: 0091 9016098640.

ABSTRACT: BACKGROUND: Context: The epidemiology of hypertension, in terms of its importance as a risk factor for cardiovascular diseases, continues to be major area of research. **Aims:** The aim of this study is to assess the prevalence of various risk factors on hypertension among adult population (20-60 years) in the rural areas. **MATERIAL AND METHODOLOGY:** The present study was a community based cross-sectional study. The study was carried out in three villages of the field-practice area of Rural Health Training Centre of Community Medicine Department, M. P. Shah Medical College, Jamnagar during the months of September to November 2011. A total of 250 adult study subjects were included by simple sampling random technique in the present study. Pre-designed, pre-tested schedule was used to collect data regarding demographic characteristics and different risk factors. Percentages, chi-square test and P-value were calculated using Epi Info software. **RESULTS:** Out of 250 study subjects, 25.60% were male while 74.40% were females. Majority of the subjects belonged to 30 to 39 years of age. Overall magnitude of hypertension was found to be 15.6%. Addiction to any form of tobacco was found among 28.40% of the study participants and out of those, almost 50% were hypertensives. 29.82% of the over-weight individuals were also found hypertensives. **CONCLUSIONS:** The prevalence of hypertension was found to be 15.6% in the rural areas in the present study, which needs attention because in current trend of migration from rural to urban area it may cause harm as a hidden disease and may give impact on other non-communicable diseases.

KEY-WORDS: Hypertension, risk factors, rural area

INTRODUCTION: Hypertension is reported to be the fourth contributor to premature death in developed countries and the seventh in developing countries.[1] Reports indicate that nearly 1 billion adults (more than a quarter of the world's population) had hypertension in 2000, and this is predicted to increase to 1.56 billion by 2025.[2] Earlier reports also suggest that the prevalence of hypertension is rapidly increasing in developing countries[3, 4] and is one of the leading causes of death and disability in developing countries. The epidemiology of hypertension, in terms of its importance as a risk factor for cardiovascular diseases, continues to be major area of research. India is a vast country with a heterogeneous and young population.

In the past, the control and prevention of communicable diseases were emphasized, but, recently, attention has shifted to the control and prevention of non-communicable diseases, including stroke, hypertension, and coronary artery disease at the national level in view of the rising incidence of these diseases.[5] Blood pressure (BP) is directly associated with risks of several types of cardiovascular disease, and the associations of BP with disease risk are continuous, indicating that large proportions of most populations have non-optimal BP values. Data on Hypertension in the rural areas are again limited, so that present study was conducted to assess the prevalence of various risk factors on hypertension among adult population (20-60 years) in the rural areas.

SUBJECTS AND METHODS: The present study was a community based cross-sectional study. The study was carried out in the field practice area of Rural Health Training Centre (RHTC) of Community Medicine Department, M. P. Shah Medical College, Jamnagar i.e. Alia village, during the months of September to November 2011. Total 250 study subjects in the age group of 20 – 60 years were included by simple random sampling technique in the present study. Oral consent was obtained from the participant prior to enroll in the study. Pre-designed, pre-tested proforma was used to collect data regarding demographic characteristics and different risk factors i.e. smoking, alcoholism etc. through house to house visits.

Blood pressure was recorded in the sitting position in the right arm to the nearest 2mmHg using the mercury sphygmomanometer. Two readings were taken 5 minutes apart and mean of two was taken as the blood pressure.[6] Hypertension was diagnosed based on drug treatment for hypertension or if the blood pressure was greater than 140/90 mmHg – Joint National Committee 7 (JNC VII) Criteria.[7] Anthropometric measurements including weight, height, waist and hip measurements were obtained using standardized techniques as given below. Height was measured with a tape to the nearest cm. Subjects were requested to stand upright without shoes with their back against the wall, heels together and eyes directed forward. Weight was measured with a traditional spring balance that was kept on a firm horizontal surface. Subjects were asked to wear light clothing and weight was recorded to the nearest 0.5 kg. Body mass index (BMI) was calculated using the formula: weight (Kg)/height $(m)^2$.[6] BMI of equal to or more than 25 was regarded as Overweight and lesser than 25 was considered as non-overweight. Percentages, chi-square test and P-value were calculated using Epi Info software.

RESULTS: Figure – 1 indicates that risk factors for hypertension were found in orders of overweight (22.80%), tobacco chewing (14.80%) and smoking (8.40%) in the study subjects.

Table – 1 shows that majority (72%) of the study subjects were in the age group of 20 – 39 years and 6.8% were in the age group of 50 – 60 years. Sex-wise distribution of study subjects shows over representation of females, which may be due to collection of data in day time when majority of male members of the family were not present in their houses. More than fifty percent were found to be illiterate. Out of the total, 66.80% of the participants were housewives followed by farmers (11.20%). Married study subjects were 87.2%, whereas 10.8% and 2% were unmarried and widow respectively.

Table – 2 shows association of non-modifiable risk factors with hypertension. Out of the total 250 study subject, 39 (15.6%) were found to be hypertensive. Significant statistical association was observed between age and hypertension. As age increases, the chance of becoming hypertensive rises. Sex-wise distribution shows that though hypertension was found

more among females, statistical test fails to prove this association ($p>0.05$). Positive family history was strongly related with hypertension ($p=0.0002$).

Table – 3 shows various modifiable risk factors associated with hypertension. 28.40% of the study subjects were addicted to tobacco in any form, whereas 71.60% subjects were non-addicted. Almost half (49.30%) of addicted subjects were hypertensive and 2.23% of non-addicted individuals were hypertensive, this difference is statistically significant ($p<0.05$). No significant association was found among various forms of addiction (smoking, tobacco chewing, snuffing viz.) and hypertension ($p>0.05$), depicts that any form of addiction is associated with hypertension. Hypertension was found almost double among married persons (16.51%) than unmarried and widow (9.38%), but the association was not statistically significant. 17.29% Illiterates was found hypertensives. Hypertension was almost equally distributed among different occupations in the range of 15 to 22%. While looking at body mass index, 17 (29.82%) overweight individuals were found hypertensive and the association between over-weight and hypertension was statistically significant ($p=0.0016$). Associated co-morbid conditions such as diabetes mellitus and coronary heart disease were also found among hypertensives.

DISCUSSION: In the present study the overall prevalence of hypertension was found to be 15.6%. Similar findings have also been reported in other studies. Comparable prevalence (15%) was found in the study conducted at squatter settlement of Karachi (Pakistan).[8] Similar prevalence of hypertension (16.9%) has also been reported in the study conducted among labour population of Gujarat.[9] A higher prevalence (20.6%) was reported in the study conducted among adult population at rural Wardha. [10] Prevalence of 23% was reported by Cielito C. in rural areas of Philippines.[11] The WHO estimates the prevalence of HTN at 20% among adult populations in several countries.[12] However a study among tribal "Oraon" population of Orissa revealed lower prevalence of hypertension (4.6/1000 population).[13] Similar finding (prevalence 5.8%) was also noted by Chadha SL et al[14] among Gujaratis residing in Delhi and prevalence of 7.8% was reported in hospital patients, Mumbai.[15] Differential rates are due to different cut-off points in determining the level of hypertension and also to the differing age groups constituting the study population.

The prevalence of hypertension rises with the advancing age i.e. it was maximum (52.94%) in the age group of 50 – 60 years (table – 2), while minimum i.e. 3.45% in the age group of 20 – 29 years. Strong statistical association was found between the age group and hypertension ($p=0.0001$). Age increase prevalence of hypertension was also reported by Todkar SS[16], Reddy SS.[17] Similar observations were found in other studies.[18,19,20] This increase in age incidence of hypertension can be explained by changes in the lifestyle, migration, stress, atherosclerotic changes in the blood vessels that happen with the age and certain genetic and environmental factors.

Addiction to any form of tobacco was found to be 28.40% in the study subjects. Prevalence of hypertension was higher among addicted in comparison with non-addicted (49.30% vs 2.23%). This difference is statistically significant. There is a plethora of studies suggesting the tobacco consumption as an important and independent risk factor for hypertension and cardiovascular diseases.[9] A positive association was observed between body mass index and development of hypertension. Persons having BMI more than or equal to 25 reported with higher risk of hypertension. The similar findings were reported by number of epidemiological studies e.g. Todkar SS et al.[16] 2009, Das et al.[21] 2005, Reddy SS and Prabhu GR[17] 2005. Associated co-morbid conditions such as Diabetes and Coronary heart disease

(CHD) were observed among hypertensives. This findings were supported by Reddy SS and Prabhu GR[17] 2005. Education, Occupation and Marital status has not been significantly associated with hypertensives as observed in the current study (p>0.05).

Thus to summarize, this study reveals that the magnitude of hypertension in the rural population is comparable to the magnitude found in the other Indian studies.

CONCLUSION: The overall prevalence of hypertension in the present study was is 15.6%. Significant association has been noted between hypertension, age and body mass index. Other modifiable risk factors were associated with hypertension but the statistical association was not found.

Limitations of the study:

1. It is likely that a systematic and larger study may give better understanding of the prevalence and the underlying risk factors among these populations.
2. Over representation of female in the current study dilutes picture of various risk factors on hypertension.

Figure 1 Distribution of participants according to risk factors of hypertension

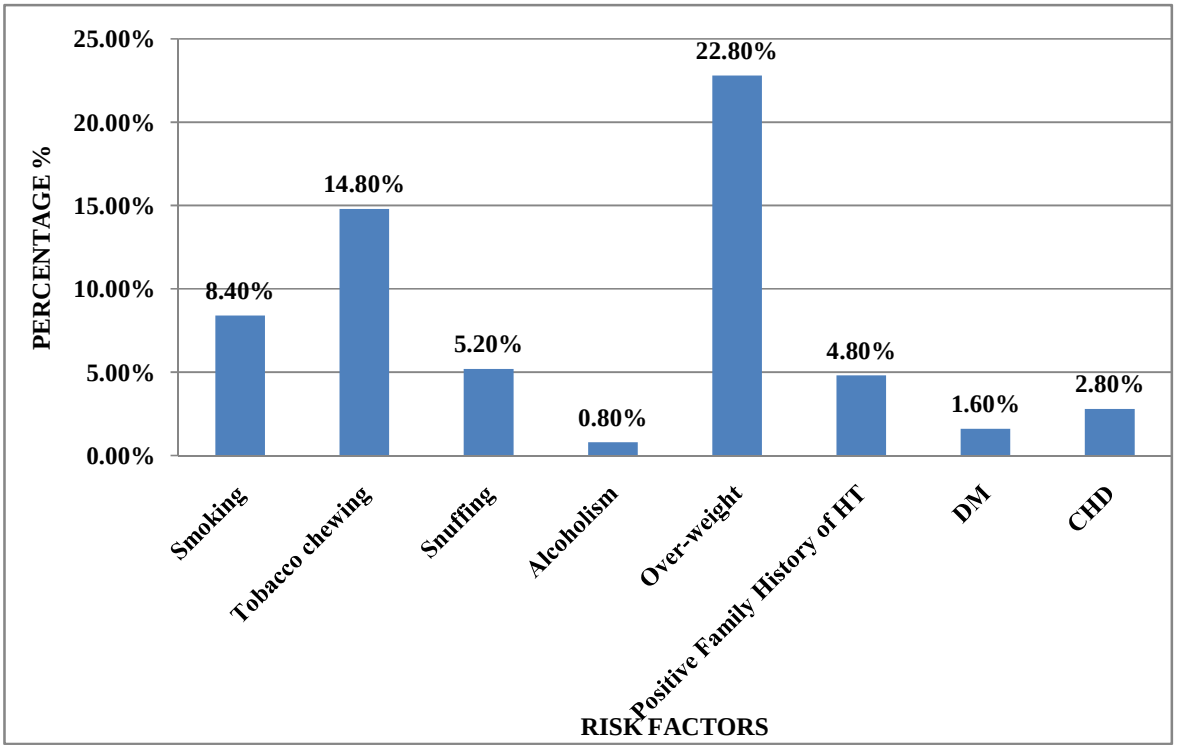


Table: 1 Demographic profile of study participants

	Number (N=250)	Percentage
AGE GROUP		
20-29	87	34.8%
30-39	93	37.2%
40-49	53	21.2%
50-60	17	6.8%
SEX		
Male	64	25.6%
Female	186	74.4%
EDUCATION		
Illiterate	133	53.2%
Primary	78	31.2%
Secondary	32	12.8%
Higher Secondary	4	1.6%
Graduate	3	1.2%
OCCUPATION		
Service	9	3.6%
Business	11	4.4%
Farmers	28	11.2%
Labourers	23	9.2%
Housewife	167	66.8%
Unemployed	12	4.8%
MARITAL STATUS		
Married	218	87.2%
Unmarried	27	10.8%
Widow	5	2%

Table: 2 Non-modifiable risk factors associated with hypertension

Risk Factors	Non-hypertensive (n=211)		Hypertensive (n=39)		TOTAL (N=250)		chisquare value	p value
	No.	%	No.	%	No.	%		
Age								
20-29	84	96.55	3	3.45	87	34.80	34.769	0.0001
30-39	81	87.10	12	12.90	93	37.20		
40-49	38	71.70	15	28.30	53	21.20		
50-60	8	47.06	9	52.94	17	6.80		
Sex								
Male	50	78.13	14	21.88	64	25.60	1.972	0.1602
Female	161	86.56	25	13.44	186	74.40		
Positive family history	5	41.67	7	58.33	12	4.80	14.240*	0.0002

*=chisquare value is calculated between Positive Family History and No positive Family History among hypertensives and Non-hypertensives

Table: 3 Modifiable risk factors affecting hypertension

Risk Factors	Non-hypertensive (n=211)		Hypertensive (n=39)		TOTAL (N=250)		chisquare value	p value
	No.	%	No.	%	No.	%		
Addiction								
Smoking	10	47.62	11	52.38	21	8.40	0.354	0.8379
Tobacco chewing	20	54.05	17	45.95	37	14.80		
Snuffing	6	46.15	7	53.85	13	5.20		
Non Addicted	175	97.77	4	2.23	179	71.60	81.975 *	0.0001
Marital status								
Married	182	83.49	36	16.51	218	87.20	0.606	0.4363
Unmarried/widow	29	90.63	3	9.38	32	12.80		
Education								
Illiterate	110	82.71	23	17.29	133	53.20	0.375	0.5405
Literate	101	86.32	16	13.68	117	46.80		
Occupation								
Service	7	77.78	2	22.22	9	3.60	1.019	0.9069
Business	9	81.82	2	18.18	11	4.40		
Farmer	23	82.14	5	17.86	28	11.20		
Labourer	18	78.26	5	21.74	23	9.20		
Housewife	142	85.03	25	14.97	167	66.80		
Body Mass Index								
Over-weight	40	70.18	17	29.82	57	22.80	9.99	0.0016
Non-obese	171	88.60	22	11.40	193	77.20		
Associated co-morbid condition								
DM	0	0.00	4	100.00	4	1.60		
CHD	0	0.00	7	100.00	7	2.80		

*=chi-square value is calculated between addicted and non-addicted.

REFERENCES:

1. Deepa R, Shanthirani CS, Pradeepa R, Mohan V. Is the 'rule of halves' in hypertension still valid?--Evidence from the Chennai Urban Population Study. J Assoc Physicians India 2003;51 153-7.
2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005; 365: 217-23.
3. Reddy KS. Hypertension control in developing countries. Genetic issues. J Hum Hypertens 1996; 10:S33-8.
4. Nissinen A, Bothig S, Granroth H, Lopez AD. Hypertension in developing countries. World Health Stat Q 1988;41:141-54.

5. Shyamal Kumar Das, Kalyan Sanyal, and Arindam Basu. Study of urban community survey in India: growing trend of high prevalence of hypertension in a developing country. Int J Med Sci. 2005;2(2):70–78.
6 Mohan V, Deepa M, Farooq S, Datta M, Deepa R. Prevalence, awareness and control of hypertension in Chennai – the Chennai Urban Rural Epidemiology Study (CURES-52). J Assoc Physicians India 2007;55:326-32.
7 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7). JAMA 2003;289:2560–71.
8 Hemna Siddiqui, Qudsia Anjum, Amir Omair, Jawed Usman, Raza Rizvi, Tabinda Ashfaq. Risk factors assessment for hypertension in a squatter settlement of Karachi. J Pak Med Assoc 2005;55:390-2
9 Tiwari RR. Hypertension and epidemiological factors among tribal labour population in Gujarat. Indian Journal of Public Health 2008; 52(3):144-146
10 Deshmukh PR, Gupta SS, Dongre AR, Bharambe MS, Maliye C, Kaur S, Garg BS. Relationship of anthropometric indicators with blood pressure levels in rural Wardha. Indian J Med Res2006; 123: 657-664.
11 Cielito C. Reyes-Gibby and Lu Ann Aday. Prevalence of and Risk Factors for Hypertension in a Rural Area of the Philippines. J Community Health 2000;25:389-99.
12 Hypertension Control Report of a WHO Expert Committee. Geneva: World Health Organization, 1996.
13 Dash SC, Sundaram KR, Swain PK. Blood pressure profile, urinary sodium and body weight in the 'Oraon' rural and urban tribal community. J Assoc Physicians India. 1994; 42: 878-80.
14 Chadha SL, Gopinath N, Ramachandran K. Epidemiological study of coronary heart disease in Gujaratis in Delhi (India). Ind J Med Res 1992, 96:115-121.
15 Joshi SV, Patel JC, Dhar HL. Prevalence of hypertension in Mumbai. Indian J Med Sci 2000;54:380-3
16 Todkar SS, Gujarathi VV, Tapare VS. Period prevalence and sociodemographic factors of hypertension in rural Maharashtra: A cross-sectional study. Indian J Community Med 2009;34:183-7
17 Reddy SS and Prabhu GR. Prevalence and risk factors of hypertension in Adults in an urban slum, Tirupati, A.P. Indian J Community Med 2005; 30: 84-6.
18 Chadha SL, Radhkrishnan S, Ramachandran V, Kaul U and Gopinath N. Prevalence, awareness and treatment status of hypertension in urban populations of Delhi. Ind J Med Res 1990; 92:233-40.
19 Bhasin SK, Chaturvedi S, Gupta P and Aggarwal P. Status of physical exercise and its association with obesity and hypertension in two urban assembly constituencies of East Delhi. Journal of Indian Medical Association 2001; 99:631-33.
20 Kalavathy MC, Thankappan KR, Sharma PS and Vasan RS. Prevalence, awareness, treatment and control of hypertension in an elderly community based sample in Kerala, India. National Medical Journal of India 2000; 13:9-15.
21 Das K, Sanyal K, Basu A. A study of urban community survey in India; Growing trend of high prevalence of hypertension in individuals. International Jr of Med Sciences 2005;2:70-8.

FLUCONAZOLE SUSCEPTIBILITY TESTING OF CANDIDA SPECIES BY DISC DIFFUSION AND AGAR DILUTION METHOD

Supriya Tankhiwale, Sunita Gajbhiye, Rajaram Powar

1. Associate Professor, Department of Microbiology, Government Medical College & Hospital, Nagpur, Maharashtra.
2. Assistant Professor, Department of Microbiology, Government Medical College & Hospital, Nagpur, Maharashtra,
3. Professor, Department of Microbiology, Government Medical College & Hospital, Nagpur, Maharashtra.

CORRESPONDING AUTHOR
Dr. Sunita Gajbhiye
OASIS, plot no 11, Anand Nagar, Atrey layout,
Nagpur-440022, Maharashtra, India
E-mail: sunitarajgaj@yahoo.com,
Ph: 0091 9325339668.

ABSTRACT: PURPOSE: Fungal infections with candida species are an important cause of morbidity and mortality[1]. Situation is further worsened by increasing resistance to antifungal drugs [2].The objective of this study was to evaluate antifungal susceptibility pattern of Candida species to fluconazole by disc diffusion and agar dilution method and compare these two methods as far as fluconazole is concerned. **MATERIAL & METHODS:** Between January 2006 to December 2006, 119 Candida species were isolated from 225 sputum samples of patients of pulmonary tuberculosis and immunocomprised patients. **RESULTS:** Of the 119 Candida isolates 33 (27.73%) were Candida albicans, 32 (26.89%) were Candida gullermondi, 27 (22.68%) Candida tropicalis, 14 (11.76%) Candida pseudotropicalis, 7 (5.88%) Candida parapsilosis and 6 (5.04%) were Candida krusei. The Candida isolates were tested for fluconazole by disc diffusion method on Muller hinton agar with 2% glucose and 0.5 microgram of methylene blue [3].Amongst the 119 Candida isolates, 17 (14.28%) isolates were resistant to fluconazole by disc diffusion method. MIC done by agar dilution method using CLSI guidelines [4]. Candida isolates showed growth of 15 (88 .23%) Candida strains upto 8 microgram / ml with 80% inhibition of growth whereas 2 (1.7%) isolates showed MIC upto 64 microgram/ ml. **CONCLUSIONS:** Candida albicans though a common species other species were isolated in significant number. The results of disc diffusion for fluconazole do not correlate with agar dilution method. To cater the need of resource constrained laboratories, it is important to have results that correspond to the clinical outcome of antifungal treatment and show co-relation to those obtained with the reference method as recommended by CLSI guidelines and also be reproducible[5]. Further critical studies are needed.

KEYWORDS: Candida, Fluconazole, MIC, Agar dilution method.

INTRODUCTION: Fungal infections have dramatically increased in recent years along with the increase in drug resistant isolates [1]. The last few decades have seen a steady increase in the incidence of fungal infections especially Candida species. Prolonged antibiotic therapy, invasive therapeutic procedures, radiotherapy and AIDS pandemic have all contributed to the rise in these infections. Other causes include recipients of solid organ transplant and cytotoxic chemotherapy[3] .Candida species are the most common pathogens as far as fungal isolates are concerned.

Candidiasis is the most common fungal disease with a wide spectrum of clinical presentation .The infection may be acute or chronic, superficial or deep involving various organs[3]. Since the Candida species are normal commensals of human skin, throat, gastro intestinal tract, female genital tract and urine of patients with indwelling catheters, its pathogenic role is to be confirmed by repeated isolation. The present study is undertaken with following objectives,

a) To study the antifungal sensitivity pattern of Candida isolates using disc diffusion method
b) To study the MIC of fluconazole by using agar dilution method

MATERIALS AND METHODS:- Sputum samples from patients with chronic lung diseases with or without immunocompromised state were collected. Patients with upper respiratory tract infection and also in patients where Candida species were not isolated for more than two times were excluded from the study. Early morning first sputum sample was collected after thorough rinsing of mouth in sterile wide mouth container and transported immediately to the microbiology laboratory. Sputum samples were processed for

a) Gram stain
b) KOH preparation
c) Culture on Sabarauds Dextrose Agar(SDA)

The Sputum samples showing pseudohyphae, large number of budding yeast cells were included in the study. Culture on SDA showing more than 25-30 colonies or confluent heavy growth of Candida was considered as pathogenic. A second sputum sample was collected for confirmation and re-isolation. The Candida isolates were subjected to germ tube test and cultured on corn meal agar. Depending on chlamydospore formation, the species were identified. Confirmation of the species was done by sugar assimilation test[6]**.** Antifungal susceptibility testing by disc diffusion method for fluconazole was done using fluconazole disc from Hi-media laboratories on Muller-Hinton agar with 2% glucose and 0.5 microgram methylene blue**.** Other drugs tested were- Ketoconazole, Clotrimazole, Nystatin and Amphotericin– B. The standard strain used as control was Candida albicans ATCC 90028 and the zone sizes[7] were noted as follows:

- Candida albicans - 28.8mm ±6.5mm
- Candida tropicalis- 27mm±8mm
- Candida gullermondi-24.7mm±7.2mm
- Candida parapsilosis-32.8mm±7.4mm
- Candida kruzei-18mm±8.5mm

The minimum inhibitory concentration for fluconazole was done by agar dilution method as per the CLSI guidelines. The MIC reading was defined as the lowest concentration inhibiting at least 50% of the growth . The isolates tested for fluconazole showing MIC of

- 8 microgram / ml were classified as susceptible,
- Those with MIC more than 64microgram / ml were labeled as resistant and
- The isolates with MIC between 16 – 32 were termed as susceptible dose dependent.[7]

OBSERVATIONS

TABLE NO. I n=119

S. NO.	CANDIDA SPECIES	NO.	%
1	C. albicans	33	27.73
2	C. gullermondi	32	26.89
3	C. tropicalis	27	22.68
4	C. pseudotropicalis	14	11.76
5	C. parapsilosis	07	5.88
6	C. krusei	06	5.04

Of the 225 sputum samples received, 119 candida species were isolated. Candida albicans was the most commonly isolated species followed by Candida gullermondi and Candida tropicalis

TABLE NO.2

Sensitivity pattern of Candida species to fluconazole by disc diffusion method (n= 119)

S.no	Candida species	Sensitive	Resistant
1	C. albicans (n=33)	31	02
2	C. gullermondi (n= 32)	29	03
3	C. tropicalis (n=27)	26	01
4	C . pseudotropicalis (n= 14)	11	03
5	C. parapsilosis (n=7)	05	02
6	C. krusei (n=6)	00	06
	Total	102	17

From the above table it is evident that maximum sensitivity to fluconazole was seen with Candida albicans. Non – albicans Candida species also showed a similar pattern of sensitivity with fluconazole. Candida krusei is inherently resistant to fluconazole, hence all Candida krusei isolates showed resistance to the drug .

TABLE NO.3
MIC of Candida species to fluconazole by agar dilution method

s. no	Candida species (n=17)	Minimum Inhibitory Concentration			
		8	16	32	64
1	C. albicans (n=2)	01			01
2	C . gullermondi (n=3)	02			01
3	C. tropicalis (n=1)	01			
4	C. pseudotropicalis (n=3)	03			
5	C. parapsilosis (n=2)	02			
6	C. krusei (n=6)	06			
	Total	15			02

MIC was done for fluconazole for all species of Candida . All the strains which were sensitive by disc diffusion method were also sensitive by agar dilution method. their MIC ranged from 0-8 microgram / ml .

TABLE NO.5
Comparison between disc diffusion method and agar dilution method for fluconazole susceptibility of candida isolates

Agar dilution method	Disc diffusion method		Total
	Sensitive	Resistant	
Sensitive	102	15	117
Resistant	0	02	02
Total	102	17	119

Results of fluconazole resistance differ in disc diffusion method and agar dilution method. Their sensitivity matches with each other.

Positive predictive value (ppv) of **sensitive test** by disc diffusion method = 100%
Positive predictive value (ppv) of **resistant test** by disc diffusion method = 11.76%

DISCUSSION: The present study was carried out to determine the in- vitro susceptibility pattern of Candida isolates to fluconazole by disc diffusion method and MIC by agar dilution method .out of 225 sputum samples, 119 Candida species were isolated .These isolates were tested for

fluconazole susceptibility using fluconazole disc on Mueller Hinton agar with 2% glucose and 0.5 microgram methylene blue[6] . In this study, Candida albicans though a common isolate, other non albicans species were isolated in significant number .The findings are comparable with a study by Khan .S. et al 2006. Fluconazole susceptibility testing using disc diffusion method showed 17 (14.28 %) strains to be resistant to the drug, whereas MIC of these strains by agar dilution method showed only 2 (1.7%) strains to be resistant . This is comparable with a study by Leibowitz L D[8] et al and Rex JH, Rinaldi MG et al.

The co- relation between these two methods is not comparable. Statistical analysis with respect to sensitivity showed that results of disc diffusion and agar dilution match with each other, however the results of resistance differ. To cater the need of resource constrained laboratories, it is important to have an antifungal susceptibility testing method that gives results which correspond to clinical outcome of antifungal treatment, show co- relation with reference method and be reproducible. The major sources of susceptibility test variation for fluconazole and other azoles in vitro have been reported to be pH, the composition of the test medium, inoculums size, temperature and duration of incubation. In addition, partial inhibition of fungal growth in vitro often takes place over a range of fluconazole concentration which can make end point determinations both difficult and subjective[9].

Further critical studies with large sample size needed.

REFERENCES:

1. Jiun-Ling Wang, Shan-Chwen Chang, Po-Ren Hsuch, Yee-Chun Chen. In vitro antifungal susceptibility testing of candida blood isolates and evaluation of e- method . J. micro. Immunol. Inf . 2004 ; 37; 335-342.
2. Rex JH, Rinaldi MG, Pfaller MA. Resistance of Candida species to fluconazole. Antimicrob. Agents Chemother 1995 ;39:1-8.
3. Lee SC, Fung CP, Lee N, See LC, Huang JS, Tsai CJ. Fluconazole disc diffusion test with methylene blue and glucose enriched Muller-Hinton agar for determining susceptibility of Candida species. J Clin Microbiol 2001;39:1615-7.
4. National Committee for Clinical Laboratory Standards. Method for antifungal disc diffusion susceptibility testing of yeast; proposed guideline. NCCLS document M44-P, Wayne PA: National committee for Clinical Laboratory Standards Press; 2003.
5. Khan S, Singhal S, Mathur T, Upadhyay DJ, Rattan A. Antifungal susceptibility testing method for resource constrained laboratories. IJMM 2006;Vol.24;No.3:171-176.
6. L.J.R Milne in Fungi in Mackie and McCartney's Practical Medical Microbiology 14th edition, J.Gerald Collee, Marmion BP, Editors Churchill Livingston 699-700.
7. Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA. Antifungal susceptibility testing: Practical aspects and current challenges. Clin Microbiol Rev 2001;14:643-58.
8. Leibowitz LD, Ashbee HR, Evans EG, Chong Y, Mallatova N, Zaidi M. Global Antifungal Surveillance Group. A two year global evaluation of Candida species to fluconazole disc diffusion. Diagn. Microbiol Inf. Dis. 2001;40:27-33
9. MA Pfaller, B Dupont, GS Kobayashi, J Muller, MG Renaldi, A Espinel-Ingroff, S Shadomy, PF Troke, TJ Walsh and DW Warnock. Standardized testing of fluconazole: an International Collaborative Study. Antimicrobial Agents and Chemotherapy 1992, 36(9), 1805-1809.

OSTEOPETROSIS – A CHALLENGE IN RARE SITUATION.

Jyoti V Kulkarni, Rashmi Bengali, Suhas Jewalikar, Anil Joshi

1. Assistant Professor, MD, Department of Anaesthesiology, Government Medical College, Aurangabad,
2. Assistant Professor, MD, Department of Anaesthesiology, Government Medical College, Aurangabad,
3. Associate Professor, MD, Department of Anaesthesiology, Government Medical College, Aurangabad.
4. Assistant Professor, MD, Department of Medicine, Government Medical College, Aurangabad.

CORRESPONDING AUTHOR
Jyoti Vasantrao Kulkarni,
7, Bharat Nagar, Opp. Indraprastha Enclave,
Jyoti nagar, Aurangabad, Maharashtra, Pin 431005,
E-mail: jyotianil.joshi71@gmail.Com,
Ph: 0091 240 2337639, 0091 9822769648, 0091 8275938053.

ABSTRACT: BACKGROUND: Osteopetrosis is a collective term used for a pathological condition with defective function of osteoclasts presented with range of sclerosing bone diseases along with skeletal, renal, haematological & neurological manifestation. It may be autosomal recessive (ARO), autosomal dominant (ADO) and X-linked. It presents with hydrocephalus, short stature and anaemia, involvement of ocular nerve or facial nerve. Hypocalcemia, tetany, seizures & secondary hypoparathyroidism is known to occur. **CASE REPORT:** Two patients of osteopetrosis were posted for orthopaedic surgery. Both patients were operated under combined spinal epidural anaesthesia. 2ml of 0.5%Bupivacaine+30mcg clonidine was given intrathecally & epidural supplementation was given with Bupivacaine 3 cc increments after two segment regression of sensory level. Postoperative analgesia was provided by epidural Bupivacaine 0.125% along with Inj. Tramadol 50 mg on patient's request. **CONCLUSION:** Even if administration of anesthesia is a challenge in patients of Osteopetrosis, regional anaesthesia can be given safely with proper preoperative preparation & intraoperative care.

KEY WORDS: osteopetrosis, femur fracture, combined spinal & epidural anaesthesia

INTRODUCTION: Osteopetrosis is a collective term used for a pathological condition with defective function of osteoclasts presented with range of sclerosing bone diseases along with skeletal, renal, haematological & neurological manifestation. It is classified as Infantile malignant or (ARO), Intermediate type or (ARO) , Adult onset or (ADO)&X-linked Osteopetrosis.

ARO is a life threatening condition manifests in first few months with life span of 6 to 10 yrs present with seizures with normal Calcium levels, renal tubular acidosis, cerebral calcification, developmental delay, hypotonia, retinal atrophy & sensorineural deafness. ADO is type I and type II. Type I is associated with reduced number & size of osteoclasts & involvement of ocular nerve while type II is associated with proliferation of large & multinucleated osteoclasts, involvement of facial nerve , bony sclerosis, renal tubular acidosis & cerebral calcification. In X-linked Osteopetrosis severe immunodeficiency is observed with ectodermal changes.

The difficulties faced by Anaesthetists are difficult intubation due to facial deformities, head & mandibular involvement cervicomedullery stenosis may lead to cord trauma during intubation difficult spinal & epidural anaesthesia due to scoliosis and short stature. Leucoerythroblastic anaemia, pancytopenia, thrombocytopenia leading to excessive bleeding

during airway instrumentation or surgery. Hepatospleenomegaly is seen due to secondary expansion of extramedullary haematopoiesis. Decreased myocardial contractility, renal tubular acidosis and mental retardation might be seen in these patients[8]. These patients are having high risk of developing hypocalcemia, tetany, seizures & secondary hypoparathyroidism. Bone marrow suppression with inhibition of medullary haematopoiesis resulting in pancytopenia. Here we want to discuss anaesthetic management of two patients of benign adult onset osteopetrosis posted for orthopaedic surgery.

CASE REPORT: Two patients of osteopetrosis were posted for open reduction & internal fixation of fracture of femur. A 22 yrs male had # left sided shaft femur while a 47 years male had right sided # subtrochantric femur. First patient had previous fracture of tibia whereas another 47yrs male had # subtrochantric femur right. Both patients were short statured height 139 cm & 135cm respectively. Both had frontal bossing, protruding eyes, facial puffiness, small sized head, short neck & inadequate mouth opening. Also both had Pigeon shaped chest, short hands &wide thumb.

Patients were investigated in detail. All blood investigations were normal except in first patient's haemoglobin was 8.2 gm% &borderline cardiomegaly on X-ray chest. Radiographs of long bones revealed diffuse sclerosis with obliterated modularly cavity. X-ray spine was suggestive of mild lumber scoliosis. The fundus examination of right eye revealed partial optic atrophy with gradual diminution of vision since 6-7 yrs. Audiometry indicated left ear deafness. He had large tongue, high arched palate & irregular dentition. His mouth opening was 3.5 cm & mentohyoid distance was 5.5 cm. In second patient micrognathia, adequate mouth opening along with mentohyoid distance 5cm was observed. In second case family history of osteopetrosis was known with death of younger sister.

Both the patients were given oral antacids Tab Pantoprazol 40mg and inj. Taxim 1gm preoperatively. After obtaining written, valid consent patients were operated under combined spinal epidural anaesthesia. Epidural anaesthesia was given in L_2-L_3 space with 18 numbered needle trough which 18 numbered epidural catheter was put Lumbar puncture was done through same space , 2ml of 0.5%Bupivacaine+30mcg clonidine was given intrathecally. Epidural supplementation was given with Bupivacaine 0.5%, 3 cc increments after two segment regression of sensory level approximately after 95 mints of intrathecal drug administration. We got difficulty for identification of epidural space in first patient.T_8 level was achieved in first patient while t_{10} level was achieved in second patient. Both patients were haemodynamically stable throughout the procedure & didn't require any vasopressors.

Eyes were protected with eye pads after instillation of natural tears & proper padding was done at pressure points. Postoperative analgesia was provided by epidural Bupivacaine 0.125% along with Inj. Tramadol 50 mg on patient's request. During surgery Ringer's lactate solution 1oooml along with 500 ml Hydroxyethyl starch& 500ml of 5% Dextrose was infused . Second patient required one unit of blood transfusion. The procedure was uneventful in both patients. There was no excessive bleeding intraoperatively but surgeon had difficulty in bone drilling & threading screws.

DISCUSSION: Osteopetrosis is a hereditary disorder involving skeleton and characterised by increased bone density on radiographs also known as Marbel Bone Disease or Osteosclerosis fragilis generalis ats[1,3]. It was diagnosed by German radiologist in 1904 hence named as Albers-Schonberg disease.[1] Multisystemic involvement like anaemia, pancytopenia,

hepatosplenomegaly, renal tubular acidosis, compressive nerve palsies and mental retardation needs modified anaesthetic technique.[1,2,3.] Facial deformities like proptosis, high arched palate, broad facies, hypertelorism, mandibular hyper or hypoplasia[2], limited mandibular movements make oral intubation difficult while bony encroachment of nasal turbinates may preclude nasal intubation.[2,4] Poor periodontal attachment leads to increased risk of intraoperative tooth loss[4.] Thrombocytopenia lead to profuse bleeding during intubation or surgery.[2]Hypocalcemia may lead to intraoperative seizures or tetany.[4] Spinal or epidural anaesthesia is difficult due to bony thickening, scoliosis & short stature in these patients. Tendency of repeated fractures needs to take proper care while positioning during surgery & protruding eyes should be protected.

It was reported that incidence of difficult oral intubation is high along with air way obstruction & arterial desaturation[5]. Anesthetic management strategies should consider the factors that cause the high frequency of adverse airway events in this patient population[6]. Awake fibreoptic intubation is necessary in severe cases[7].we preferred combined spinal epidural anaesthesia as in both patients. We did not have any difficulty in giving combined spinal epidural anaesthesia in one patient while another patient needed multiple pricks. Access of neuroxial block may be technically difficult due to chronic compression fracture of lumbar or thoracic spine & potential for trauma to abnormal bone must be considered in these patients[7]. Besides these problems there is a possibility of interosseous injection with high serum levels of local anaesthetic solution[7]. So it is advisable to administer titrated doses of local anaesthetic drugs. Regional anaesthesia should be given after correction of thrombocytopenia if present. Peripheral nerve blocks can be given safely but for lower limb high doses of local anaesthetic drug may be needed.

Funnel like appearance known as Erlenmeyer flask deformity,[1] extremely radio dense vertebrae with alternate bands known as “rugger-jersey” sign are diagnostic of osteopetrosis[3] which was seen in our patient. Bone within bone appearance “Endobone” is due to defect in metaphyseal bone remodelling resulting in greatly thickened cortices &obliteration of medullary space[8].

Neurological effects of osteopetrosis results from restricted growth of foramina leading to Optic, Facial & Trigeminal neuropathies, strabismus, hearing loss, dysarthria, hydrocephalus, cerebral atrophy& developmental delay.[9]Short stature might be due to impaired longitudinal growth. Both patients were belong to intermediate type osteopetrosis so there was no evidence of hepatosplenomegaly, anaemia, renal tubular acidosis & pancytopenia. There was evidence of involvement of Abducent nerve & Optic nerve in one patient without raised intracranial pressure in one patient.

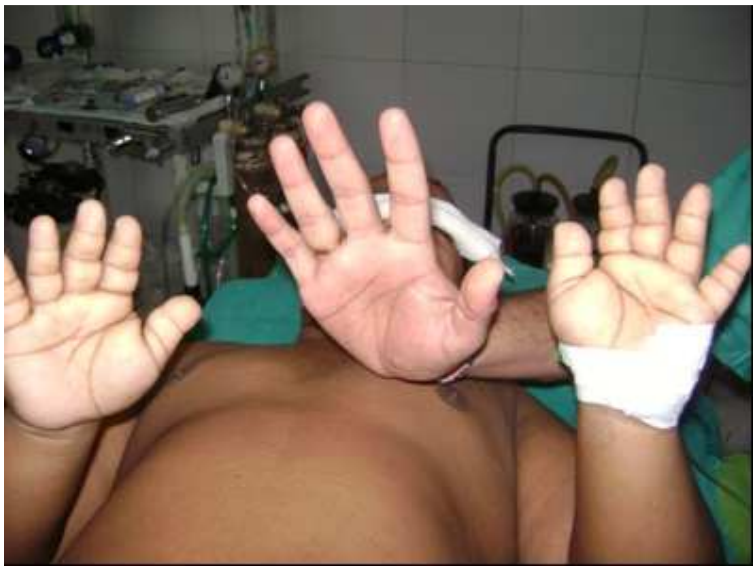
Deficiency of carbonic unhydrase in osteoclasts causes defective hydrogen ion pumping & hence defective bone resorption results. Carbonic anhydrase II has activity in bone, kidney and brain. These Patients may present with slightly acidic blood with high chloride concentration. It is due to excessive leakage of bicarbonates from renal tubules.[10]

Infantile osteopetrosis is severe form of disease presents in early childhood. The life threatening complication is bone marrow failure leading to anaemia, hepatosplenomegaly, pancytopenia with increased susceptibility to infection[1, 2,3] Benign type of osteopetrosis is diagnosed in late adolescent or adulthood. Bone marrow function is not compromised in these

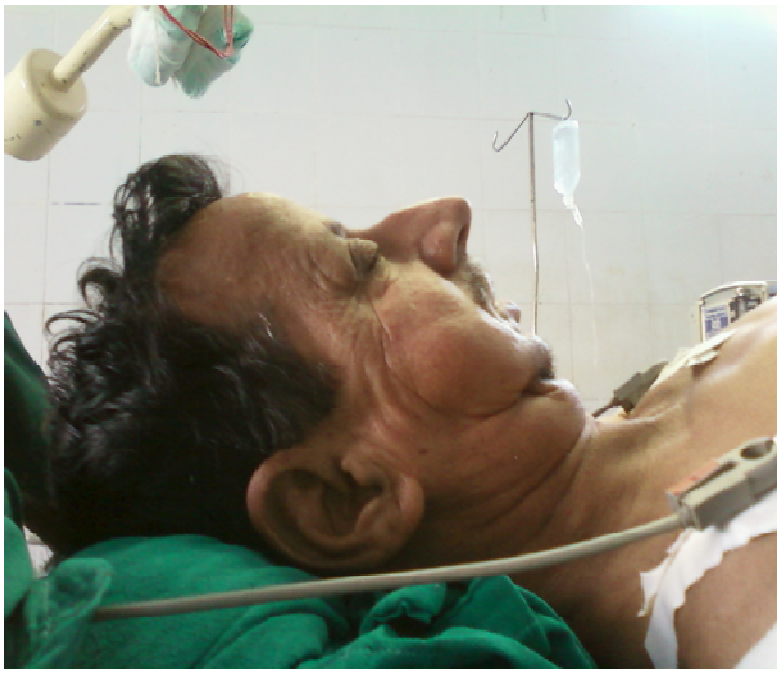
patients.[3] Steroids, high-dose calcitriol, or interferon-gamma therapy may be beneficial, and bone marrow transplantation has been advocated.

CONCLUSION: For successful management of patient anaesthesiologist must be aware of etiopathogenesis of osteopetrosis & prepared for difficult intubation & regional block. Preoperative correction of anaemia & thrombocytopenia, proper positioning & protection of eyes is must. Regional anaesthesia can be given safely in milder osteopetrosis without difficulty.

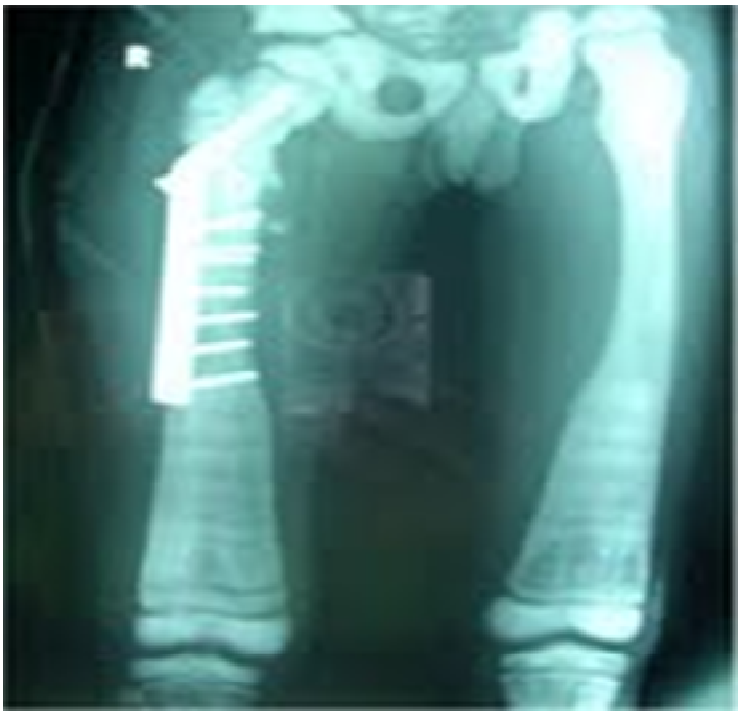
Photograph showing abnormal facies



Photograph showing short hands



Photograph showing abnormal Facies



Radiograph showing funnel shaped femur

REFERENCES:

1. Zornitza Stark and Ravi Savarirayan, Osteopetrosis, Orphanet journal of rare diseases2009,4:5
2. Napoleon Burt,Gary R.,Haynes,MelindaK.Bailey.,Patients with malignant osteopetrosis are at high risk of anaesthetic morbidity and mortalityAnesthesia&Aalgesia June 1999 vol. 88 no. 6 1292
3. R. Rajendran, ch-17, Diseases of Bone & Joints, Shafer's Textbook of Oral Pathology, 6th edition, 681-753.
4. Victor C Baum,JenniferE,O'Flaherty. Ch-2 Syndromes listed alphabetically Anaesthesia for genetic metabolic and dysmorphic syndromes of childhood. 2ndEdition.Page no.8-415.
5. AmarpreetKaur, Ctherine A, Billups , Mary Edna Prish RN, Roland N.Kaddoum et al. Complications of Anaesthesia for Malignant infantile osteopetrosis before & after haemopiotic Stem Cell Transplant. Paediatric Anaesthesia vol20, issue11,page no-1046-51 Nov 2010.
6. Burt N, Haynes GR, Bailey MK Patients with malignant osteopetrosis are at high risk of anesthetic morbidity and mortality.AnesthAnalg. 1999 Jun; 88(6):1292-7.
7. JohETEt ZLAFF, Skin & Bone Disorder, Ch no. 10, Anesthesia& uncommon diseases, 5th edition by Lee A Fleisher, 2006 (327-358)
8. REGEZI,SCIUBBA,JORDAN, CH-15, Metabolic &Genetic Diseases, Oral Pathology,Clinical pathological correlation, 5th edition, page no 335-358
9. 9) M. L. Kulkarni,S.N. Marakkanvar,S, Sushant,N.Pradeep,C.Ashok, M.D. Balaji,etal, Osteopetrosiswith Arnold Chiari Malformation Type I & Brain Stem Compression, Indian Journal Pediatr 2007;74(4)'412-415
10. WhyteMP,MurphyWA,FallonMD,et al Osteopetrosis:renal tubular acidosis and basal ganglion calcification in three sisters.AM J Med 1980;69:64-74.

DIAGNOSTIC ACCURACY OF SQUASH SMEAR TECHNIQUE IN BRAIN TUMORS

Sanjay Kumar Nigam, Nilam Nigam, Anand Mishra, Nitu Nigam

1. Associate Professor, Department of Pathology, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.
2. Associate Professor, Department of Pharmacology, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.
3. Associate Professor, Department of Biochemistry, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.
4. Assistant Professor, Department of Microbiology, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.

CORRESPONDING AUTHOR
Dr. Sanjay Kumar Nigam,
House no. 308, Rama Medical College,
Mandhana, Kanpur, 209217.
E-mail: sknigam@yahoo.com,
Ph: 0091 9411918206.

ABSTRACT: Background & Objectives: Primary tumors of central nervous system constitute less than 2% of overall human cancers in adults however; they constitute second most common frequently encountered tumors in children after leukaemia. Use of smear technique has been described for many years on specimens obtained with stereotactic devices, which allow multiple sampling for the diagnosis of deep seated lesions. Squash smear technique saves time and amount of tissue needed. **METHODS:** A total of 75 cases of intracranial space occupying lesions were studied. The squash smear technique was used to diagnose the brain Tumors and was compared with histopathological examination of same tumor. **RESULTS:** Out of 75 cases in 8 (10.7%) cases discordance was observed between squash smear diagnosis and histologic diagnosis. In 67 (89.3%) cases squash smear diagnosis was in accordance with the final histologic diagnosis. Thus the diagnostic accuracy of squash smear technique in this study is 89.3%. **INTERPRETATION & CONCLUSIONS:** Thus the value of squash smear technique in rapid preoperative diagnosis of neurosurgical biopsies is corroborated by above study and the accuracy of this study matched with the other studies done in the past on squash smear technique and it should be used regularly for rapid intra-operative diagnosis of intracranial space occupying lesion.

KEY WORDS: squash smear technique, intracranial space occupying lesions, histopathological examination.

INTRODUCTION: Primary tumors of central nervous system constitute less than 2% of overall human cancers in adults however; they constitute second most common frequently encountered Tumors in children after leukaemia. (1)

As the biological behaviour or prognosis is partly dependent on the constituent cell type, it is of utmost important to classify these tumors. Equally important is to recognize certain primary brain tumors, which respond to aggressive radiotherapy or chemotherapy. Rapid preoperative diagnosis is of immense value to select treatment options. (2, 3)

Clinical diagnosis has improved with the advent of high resolution imaging techniques. However a definite diagnosis can only be obtained by cytological or histopathological

examination of the tumor tissue. Use of smear technique has been described for many years on specimens obtained with stereotactic devices, which allow multiple sampling for the diagnosis of deep seated lesions. The technique is also helpful to identify various non neoplastic space occupying lesions and radiation induced necrosis versus recurrent Tumors. (4)

ADVANTAGES OF SQUASH SMEAR PREPARATIONS –

The economy of time and amount of tissue needed. (5)
There is better preservation of cellular details.
The background of smear may contribute valuable information about the nature of a tumour.

DISADVANTAGES OF SMEAR PREPARATION –

Loss of cyto-architectural details.
Vascular tumors where stromal componen resist squashing.
Difficulties in grading the tumors.

AIMS AND OBJECTIVES-

To provide rapid diagnosis of central nervous system tumors.
To evaluate the diagnostic accuracy of squash smear technique.

MATERIAL AND METHODS: Present study was conducted in the pathology department of Rama Medical College and Hospital Research Centre Mandhana Kanpur on patients admitted in the department of surgery. A total of 75 cases of intracranial space occupying lesions were studied.

In every case age, sex, clinical history, duration of illness, findings on smear examination, radiological findings (CT/MRI) and operative notes were recorded.

METHODS OF PREPARING SQUASH SMEARS: Unfixed tissue in normal saline was taken from O.T. Small bits of tissue about 1-2 mm in size were selected and squashed between the two glass slides and smears prepared. (6)

Smears were fixed in absolute alcohol for 2 minutes, washed in water for one minute and stained with Harri's hematoxylin for five minutes. Smears were kept in 0.5% aqueous hydrochloride for ten to twenty seconds and rinsed in water for two minutes. Finally smears were stained with eosin and washed in absolute alcohol, dried and then mounted in DPX.

Depending upon the cyto-morphological features, in squash smears, the cyto-diagnosis of all cases was recorded in a predesigned proforma.

Histological sections were evaluated and final histological diagnosis was recorded in a predesigned proforma. Both cytologic (squash smears) diagnosis and final histopathologic diagnosis were compared to assess the diagnostic accuracy of squash smears in central nervous system Tumors. Special stains were used as and when required. During the entire study need for Immunohistochemical stains or cytogenetics study was not felt.

OBSERVATIONS AND RESULTS: In the present study role of the squash smear technique in the diagnosis of central nervous tumors and its correlation with histopathology has been evaluated. A total of 75 cases of central nervous system tumors attending surgery department during the

period of one year at Rama Medical College and Hospital Research Centre Mandhana Kanpur U.P. were selected for this study. Of these cases, 72 (96%) were intracranial space occupying lesions and 3 (4%) were spinal lesions.

AGE AND SEX DISTRIBUTION- (TABLE 1): Peak incidence of brain Tumors was observed in middle life and they comprised 17 (22.7%) of tumors examined. Second peak was observed in second decade of life and male / female ratio was 5.5:1. Male/female ratio of all patients irrespective of age group was 2.12:1. Age of youngest patient was 5 months. Age of the oldest patient was 75 years.

Table-1 age and sex distribution of patients (n=75)

Age distribution (years)	No. Of cases.	%	Male	Female
<10	05	6.6	01	04
11-20	13	17.3	11	02
21-30	09	12.0	05	04
31-40	15	20.0	11	04
41-50	17	22.7	12	05
51-60	10	13.3	07	03
61-70	04	5.3	03	01
>70	02	2.6	01	01
			51	24

Following lesions were diagnosed by examining morphology of individual lesion on squash smears – as shown in table No. 2

Astrocytoma, Oligodendroglioma, Ependymoma, Pituitary adenoma, Medullobalstoma, Meningioma, Schwannoma, Neurocytoma, Craniopharyngioma, Lymphoma, Metastatic carcinoma and Epidermoid cyst. Though heamangioblastomas were difficult to squash because of hemorrhage and fibrosis hence on squash smears a diagnosis of benign mesenchymal tumor was given.

Table 2 Cytological evaluation of squash smears – (n=75)

Diagnosis of squash smears	No. Of cases	%
Astrocytoma low grade	12	16.0
Malignant glioma	13	17.3
Oligodendroglioma	01	1.3
Ependymoma	01	1.3
Pituitary adenoma	03	4.0
Medullobalstoma	08	10.7
Neurocytoma	01	1.3
Meningioma	16	21.3
Schwannoma	09	12.0
Craniopharyngioma	02	2.6
Benign mesenchymal tumor	O3	4.0
Small round cell tumor	01	1.3
Lymphoma	01	1.3
Metastasis	01	1.3
Epidermoid cyst	03	4.0

Histopathological evaluation of biopsies from 75 cases revealed that 21 (28.0%) cases had morphological features of astrocytomas (low grade and high grade), of which 8 were grade II astrocytomas, 2 were grade III astrocytoma and 11 were glioblastoma. In 2 (2.6%) cases diagnosis of oligodendroglioma was given, In 2 (2.6%) cases diagnosis of ependymoma was given, In 16 (21.3%) cases diagnosis of meningioma was given, in 9 (12%) cases diagnosis of Schwannoma was given, in 8 (10.7%) cases diagnosis of Medullobalstoma was given, in 3 (4.0%) cases diagnosis of pituitary adenoma was given, and in 2 (2.6%) cases diagnosis of Craniopharyngioma was given. In 3 (4.0%) cases diagnosis of hemangioblastoma was given, and in 3 (4.0%) cases diagnosis of Epidermoid cyst was given. One case each from oligoastrocytoma,

neurocytoma, anaplastic oligodendroglioma, paraganglioma, lymphoma and metastatic carcinoma was recorded.

Table – 3 Histopathological evaluation of lesions (n = 75) -

Histopathological diagnosis	No. Of cases	%
Astrocytoma grade II	08	10.7
Astrocytoma grade III	02	2.6
Glioblastoma	11	14.7
Mixed oligo astrocytoma	01	1.3
Oligodendroglioma	02	2.6
Anaplastic oligodendroglioma	01	1.3
Ependymoma	02	2.6
Neurocytoma	01	1.3
Pituitary adenoma	03	04
Medullobalstoma	08	10.7
Meningioma	16	21.3
Schwannoma	09	12.0
Craniopharyngioma	02	2.6
Paraganglioma	01	1.3
Hemangioblastoma	03	4.0
Lymphoma	01	1.3
Metastatic carcinoma	01	1.3
Epidermoid cyst	03	4.0

Of these 75 cases, 8 (10.7%) cases showed discordant findings on cytologic and histological examination. Of these, 3 cases were diagnosed as low grade astrocytoma on squash smears, were subsequently diagnosed as ependymoma, mixed oligoastrocytoma and oligo dendroglioma on histological examination where as one case was diagnosed as malignant glioma on squash smears and was subsequently diagnosed as anaplastic oligodendroglioma on histologic examination.

In three cases diagnosis of benign mesenchymal tumor was given on squash smears and final histologic diagnosis of hemangioblastoma was given in all 3 cases.

In one case diagnosis of small round cell tumor was given on squash smears and final histologic diagnosis of paraganglioma was given.

In rest of the other tumors like pituitary adenoma, medulloblastoma, meningioma, schwannoma, craniopharyngioma, neurocytoma, lymphoma, and metastatic carcinoma cytologic (squash smear diagnosis) was in accordance with the final histologic diagnosis.

In 8 (10.7%) cases discordance was observed between squash smear diagnosis and histologic diagnosis. In 67 (89.3%) cases squash smear diagnosis was in accordance with the final histologic diagnosis. Thus the diagnostic accuracy of squash smear technique in this study is 89.3%.

Table – 4 comparative analysis of cytologic and histologic diagnosis

Diagnosis	Squash smear diagnosis	Histopathological diagnosis	% accuracy
Astrocytoma	25	21	84
Oligodendroglioma	01	03	33.3
Ependymoma	01	02	50
Oligoastrocytoma	-	01	-
Neurocytoma	01	01	100
Pituitary adenoma	03	03	100
Medulloblastoma	08	08	100
Meningioma	16	16	100
Schwannoma	09	09	100
Craniopharyngioma	02	02	100
Paraganglioma	-	01	-
Hemangioblastoma	-	03	-
Lymphoma	01	01	100
Metastatic carcinoma	01	01	100
Epidermoid cyst	03	03	100

Table –5 discordant results (8 cases)

Squash smear diagnosis	Final histologic diagnosis
Low grade astrocytoma	Ependymoma
Low grade astrocytoma	Mixed oligoastrocytoma
Low grade astrocytoma	Oligodendroglioma
Malignant glioma	Anaplastic oligodendroglioma
Small round cell tumor	Paraganglioma
Benign mesenchymal tumor (3 cases)	hemangioblastoma

DISCUSSION: The intra-operative cytology preparation was first introduced by Eisenhardt L in 1930 and by Badt in 1937. (7)

Present work has been undertaken to study the diagnostic accuracy of squash smear technique for rapid per-operative diagnosis of neurosurgical biopsies. A total of 75 lesions were examined in this study. In all cases history, laboratory investigations, radiological findings and previous investigation if any were recorded. Biopsy material was obtained by craniotomy from these cases intra-operatively and was subjected to squash smear technique followed by subsequent histopathological examination of the remaining material. (8)

This study showed that peak incidence of brain Tumors was in the middle life and they constituted 17 (22.7%) of all the Tumors examined with Second peak in 2nd decade of life and male/female ratio was 5.5:1 in this age group. Age of the youngest patient was 5 months old baby and age of the oldest was 75 years. Male/female ratio of all patients irrespective of age group was 2.12:1. Thus male showed a greater incidence of brain Tumors as compared to females. (9)

In the present study, accuracy rate of 89.3% is comparable to the other studies conducted in the past. (10, 11, 12, 13, 14, 15)

CONCLUSION: Thus the value of squash smear technique in rapid preoperative diagnosis of neurosurgical biopsies is corroborated by above study. The accuracy of this study (89.3%) matched with the other studies done in the past on squash smear technique. So we recommend using this technique regularly for rapid intra-operative diagnosis of intracranial space occupying lesion. (10, 11, 12, 13, 14, 15)

BIBLIOGRAPHY:

1. Lannering B, Sandström PE, Holm S, Lundgren J, Pfeifer S, Samuelsson U, Strömberg B, Gustafsson G; Swedish Childhood CNS Tumor Working Group Classification, incidence and survival analyses of children with CNS Tumors diagnosed in Sweden 1984-2005. Acta Paediatr. 2009 Oct;98(10):1620-7. Epub 2009 Jul 7.

2. (2). Asha T, Shankar SK, Rao TV, Das S. Role of squash-smear technique for rapid diagnosis of neurosurgical biopsies--a cytomorphological evaluation. Indian J Pathol Microbiol. 1989 Jul;32(3):152-60.
3. (3)N. Krishnani, N. Kumari, S. Behari, C. Rana and P. Gupta Intraoperative squash cytology: diagnostic accuracy and its impact on immediate surgical management of central nervous system Tumors Cytopathology. 2011 Aug 15. doi: 10.1111/j.1365-2303.2011.00905.x.
4. Collaço LM, Tani E, Lindblom I, Skoog L. Stereotactic biopsy and cytological diagnosis in solid and cystic intracranial lesions. Cytopathology. 2003;14:131–5.
5. Olasode BJ, Ironside JW. The brain smear, a rapid affordable intraoperative diagnostic technique for brain Tumors appropriate for Africa. Trop Doct. 2004;34:223–5.
6. Silverberg SG, Delellis RA, Frable WJ: Principle and practice of surgical pathology and cytopathology, vol.3, 3rd edn.1997.
7. Eisenhardt L, Cushing H. Diagnosis of Intracranial Tumors by Supravital Technique. Am J Pathol. 1930 Sep;6(5):541-552.7.
8. Nandita Ghosal, A S Hegde, Ganesh Murthy, Sunil V Furtado Smear preparation of intracranial lesions: a retrospective study of 306 cases Diagnostic Cytopathology (impact factor: 1.3). 08/2011; 39(8):582-92.
9. Shibui S. The present status and trend of brain tumors based on the data of the Brain Tumor Registry of Japan. Brain Nerve. 2012 Mar;64(3):286-90.
10. Willems JG, Alva-Willems JM. Accuracy of cytologic diagnosis of central nervous system neoplasms in sterotactic biopsies. **Acta Cytol**. 1984 May-Jun;28(**3**):243-9.
11. Mc Mennemey WH: An appraisal of smear diagnosis in neurosurgery. Am. J. Clin. Pathol. 33:471,1960
12. Berkeley BB, Adams JH, Doyle D, Graham DI, Harper CG. The smear technique in the diagnosis of neurosurgical biopsies. N Z Med J. 1978 Jan 11;87(603):12-5.
13. Iqbal M, Shah A, Wani MA, Kirmani A, Ramzan A. Utility of Crush Smear Cytology in Intraoperative Diagnosis of Central Nervous System lesions. Acta Cytol 2006; 50(6): 608-16.
14. Shukla K, Parikh B, Shukla J, Trivedi P, Shah B. Accuracy of cytologic diagnosis of Central Nervous system Tumors in crush preparation. Indian J Pathol Microbiol. 2006;49:483–6.
15. Roessler K, Dietrich W, Kitz K. High diagnostic accuracy of cytologic smears of central nervous system Tumors. A 15-year experience based on 4,172 patients. Acta Cytol. 2002; 46:667–74.

NEW TRENDS IN PERIODONTICS.

Kiran Kumar N, Chandhra Mohan P. Ramesh Babu, Srikanth C, Arpita Paul R.

1. Professor, Department of Periodontics, Mamata Dental College, Khammam, Andhra Pradesh.
2. Reader, Department of Periodontics, Mamata Dental College, Khammam, Andhra Pradesh.
3. Reader, Department of Periodontics, Mamata Dental College, Khammam, Andhra Pradesh.
4. Professor & HOD, Department of Periodontics, Mamata Dental College, Khammam, Andhra Pradesh.
5. Reader, Department of Periodontics, Mamata Dental College, Khammam, Andhra Pradesh.

CORRESPONDING AUTHOR
Dr Ramesh Babu M,
Dept. of Periodontics,
Mamatha Dental College,
Khammam, Andhra Pradesh,
E-mail: ramesh_8353@yahoo.com,
Ph: 0091 9908760827.

ABSTRACT: New research is demonstrating that a person's total health is indeed related to their oral health. Elimination of all oral infections, including gingivitis and periodontitis, is important to overall health. The article reviews various trends in nonsurgical and surgical therapy that will successfully arrest periodontal infections. Opportunities for early diagnosis and prevention will play an increasing role in dental practice in the future as patients understand the importance of oral health to overall health. There is an urgent need to educate the public as to the importance of periodontal health. All of these findings indicate that periodontal disease must be viewed from a whole new perspective, particularly since some form of periodontal disease is present in a large percentage of the population. A prospective approach of prevention and early intervention in treating the disease is more important than ever before.

KEY WORDS: Probiotics, vaccines, microsurgery, lasers, stem cells, gene therapy.

INTRODUCTION: Advances in periodontal science and practice over the last decade have radically changed the understanding of periodontal diseases and have opened new, exciting prospects for both non-surgical and surgical therapy of periodontal diseases. Mechanical methods of subgingival debridement accomplished by thorough scaling and root planing, accompanied by oral hygiene procedures, have served as the gold standard of periodontal therapy for decades.

A practical, convenient, and pragmatic way to treat periodontal disease has been the dream of every clinician. However, realization of the dream seems to be elusive. The prime concern in any periodontal treatment is a control over the errant microorganisms and resolution of soft tissue inflammation and restoration of lost alveolar support. Resolution of soft tissue inflammation appears to be an established accomplishment after scaling, root planing (SRP) and oral hygiene instructions.

Various treatment options are available in the armory of a periodontist including surgical and non-surgical therapy. Not satisfied with traditional weapons to combat oral microbiota and restore lost alveolar bone support, ingenious clinicians, scientists, and researchers have turned toward novel and sometimes exotic avenues and explored new

frontiers of physical laws, pharmacological molecules, and new concepts as available for other medical situations. The newer trends may be listed as follows

1. Probiotics
2. Ozone Therapy
3. Periodontal Vaccine
4. Microsurgery
5. Lasers
6. Waterlase
7. Stem cells
8. Tissue Engineering
9. Photodynamic Therapy (PDT)
10. Gene therapy
11. RNA interference
12. Nanotechnology
13. Perioprotect
14. Tri immune phasic therapy (TIP)
15. Bone One Session Treatment (BOST)
16. Use of Newer Molecules to resolve inflammation
17. Therapeutic approaches recently available to control inflammation and bone resorption.

PROBIOTICS: The World Health Organization (WHO) has defined Probiotics as live organisms, which, when administered in adequate amounts, confer a health benefit on the host. Probiotics repopulate beneficial bacteria which can help to kill the pathogenic bacteria and fight against infection. They are also called "friendly bacteria" or "good bacteria". [1]

PREBIOTICS: "Non-absorbable food components that beneficially stimulate one or more of the beneficial microbe groups and thus have a positive effect on human health"[2]

SYNBIOTICS: "Combined administration of specific Prebiotics with Probiotics to provide definite health benefits by synergistic action"[2]

Probiotics can be bacteria, moulds, yeast. But most probiotics are bacteria. Among bacteria, lactic acid bacteria are more popular. Lactobacillus acidophilus, L. casei, L. lactis, L. salivarius, L. plantrum, L. bulgaricus, L. rhamnosus, L. reuteri, Streptococcus thermophilus, E. faecalis, Bifidobacterium bifidum are commonly used bacterial probiotics. Probiotics can be in powder form, liquid form, gel, paste, granules or available in the form of capsules, sachets, etc.[3]

PHYSIOLOGICAL EFFECTS: The physiological effects attributed to Probiotic bacteria include[4]-

The reduction of gut pH

Production of antibacterial substances, e.g., organic acids, bacteriocins, hydrogen peroxide, diacetyl acetaldehyde, lactoperoxidase system, lactones, and other unidentified substances

Reconstruction of normal intestinal microflora after disorders caused by diarrhoea, antibiotic therapy, and radiotherapy

Reduction of cholesterol level in the blood

Stimulation of immune functions.

PROBIOTICS AND PERIODONTAL HEALTH: Probiotics lower the pH so that plaque bacteria cannot form dental plaque and calculus that causes the periodontal disease. They make an excellent maintenance product because they produce antioxidants. Antioxidants prevent plaque formation by neutralizing the free electrons that are needed for the mineral formation. Probiotics are able to breakdown putrescence odours by fixating on the toxic gases (volatile sulphur compounds) and changing them to gases needed for metabolis.

Lactobacilli can produce different antimicrobial components including organic acids, hydrogen peroxide, low molecular weight antimicrobial substances, bacteriocins and adhesion inhibitors and have gained prominence as Probiotics. A majority of the strains including L. salivarius were shown to suppress the growth of Aggregatibacter actinomycetemcomitans, P. gingivalis and Prevotella intermedia. Probiotic strains included in periodontal dressings at optimal concentration of 108 CFU/ml were shown to diminish the number of most frequently isolated periodontal pathogens.[5]

OZONE THERAPY: Oxygen/ozone therapy has a long history of research and clinical application with humans. The German chemist, C.D. Schonbein, first discovered ozone in 1840. The first medical application was in 1870 when Dr. C. Lender purified blood in test tubes. Medical applications became widespread throughout Europe and America. As of 1929, more than 114 diseases were listed for treatment with oxygen/ ozone therapy. Interestingly enough, in 1930, a German dentist, Dr. E.A. Fisch, used ozone on a regular basis in his dental practice in Zurich, Switzerland, and published numerous papers on the subject. Dr. Fisch influenced the work of Dr. Erwin Payr, a renowned surgeon. Dr. Payr's work set the stage for mainstream use of oxygen/ozone therapy in medicine.

Ozone is a powerful oxidizer. It effectively kills bacteria, fungi, viruses, and parasites at a dramatically lower concentration than chlorine, with none of the toxic side effects. One molecule of ozone is equal to between 3,000 to 10,000 molecules of chlorine and it kills pathogenic organisms 3,500 times faster!

APPLICATION OF OZONE THERAPY:

Three basic forms of application to oral tissue are applied —

1) Ozonated water,
2) Ozonated olive oil, and
3) Oxygen/ozone gas.

ADVANTAGES

Safe and efficacious with no toxicity or side effects.

PERIODONTAL VACCINE[6]: The primary role of any periodontal vaccine would be to eradicate the global periodontal disease burden with the ultimate purpose of lowering periodontal disease associated morbidity in humans.

As an innovative strategy, vaccines using cross-reactive immunodominant epitopes as antigenic molecules in an attempt to stimulate antigen-specific regulatory T-cells (Tregs, CD4+, CD25+, FoxP3+), secreting IL-10 and TGF-β, may provide new clues for periodontal disease prevention, through the induction of either immune tolerance or an effector function.

Periodontal disease as a multifactorial and polymicrobial disease requires a sophisticated vaccine design regimen targeting multiple pathogenic species. Vaccine regimens

including the commonly shared antigens by selected periodontopathogenic species would be considered an innovative strategy.

MICROSURGERY[7]: In 1980, Serafin described microsurgery as a methodology, a modification and refinement of existing surgical techniques using magnification to improve visualization that had implications and applications to all specialties.

There are two types of magnifications available to dentist's, magnification loupes and the operating microscope.

ADVANTAGES OF MICROSURGERY:

1. Gentle handling of soft and hard tissues with the same universally accepted surgical principles.
2. Extreme and accurate wound closure.
3. Healing by primary intention.
4. Clinical horizons will continue to improve with operator experience and willingness to employ previously unused basic optic magnification and ergonomic techniques and technology.
5. Eliminate patient pain and morbidity.

LASERS[8]: The word **LASER** is an acronym for **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation. Light is a form of electromagnetic energy that behaves as a particle and a wave. The basic unit of this energy is called a photon.

CO_2, Nd: YAG, Diode and Er: YAG Lasers are the commonly used ones in Periodontics

All lasers work by delivering energy in the form of light. When used for surgical and dental procedures, the laser acts as a cutting instrument or a vaporizer of tissue that it comes in contact with. When used for "curing" a filling, the laser helps to strengthen the bond between the filling and the tooth. When used in teeth whitening procedures, the laser acts as a heat source and enhances the effect of tooth beaching agents.

LASER INDUCED INTERACTION BETWEEN LIGHT AND TISSUE[9]: Lasers produce light energy within a narrow frequency range. For most practical purposes, the light produced by lasers can be considered to be monochromatic. Typically, lasers are named according to the active element within them that goes through the stimulated quantum transitions, which create the light. The wavelength of the light that a laser produces is characteristic of the particular active element. For e.g. Nd: YAG lasers, of similar design produce light with a wavelength of 1.064 micrometers. All CO_2 lasers of similar design produce light with a wavelength of 10.6μm.

LASER ENERGY PENETRATION: The wavelength of the light is the primary determinant of the degree to which the light is absorbed in the target material (oral tissues). Depending on the tissue, some lasers penetrate deeper than others. By contrast other laser wavelengths are limited to a shallow penetration and have a surface effect on tissue. The deeper the laser energy penetrates, the more it is scattered and distributed throughout the tissue. The degree to which this occurs also is affected by the power of the laser and exposure duration, but wavelength is the prime factor. For eg.CO_2 laser penetrates only about 0.03 to 0.1 millimeter in to tissue, whereas Nd: YAG laser penetrates 2 to 5 millimeters into tissues.

USES OF LASERS IN PERIODONTICS:

Sulcular debridement
Soft tissue ablation, removal of large masses of tissue.
Curettage
De- epithelialization
Incision
Root desensitization
Second stage implant surgery
Osseous ablative surgery
Soft tissue crown lengthening
Frenectomy

ADVANTAGES:

Reduced need for anesthesia
Greater comfort during and after surgery.
Hemostasis and reduced risk of blood borne pathogens
High patient acceptance of treatment.
Reduced stress and fatigue for the practitioner and staff.

LIMITATIONS:

All lasers require specialized training and attention to safety precautions.
Slower than traditional methods.

WATERLASE[10]: Erbium-Chromium doped: Yittrium-Selenium- Gallium-Garnet (Er, Cr: YSGG) laser is commercially available as WaterLase. It uses a patented combination of laser energy and water by a process called Hydro photonics, to perform a wide range of dental procedures.

MECHANISM: The Pain-free WaterLase Laser is from BIOLASE Technology, the world's leading maker of dental lasers. Tooth enamel naturally contains up to 5% water; dentin and bone up to 25%. Years of BIOLASE research led to discovery of a water-energizing 2,780 nm YSGG laser and a hand piece that delivers air and water in precise proportions – both BIOLASE patented – that combine to symbiotically excite water molecules from both the hand piece spray and inside the target tissue. The result is an effective pain-free biological micro-ablation of tooth structure. The atomized spray of water and air continually re-hydrates the tooth, preventing heat and pain.

USES OF WATERLASE IN PERIODONTICS

Full thickness flap
Partial thickness flap
Split thickness flap
Laser soft tissue curettage
Laser removal of diseased, infected, inflamed and necrosed soft tissue within the periodontal pocket
Removal of highly inflamed edematous tissue affected by bacteria penetration of the pocket lining and junctional epithelium
Removal of granulation tissue from bony defects

Sulcular debridement (removal of diseased or inflamed soft tissue in the periodontal pocket to improve clinical indices including gingival index, gingival bleeding index, probe depth, attachment loss and tooth mobility)
Osteoplasty and osseous recontouring (removal of bone to correct osseous defects and create physiologic osseous contours)
Ostectomy (resection of bone to restore bony architecture, resection of bone for grafting, etc.)
Osseous crown lengthening.

CLINICAL BENEFITS/ADVANTAGES WITH WATERLASE:
1. Painless dentistry (WaterLase, does not generate heat, vibration or pressure, many dental procedures can be performed pain-free with fewer shots, less need for anesthesia).
2. Accuracy and Precision: (WaterLase, periodontists able to remove bone and gum tissue precisely while leaving surrounding areas unaffected. This conserves and allows you to keep more of the healthy tooth structure.)
3. Reduced Trauma (WaterLase, reduces damage to healthy portions of the tooth and minimizes trauma).
4. Less Bleeding and Swelling(Due to its conservative, gentle cutting action and coagulating capabilities, the WaterLase, performs many soft tissue (gum) procedures with little or no bleeding and less post-op swelling.)
5. Versatility (The WaterLase, is extremely versatile. It can be used for a wide range of hard and soft tissue procedures. From decay removal, cavity preparation, root canals, smile design, gum and bone surgical procedures and many others.)

DRAWBACKS: As the Waterlase is quite expensive, it may not make it into many smaller dental practices.

STEM CELLS[11]: Stem cells are defined as cells that have clonogenic and self-renewing capabilities and differentiate into multiple cell lineages. All stem cells regardless of their source have three general properties: they are capable of dividing and renewing themselves for long periods, they are unspecialized, and they can give rise to specialized cell types. Depending upon the intrinsic signals modulated by extrinsic factors in the stem cell niche, these cells may either undergo prolonged self-renewal or differentiation?

Stem cells are defined as totipotent progenitor (clonogenic) cells capable of self renewal and multilineage differentiation. On the basis of Cell Maturity they can be divided into

A) Embryonic Stem Cells

B) Adult Stem Cells

As adult stem cells are not totipotent they can be further classified depending on their origin and their differential potential into:

1. Haematopoietic Stem Cells .
2. Non-haematopoietic Stem Cells Or Mesenchymal Stem Cells.

THE ADULT PERIODONTAL LIGAMENT STEM CELLS (PDLSC): The PDL cell population is heterogeneous, consisting of two major mesenchymal lineages, fibroblastic and mineralizing tissues, further divided into osteoblastic and cementoblastic subsets. The concept that the stem cells may reside in the periodontal tissues was proposed approximately 20 years ago by Melcher, who queried whether the three cell populations of the periodontium (cementoblasts,

osteoblasts, and periodontal ligament fibroblasts) were derived from a single population of ancestral cells or stem cells. The most compelling evidence that these cells are present within the periodontal tissues has been provided by the studies of McCulloch in 1987, who identified a small population of progenitor cells adjacent to blood vessels within periodontal ligament. These cells demonstrated some classical cytological features of stem cells, including small size, responsiveness to stimulating factors and slow cycle time. More recently, Seo in *2004* , isolated PDLSCs from normal impacted third molars, and using cloning techniques verified that only some of the progenitor cell strains of periodontal ligament can be considered stem cells. These periodontal adult stem cells have the morphological, phenotypic, and proliferative characteristics of adult Mesenchymal Stem Cells. Only these cells are capable of promoting turnover and tissue homeostasis, serving as a source of renewable progenitor cells generating cementoblasts, osteoblasts, and fibroblast throughout the adult life. In general, these cells require a suitable scaffold such as hydroxyapatite/ tricalcium phosphate to induce the formation of bone, cementum, and bone *in vivo*.[1]When *ex vivo* expanded PDLSCs are implanted *in vivo* with a suitable scaffold, atypical cementum/PDL like structure forms.

Periodontal regeneration requires consideration of many features that parallel periodontal development, including the appropriate progenitor cells, signaling molecules, and matrix scaffold in an orderly temporal and spatial sequence. It is clear that the current regenerative procedures are less than ideal but the identification of stem cells in human dental tissues in recent years holds promise to the development of novel, more effective approaches to periodontal regeneration and reconstructive therapy. With the identification of adult human stem cell populations residing in the periodontal ligament, the next phase is to determine the clinical utility of the cells.

USES / CLINICAL APPLICATIONS OF MESENCHYMAL STEM CELLS (MSC)

1. Dental and craniofacial tissue engineering.
2. Dental pulp applications.
3. Creation of artificial embryonic teeth primordia from cultured cells.
4. Cementoblast like cells applications.
5. Periodontal regeneration.

TISSUE ENGENEERING[12]: Tissue engineering is a contemporary area of science based on the principles of cell biology, developmental biology and biomaterials science to develop new procedures and biomaterials to replace lost or damaged tissues.

The three key elements for dental tissue engineering are

1. Signals for morphogenesis- BMPs, FGFs
2. Progenitor/stem cells- cells derived from marrow, dental pulp and PDL-derived cells.
3. Scaffolds of extracellular matrix components- collagens, fibronectin and proteoglycans.

PHOTODYNAMIC THERAPY (PDT)[13] :Photodynamic therapy is based on the principle that a photoactivatable substance (the photosensitizer) binds to the target cell and can be activated by light of a suitable wavelength. During this process, free radicals are formed (among them singlet oxygen), which then produce an effect that is toxic to the cell.

MECHANISM OF ACTION: Briefly, upon illumination, the photosensitizer is excited from the ground state to the triplet state. The longer life time of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of the cytotoxic species takes place during PDT while in this state only. The cytotoxic product, usually O2 cannot migrate >0.02mm after its formation, thus making it ideal for the local application of PDT without endangering distant molecules, cells, or organs.

ADVANTAGES: PDT is also beneficial during the maintenance of periodontal therapy because it may act on the biofilm and eliminate the need for the removal of additional root substance by mechanical retreatment. Thus, the patient may experience less dentinal hypersensitivity.

This therapy also serves as an adjunct to mechanical therapy in sites with difficult access.

ADVERSE EFFECTS: PDT has the potential of phototoxic and photo-allergic unwanted side effects

There may be impairment of benign oral flora which may lead to an overgrowth of a single resistant species

Histologically scar formation is evident

Has the potential of promoting genotoxic effects

GENE THERAPY[14]: Application of growth factors or soluble forms of cytokine receptors by gene transfer provides a greater sustainability than that of single protein application. Gene therapy may achieve greater bioavailability of growth factors within periodontal wounds, which may provide greater regenerative potential.

GENE DELIVERY METHODS: Genetic engineering approaches generally consist of two modalities: in vivo and ex vivo gene delivery. In the former, gene constructs, such as expression plasmid DNA or a viral particle, are physically entrapped within a scaffold or matrix. When the scaffold containing the gene constructs is implanted into the tissue defect, the host cells migrate into the implant, take up the
Gene constructs and starts to produce the encoded protein.

In the latter approach, cultured cells are transfected (in Nonviral delivery systems) or transduced (in viral delivery systems) with gene constructs in vitro before they are transplanted into the tissue defect.

GENE ACTIVATED MATRIX (GAM): It has been produced based on the principle of combination of naked DNA with a biodegradable carrier.

LIMITATION: Difficulties in the preparation of collagen based GAMs and the enormous amount of plasmid required may limit its clinical use.

Gene therapy studies utilizing bone morphogenetic proteins (BMPs) have also been performed and bone morphogenetic protein-7 (BMP-7) transduced fibroblasts were used to stimulate repair of alveolar bone wounds and represent the first successful evidence of periodontal tissue engineering employing ex vivo gene transfer of bone morphogenetic proteins.

ADVANTAGES: Safer and more cost-effective than cell-based therapies.

Mimic the complex natural process of periodontal tissue formation, because multiple genes, and multiple factors, can be delivered within the bone defect

RNA INTERFERENCE[14]: A conserved biological response to double-stranded RNA, known variously as RNA interference (RNAi) or post-transcriptional gene silencing, mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes. RNAi has been cultivated as a means to manipulate gene expression experimentally and to probe gene function on a whole-genome scale.

The phenomenon of RNAi was first discovered in the nematode worm *Caenorhabditis elegans* as a response to double-stranded RNA (dsRNA),which resulted in sequence-specific gene silencing. Following on from the studies of Guo and Kemphues, who had found that sense RNA was as effective as antisense RNA for suppressing gene expression in worms, Fire, Mello and colleagues were attempting to use antisense RNA as an approach to inhibit gene expression.

The RNA-mediated silencing process is defined as RNAi, a discovery for which Fire and Mellow received the 2006 Nobel Prize.

RNAi works through small RNAs of approximately 20 to 30 nucleotides that guide the degradation of complementary or semi complementary molecules of messenger RNAs (posttranscriptional gene silencing) or interfere with the expression of certain genes at the promoter level (transcriptional gene silencing).

RNAI IN PERIODONTAL REGENERATION: Through the silencing of genes that negatively control cell proliferation and cell differentiation or genes that induce inflammation or apoptosis, RNAi may favor tissue regeneration.

Tumor necrosis factor-α–targeted siRNA can suppress osteolysis induced by metal particles in a murine calvaria model, opening the way to the application of RNAi in orthopedic and dental implant therapy.

NANOTECHNOLOGY[15] : The term “nanotechnology” was coined by Prof. Kerie E. Drexler, a lecturer and researcher of nanotechnology. «Nano» is derived from the Greek word for “dwarf”. Nanotechnology is the science of manipulating matter measured in the manometer, roughly the size of 2 or 3 atoms. The basic idea of nanotechnology, used in the narrow sense of the world, is to employ individual atoms and molecules to construct functional structures.

"The science and technology of diagnosing treating and preventing disease and traumatic injury of relieving pain, and of preserving and improving human health, through the use of nanoscale structured materials, biotechnology and genetic engineering and eventually complex molecular machine system and Nanorobots".

NANOROBOTICS IN DENTISTRY: Nanorobots induce oral analgesia, Desensitize tooth, manipulate the tissue to re-align and straighten irregular set of teeth and to improve durability of teeth.

NANODENTISTRY: Nanodentistry will make possible the maintenance of comprehensive oral health by employing nanomaterials, including tissue engineering, and ultimately, dental nanorobots. New potential treatment opportunities in dentistry may include: local anaesthesia, dentition renaturalization, and permanent hypersensitivity cure, complete orthodontic realignments during a single office visit, covalently bonded diamondised enamel, and

continuous oral health maintenance using mechanical dentifrobots. When the first micro-size dental nanorobots can be constructed, dental nanorobots might use specific motility mechanisms to crawl or swim through human tissue with navigational precision, acquire energy, sense, and manipulate their surroundings, achieve safe
cytopenetration and use any of the multitude techniques to monitor, interrupt, or alter nerve impulse traffic in individual nerve cells in real time. These nanorobot functions may be controlled by an onboard nanocomputer that executes preprogrammed instructions in response to local sensor stimuli. Alternatively, the dentist may issue strategic instructions by transmitting orders directly to *in vivo* nanorobots via acoustic signals.

NANOTECHNOLOGY IN PERIODONTICS[16]

MECHANISM OF ACTION: Functions may be controlled by an onboard nanocomputer executing programmed instructions in response to local sensor stimuli. Alternatively, the dentist may issue strategic instructions by transmitting his orders directly to in vivo nanorobots via acoustic signals (e.g. ultrasound) or by other means.

TREATMENT OPPORTUNITIES IN PERIODONTICS:

Tooth Repair
Hypersensitivity Cure
Nanorobotic Dentifrice (Dentifrobots)

Nanotechnology will change dentistry, healthcare, and human life more profoundly than many developments of the past. As with all technologies, nanotechnology carries a significant potential for misuse and abuse on a scale and scope never seen before. However, they also have potential to bring about significant benefits, such as improved health, better use of natural resources, and reduced environmental pollution. Current work is focused on the recent developments, particularly of nanoparticles and nanotubes for periodontal management, the materials developed from such as the hollow nanospheres, core shell structures, nanocomposites, nanoporous materials, and nanomembranes will play a growing role in materials development for the dental industry.

PERIOPROTECT[17]: PerioProtect is a comprehensive method that is customized for individual patients to help manage biofilms, growing in the spaces or pockets between teeth and gum tissue. The overall goal of the Perio Protect Method is to manage oral biofilm with minimally invasive dentistry for lasting oral health.

The Method is a combination of treatments, including a non-invasive chemical debriding therapy used in conjunction with traditional mechanical debriding procedures. The chemical therapy involves a tray delivery of doctor-prescribed solutions to chemically debride biofilm from the periodontal pocket and alter the pocket's microbiological environment to disrupt biofilm growth.

MECHCANISM: The use of non-specific chemical agents to kill all of the planktonic cells, and some of the biofilm cells, in a particular ecosystem. This strategy, which has proven to be very successful in industrial applications, and in the protection of various catheters from bacterial colonisation and consequent infection, depends on the alteration of the microbial ecology of an ecosystem so that biofilm formation is minimised. Biofilm bacteria show the same susceptibility to non-specific oxidising agents as their planktonic counterparts, so that industrial biocides and

"catheter lock" solutions kill all of the planktonic cells and as many of the biofilm cells as their stochiometry allows. The regular application of 0.5 % bleach in the "Y" sets used to protect Tenchoff catheters from colonisation and infection has been successful for several years, in spite of the extreme susceptibility of the peritoneum to bacterial incursions.

The PerioProtect therapeutic system uses this concept, because it delivers peroxide and an antioxidant to the periodontal space at regular intervals, and kills the planktonic bacteria and enough of the biofilm bacteria to gradually alter the microbial ecology of this ecosystem. The direct result of this alteration in the microbial ecology of the periodontal space is a sharp reduction in the rate at which available surfaces are colonised; the relevant measurement is the rate of colonisation of an inert carrier material introduced into the area for a specified length of time. This measurement is clinically relevant because it is the inflammatory response of the gingival tissues to the presence of planktonic and detached biofilm cells that lies at the base of the aetiology of this, and all other, chronic biofilm infections.

Very Recent study[18] done by Mark S. Putt in 2012,showed that, the adjunctive use over three months of 1.7% hydrogen peroxide gel, locally administered using PerioProtect (prescription customized trays) in the treatment of subjects with moderate to advanced periodontitis, demonstrated statistically significant clinical improvements in pocket depths and bleeding when compared with SRP alone.

TRI-IMMUNOPHASIC THERAPY[19]: William Hoisington has developed a new technique for treatment that allegedly tackles the issue of periodontal disease in an entirely new way.

DEVELOPMENT OF TIP AND BOST: TIP and BOST (Bone One Session Treatment) have been elaborated and tested over the past several years on over 2,500 patients with remarkably consistent success in saving teeth thought lost, and limiting anaerobic bacteria generated inflammation to an acceptable minimum.

TECHNIQUE: First DNA test should be done to identify the bacteria to choose the antibiotic of choice.

Gingiva is intentionally stretched with the rounded back of curettes in a three stage process. The tissue becomes more stretched as the instruments advance down to the bone level.

Here the curettes are inverted to allow the rounded tip of the curettes to plasty the surface of the bone and remove any attached granulation tissue or degenerated attachment. The goal is a smooth, regular bone surface and fresh bleeding to flush out bacteria and toxins from the porosities, right where the immune reaction of inflammation can change into one of regeneration. This type of healing happens with the help of the stem cells from the periodontal ligament.

The clot that is firmly attached to the clean bone serves as a scaffold. The stem cells can move along it and up the root surfaces at the rate of 0.5mm per day for eight days and thicken the layer on the clot. To permit this activity it is also important to keep the epithelial attachment away from the roots. This is done with the oral hygiene technique that keeps the pocket open and also inhibits the reformation of the sticky layer.

As healing time increases, the pockets gradually fill in from the bottom with very dense, partially mineralised connective tissue in about four to six weeks, and finally will become acellular.

USE OF NEWER MOLECULES TO RESOLVE INFLAMMATION:

Resolvins (resolution - phase interaction products) are endogenous mediators that have an anti-inflammatory role. They have been found to be effective in preclinical models of asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, retinal disease etc. They offer an entirely novel biological approach in treatment of several inflammatory diseases. They reduce neutrophil trafficking, regulate production of cytokines and reactive oxygen species and have been successfully evaluated in ligature induced P gingivalis provoked animal model periodontitis. Prior application of Resolvin E1 prevented animal models from developing periodontitis in contrast to unprotected animals which showed progressive disease.[20]

THERAPEUTIC APPROACHES RECENTLY AVAILABLE TO CONTROL INFLAMMATION AND BONE RESORPTION.

Recently several medications targeted to suppress inflammation and bone resorption have been evolved and some of these are primarily directed at control of osteoporosis and rheumatoid arthritis. They include TNF-α inhibitors (Adalimumab, Etanaracept, Golimumab, and Infliximab), anti-cytokine agents (Anakinra, Tocilizumab, and AMG714) and inhibitor of RANK/RANKL (Denosumab). The use of some of these agents has been restricted to animal studies where protective effect on experimentally induced periodontitis lesions was observed. [21.]

CONCLUSION: Clinicians should continue to develop and enhance their diagnostic skills, assess factors that affect diagnosis and prognosis, formulate a comprehensive treatment plan, render appropriate treatment, evaluate the outcome and determine when periodontal care is indicated.

REFERENCES:

1. Wim Teughels, Mark Van Essche, Isabelle Sliepen, Marc Quirynen, Probiotics and oral healthcare. Periodontology 2000 2008;48:111–147
2. Harish K, Varghese T. Probiotics in humans – evidence based review. Calicut Medical Journal 2006;4(4):e3.
3. Suvarna VC and Boby VU. Probiotics in human health: A current Assessment. Current Science 2005;88(11):1744–1748.
4. Rajiv Saini, Santosh Saini, Sugandha. Probiotics: The Health Boosters. Journal of Cutaneous and Aesthetic Surgery 2009;2(2):112.
5. Deepa D, Mehta DS. Is the role of probiotics friendly in the treatment of periodontal diseases!! Journal of Indian Society of Periodontology 2009;13(1):30–31.
6. Choi1 & J. Seymour Vaccines against periodontitis: a forward-looking review J Periodontal Implant Sci 2010;40:153-163
7. Tibbetts & Shanelec Principles And Practice Of Periodontal Microsurgery Int J Microdent 2009;1:13-24.
8. Academy Report Lasers in Periodontics J Periodontal 2002;73:1231-1239
9. Douglas N, Dederich BS, Ronald D. Lasers in dentistry.JADA,2004:135:204-211.
10. Nitin Tomer New Innovative Technology: Waterlase in Periodontics People's Journal of Scientific Research Vol.3(1), Jan 2010
11. Buch A & Choksi D. Clinical Applications Of Stem Cells In Dentistry, J of Dental Science . 2010:1(1):26-29.

12. Grover et al. Future of Periodontal Regeneration. J Oral Health Comm Dent 2010;4:38-47
13. Malik et al. Photodynamic Therapy- A Strategic Review Indian J Dent 21(2):2010
14. Gregory J Hannon. RNA Interfernce. Review. Nature J.2002;418(11):244-251.
15. Jhaver H.M.Nanotechnology:The future of dentistry.Journal Nanoscience and Nanotechnology.2005:5:15-17
16. Anna V. Rabychuk, Ivan S. Chekman, Tetyana Yu. Nebesna.Nanotechnology and Nanoparticles in dentistry.J of Pharmacology and Pharmaceutics.2009:1:18-20.
17. Schaudinn C, Gorur A, Keller D, Sedghizadeh P, Costerton W. Periodontitis: an archetypical biofilm disease. J Am Dent Assoc 2009;140:978-86.
18. Mark S.Putt, Howard M.Proskin. Custom Tray Application of Peroxide Gel as an Scaling and Root Planing in the Treatment of Periodontitis:A Randomized, Controlled Three-Month Clinical Trial.J Clin Dent 2012:23:48-56.
19. William Hoisington New developments in perio: Tri-Immuno-Phasic therapy. Preventive Dentistry 2006 : 1 (2) :30-34.
20. Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, *et al*. RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis. FASEB J 2006;20:401-3.
21. Jayakumar.A.,Naveen.A, Haritha A.Novel and often bizarrae strategies in the treatment of periodontal diseases.Journal of Indian Society of Periodontology 2012:16:1:4-10.

A STUDY ON THE ADVERSE EFFECTS OF FORMALDEHYDE ON THE STAFF OF ANATOMY DEPARTMENTS WORKING IN VARIOUS MEDICAL COLLEGES

Krishnaiah M, Mrudula C.

1. Assistant Professor, MD, Department of Anatomy, Deccan College of Medical Sciences.
2. Assistant Professor, MD, Department of Anatomy, Apollo institute of Medical Sciences and Research.

CORRESPONDING AUTHOR
Dr. Mrudula,
Flat No-103, Plot No 592
Mountain view apt Aurora cly,
Rd no-3, Banjara Hills.
E-mail: drmrudula4@yahoo.com,
Ph: 0091 9849061616.

ABSTRACT: Formaldehyde is the widely used chemical for embalming cadavers, for fixation of tissues in histology, for fumigation of operation theatres and sterilization of surgical instruments . Formaldehyde cannot be absorbed through the skin but becomes vapor at room temperature. Anatomists, pathologists, forensic surgeons, staff of dissection hall , embalming laboratory , histology laboratory and medical students will get exposure of formaldehyde gas.

It is estimated that an anatomist gets exposed to formaldehyde (gas) for about 30,000 - 60,000 hours in his / her life time. Formaldehyde is the "drug of choice" for above purpose if it is used with proper precautions , but it can produce harmful effects on many systems as on skin, respiratory system, central nervous system , gastro intestinal system and produce minor to major problems ranging from burning of eyes to carcinomas.

A cross sectional survey is conducted by selecting 120 members of anatomy staff (Both teaching and nonteaching) who are working in colleges in and around Hyderabad city. The effect of formalin on above group is studied. Results of this survey are grouped system wise and known protective measures are discussed.
KEY WORDS: Formalin, anatomy staff, systemic effects.

INTRODUCTION: Formalin, an aqueous solution of formaldehyde, is the chemical most commonly used for embalming. In 1867, the German chemist August Wilhelm von Hofmann identified formaldehyde, which is a colorless, flammable gas that is quite soluble in water . Formaldehyde is colorless at room temperature and has an irritating, pungent smell. It is commercially obtainable as formalin, which contains 37% by weight or 40% by volume of formaldehyde gas in water. In the body, formaldehyde quickly metabolizes to formic acid. The measurement of formate (formic acid minus 1 hydrogen ion) levels indicates the severity of formaldehyde intoxication.

Faculty of anatomy, staff of histology and embalming laboratory, medical students are getting exposure of formaldehyde gas. Gas evaporated from embalmed bodies in an important factor which is responsible for its harmful effects.

Concentration of this formaldehyde gas will be highest as the bodies are removed from the tanks and it slowly decreases with in 30mts to 1 hr. Authorities of America have

set a ceiling of 0.3 ppm for formaldehyde gas and authorities of Japan set the ceiling between 0.008 to 0.3 ppm. The ceiling levels are assessed by taking air samples from different places by using a diffusive sampling device for organic carbonyl compounds and analyzed through high performance liquid chromatography . The recommended airborne exposure limits are 0.016 ppm averaged over a 10- hour work shift and 0.1 ppm not to be exceeded during any 15- minute work period (Agency for Toxic Substances and Disease Registry [ATSDR], 1999).

Formaldehyde gas produces its effects on many systems. It produces various effects like

(1) Irritation of eyes
(2) Irritation of air passage mainly upper respiratory tract
(3) Contact allergic dermatitis
(4) Vertigo
(5) Convulsions
(6) Pain abdomen nausea, vomiting
(7) Renal failure

A cross sectional survey is conducted on anatomy staff and medical students of various colleges of Hyderabad and results as per system are grouped, analyzed to assess the magnitude of the problem.

MATERIAL AND METHOD: A group of 120 members are selected from Deccan College of Medical Sciences, osmania medical college and Gandhi Medical Colleges. This group Is composed of 40 Teaching staff members, 20 Non teaching staff members and 60 Medical students. They are working in anatomy department for a period ranging 1 year to 25 years.

A format as shown was prepared. It includes

1) Preliminary data
2) Protective measures practicing at the both departmental and individual levels
3) Effects of formalin on various systems like mucosa of the eye, nose, skin, gastro intestinal, renal and reproductive systems.

Maximum care is taken to exclude problems arising from other causes and importance is given to treatment history. General and systemic examinations are conducted for required members. Percentages of effects of formaldehyde on various systems are calculated and presented.

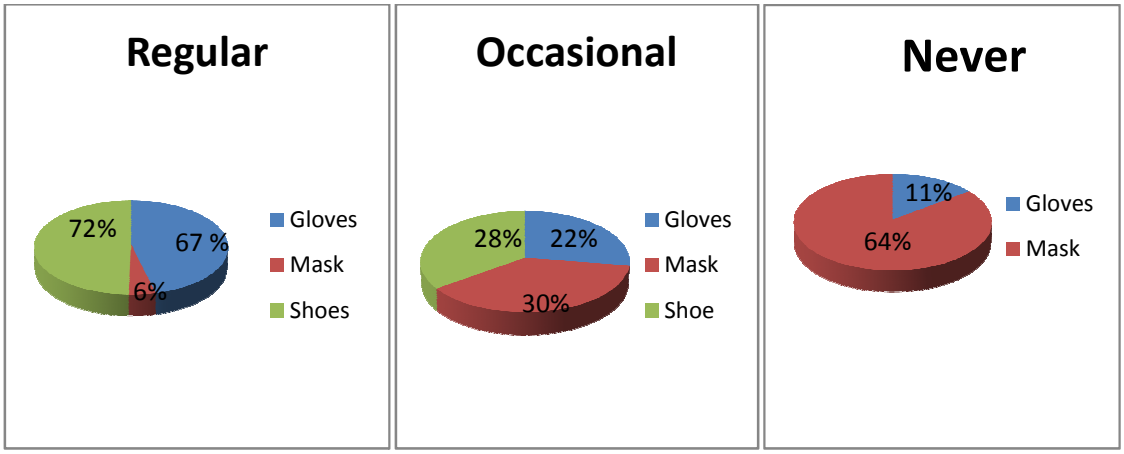
OBSERVATIONS:

Departmental protective measures practicing

1.Measures against fire risk	No
2.Checking of formalin gas levels in dissection hall	No
3.Checking of formalin gas levels individually	No

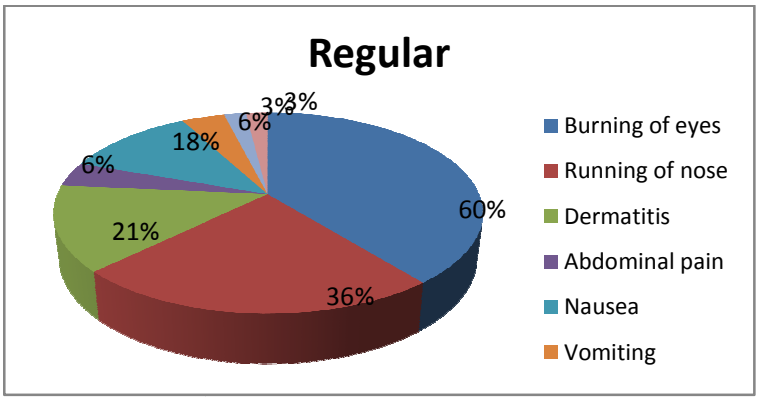
Personal protective measures practicing

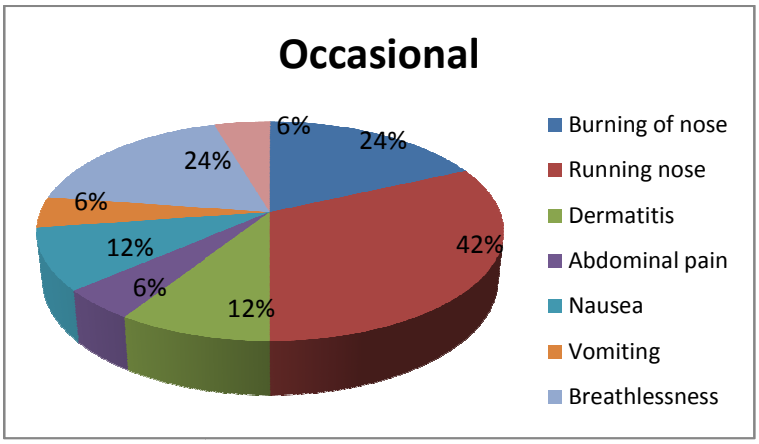
	Regular	Occasional	Never
1.Use of gloves	67%	22%	11%
2.Use of mask	6%	30%	64%
3.Use of shoes	72%	28%	0

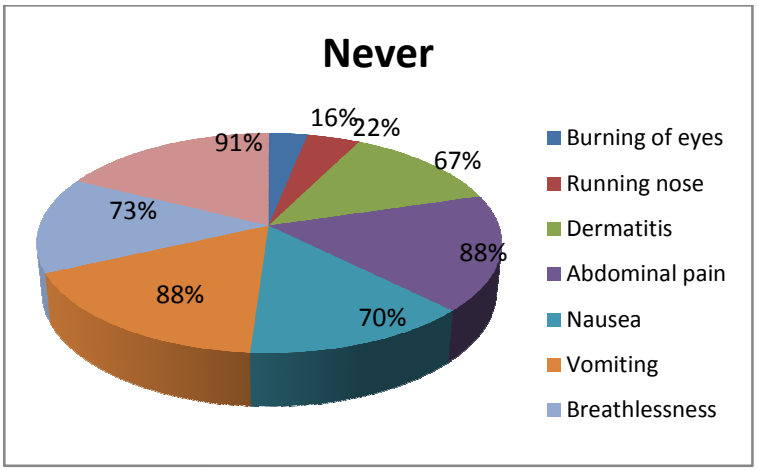


SYMPTOMATIC HISTORY

	Regular	Occasional	Never	Treatment
1.Burning of eyes	60%	24%	16%	
2.Running nose	36%	42%	22%	
3.Skin Contact allergic dermatitis	21%	12%	67%	+
4.Gastro intestinal system:				
a)Abdominal pain	6%	6%	88%	
b)Nausea	18%	12%	70%	
c)Vomiting	6%	6%	88%	
5.Respiratory system: Breathlessness	3%	24%	73%	
6.Renal system: a)Urine output	Normal			
b)Renal pain	0	0	0	
7.Central nervous system:				
a)Vertigo	3%	6%	91%	
b)Convulsions	0	0	0	







DEPARTMENTAL PROTECTIVE MEASURES PRACTICING: All dissection halls are having exhaust fans and windows for ventilation but no institution(Deccan college of medical sciences, Osmania medical college and Gandhi medical college where the study is done) is having facilities for estimation of formaldehyde gas levels in the hall or personal exposure levels. No institution is having preventive measures against fire risk, but no fire accident is noted till now.

PERSONAL PROTECTIVE PRACTICING: Most of the members are using shoes, 67% are using gloves, but usage of masks is very less i.e. 6% regularly.

SYSTEMIC EFFECTS:67% members are suffering from burning eyes and 36% are troubled by running nose regularly.

Abdominal pain, nausea, vomiting are troubling 6%, 18%, and 6% of staff respectively.

Dermatitis is causing a problem in 21% of members regularly and 12% of members occasionally. They are given history of taking treatment repeatedly.

About 3% of members are suffering from symptoms of breathlessness immediately after exposure to formaldehyde gas.

One person had corneal ulceration due to direct fall of formaldehyde in to eyes and had undergone surgery for the same.

10% of members suffering from sinusitis gave the history of exacerbations of symptoms by exposure to formaldehyde gas.

DISCUSSION: Formaldehyde gas produces many harmful effects on various systems. This is studied in 100 members including staff and medical students of various colleges of Hyderabad. The findings are tabulated and discussed.

- Pabst observed Squamous cell carcinoma in rats and mice in experiments by high exposure of formaldehyde gas , but epidemiological studies didn't provide evidence for carcinogenicity in man.
- Miziuki M T Proved atopic individuals show exacerbation of basic allergic symptoms by exposure of formaldehyde which is seen in most of the staff members in our study.
- Tanake measured formaldehyde concentration in dissection hall and found it was high (0.062 ppm after l0min) and low (0.11ppm after 30 min)
- Ohnichi K et al compared formaldehyde exposure in dissection hall and with personal exposure level and found out personal exposure level is higher. It is described that the indoor concentrations varied depending on the contents of laboratory sessions and seemed to increase when body cavity or deep structures were being dissected.
- White head mc, Savoia mc- Evaluated methods to reduce formaldehyde in the dissection hall by using "infutrance" and "perfect" solutions along with formaldehyde solution.
- Medical Management Guidelines for Formaldehyde gives permissible exposure limit as 0.75 ppm (averaged over an 8- hour work shift) and short -term exposure limit as 2 ppm (15 minute exposure) . The health effects may be

narrowing of the bronchi and an accumulation of fluid in the lungs ,corrosive injury to the gastrointestinal tract, especially the pharynx , epiglottis , esophagus , and stomach. The systemic effects are primarily due to its metabolic conversion to formate , and may include metabolic acidosis, circulatory shock, respiratory insufficiency , and acute renal failure. It is a potent sensitizer and a probable human carcinogen.

Present study shows 84% are suffering from mucosal irritation (eye and nose) , 21% are suffering from skin allergy, 30% are suffering from gastro intestinal symptoms regularly , 3 % are complaining of shortness of breath and 3% are suffering from giddiness immediately after exposure to formalin gas.

It is noticed that a staff member suffered with corneal ulceration by direct fall of formaldehyde into eyes who is been operated for the same.

CONCLUSION: Formaldehyde gas is undoubtedly showing its harmful effects on various systems which can be prevented by some simple and some difficult preventive measures as

- Taking out the cadavers from tanks well before starting of dissection (As peak levels of formaldehyde gas before 30 min).
- By keeping windows open and exhaust fans on. The gross anatomy laboratory should have a standard ventilation system. According to the American Conference of Governmental Industrial Hygienists (2001), the ventilation rate should exceed 15 room changes per hour.
- By using masks containing activated carbons.
- By taking care formalin not to fall into eyes.
- Adopting special care by atopic individuals.
- Using perfect solution and infutrance solutions for embalming the bodies in addition to formaldehyde.
- Having facilities to check formalin gas levels in dissection halls and individual exposure levels.
- The bucket at the end of the cadaver table should be emptied frequently.

REFERENCES:

1. Mizuki K T suda T (2001) Relationship between atopic factors & physical symptoms induced by formaldehyde gas by exposure to it in dissection course. Arerugi. 2001 Jan ;50 (1) :21 - 8
2. Ohinchi K et al (2006) Formaldehyde in gross anatomy lab – personal exposure level is higher than indoor concentration. Environ Sci Pollut Res Int. 2006 Mar; 13 (2):120-4
3. Pabst R (1987) Exposure to formaldehyde in anatomy - an occupational hazard, Anat Rec 1987 Oct; 219 (2): 109-12
4. Tanake (2003) Formaldehyde exposure levels and exposure control measures during anatomy dissecting course, Kaibogaku Zasshi 2003 Jun ;78 (2) ;43 -51
5. White head mc, savoia mc –evaluation of methods to reduce formaldehyde levels of cadavers in the dissection hall. Clin Anat. 2008 Jan ;21(1): 75-81
6. Balmes , J. (2004). Formaldehyde. In K . R. Olson (Ed.), Poisoning and drug overdose (5th ed., pp. 206-208). New York : Mc Graw Hill.
7. Agency for Toxic Substances and Disease Registry. (2008). Medical management guidelines for formaldehyde (HCHO). Atlanta: Division of Toxicology and Environmental Medicine.
8. Levels of formaldehyde, phenol and ethanol in dissection Room air and measures for reduction: japanese journal of occupational medicine and traumatology : Vol. 54 no. 1 january 2006

CLINICO-PATHOLOGICAL PROFILE OF MASTALGIA IN AND AROUND KANPUR

S. K. Katiyar, Sanjay Kumar Nigam, P. K. Omar

1. Associate Professor, Department of General Surgery, Rama Medical College Hospital & Research Centre, Kanpur.
2. Associate Professor, Department of Pathology, Rama Medical College Hospital & Research Centre, Kanpur.
3. Assistant Professor, Department of Anaesthesia, Rama Medical College Hospital & Research Centre, Kanpur.

CORRESPONDING AUTHOR
Dr. S. K. Katiyar,
FIAGES, 675,
Singhpur, Kanpur-208017,
E-mail: drskkatiyar59@rediffmail.com,
Ph: 0091 9415044878.

ABSTRACT: Mastalgia is a general term used to describe number of conditions, in which pain is present in one or both breast (s). It is the commonest breast symptom presenting in surgical clinics. Most patients come to the Doctor, because of the fear of malignancy. However the ratio of benign to malignant cases in clinics is 10:1. Simply 3 major type of breast pain may be recognized Cyclical, Non-cyclical pain; extra mammary pain Aim of This study was focused to study the cause of mastalgia i.e. to correlate the clinical diagnosis to the histological examination and conservative management. Female patients between 15 years – 55 years of age with complain of pain in breast were accepted for study. A thorough general as well as local examination was done. Mammography was done in patient above 35 years of age.

The treatment protocol is decided, all patient coming to OPD in first 15 Days of month were given Evening primrose oil in dose of 500 mg 2 cap thrice a day for a period of two months. All those coming in next half were treated with danazol 200mg /day for two months. Patients were called to OPD on every 15th day to assess the response and also to check that the patient was appropriately following the given treatment. The exact cause of mastalgia is still unknown. Severe mastalgia can however disrupt normal life by interfering with sleep, relationship with husband and children and may also affect occupation of working women. In our study we have tried to treat patient with cyclical mastalgia and true mammary non cyclical mastalgia patients using, Evening primrose oil (EPO) and danazol as line drugs. Those not responding were treated with Tamoxifen or Bromocriptine randomly (IInd) line therapy. Danazol and EPO were used again by crossover as IIIrd line therapy.

Mastalgia is a common symptom reported in surgical OPD. The highest incidence of mastalgia was found in patients of 36-45 age groups. Cyclical mastalgia was the commonest type constituting 63.75%. Mastalgia is more commonly seen in left than right breast (40% vs 28.75%). Mastalgia was more commonly observed in multiparous we conclude that Danazol seems to be best available drug for both cyclical and non cyclical mastalgia. However, Tamoxifen approaches the same efficacy in both groups; in fact it has a better response in non cyclical mastalgia group. EPO is better drug for young and for women who do not want their period to be disturbed.

KEY WORDS: Mastalgia, Tamoxifen, Danazol.

INTRODUCTION: Mastalgia is a general term used to describe number of conditions, in which pain is present in one or both breast (s). It is also called Muzodynia. It is one of the most common presenting symptoms of benign breast diseases, which may be cyclical or continuous. **Morrow M** [1] in a study experienced the prevailing apathy towards females with mastalgia. It is the commonest breast symptom presenting in surgical clinics. Most patients come to the Doctor, because of the fear of malignancy. However the ratio of benign to malignant cases in clinics is 10:1. **Adner DN et al** [2] reported that patients with severe mastalgia interfered with sexual activity, physical activity and with social activity. The clinical picture can be divided into cyclical and non- cyclical patterns of pain by studying the simple clinical history. Despite the lack of concrete evidence, it seems likely that breast pain with cyclical pattern is of hormonal origin. Breast pain especially cyclical mastodynia is rarely associated with malignant neoplasm of breast. Patients with mastalgia require treatment but because of spontaneous resolution of pain due to hormonal events like pregnancy and menopause the results of therapeutic response is difficult to assess.

Various therapies ranging from local support vitamin B_2, B_6 E and C, diuretics, NSAIDs, progestational agents thyroxine, plant extracts like vitex agnus castus, evening primrose oil (EPO), Danazol, Bromocriptine, Tamoxifen and lately LHRH analogues have been used to treat mastalgia. There still exists a small but significant group which does not respond to any form of medical treatment this may be due to resistant from or poor diagnostic classification. **Aim** of This study was focused to study the cause of mastalgia i.e. to correlate the clinical diagnosis to the histological examination and conservative management. In order to randomize treatment it was suggested that clinical entities can be distinguished as: **Preece et al** [3]

1. Cyclical pronounced
2. Duct ectasia/ periductal mastitis
3. Tietze's syndrome
4. Trauma
5. sclerosing adenosis
6. Cancer
7. Idiopathic
8. Miscellaneous.

Fentiman [4] found that simply 3 major type of breast pain may be recognized.

a). Cyclical
b) Non-cyclical pain
c) Extra mammary pain

MATERIAL AND METHODS: Female patients between 15 years – 55 years of age attending the Outpatient Department of surgery, Rama medical college & hospital with complain of pain in breast were accepted for study. A detailed history was taken and noted. It was made sure that the patient had not taken hormonal therapy for the last 6 weeks. Also that the patient's were not pregnant and did not wish to become so within a period of 6 months. Patient who had undergone hysterectomy were excluded from study. For our study the patient had to fulfill all /any of the following criteria.

- The pain had to be of such severity that the physical activity of patient was curtailed.
- The patient had persistent marked pain throughout menstruation cycle.

- The pain was not relieved by mild analgesic (NSAID)
- The pain had to be episodic and exacerbated during the cycle.

A thorough general as well as local examination was done. Mammography was done in patient above 35 years of age. If there was any suspicion a Fine Needle Aspiration Cytology was asked for. If all clinical as well as investigative procedures confirmed that we were dealing with a Benign intramammary disease causing mastalgia the case was deemed fit for our study. We divided the patients into three groups.

Group I cyclical mastalgia
Group II Non-cyclical mastalgia
Group III Post menopausal mastalgia.

The patients were asked to fill up a linear pain scale to the amount of pain they at every 15 days interval for 3 months. The total score was kept 16. The patients were examined each month to study the linear pain scale and to look for any side effect.

TREATMENT: The treatment protocol is decided, all patient coming to OPD in first 15 Days of month were given Evening primrose oil in dose of 500 mg 2 cap thrice a day for a period of two months. All those coming in next half were treated with danazol 200mg /day for two months. Patents with extra mammary pain were offered NSAIDs and local anaesthetic. Patient were asked to make a response chart for their symptoms during the therapy. Keeping CBS scoring system.

CBS I Excellent response no residual symptom
CBS II Substantial response leading some pain considered tolerable.
CBS III Poor response – Substantial pain
CBS IV No response.

Patients were called to OPD on every 15th day to assess the response and also to check that the patient was appropriately following the given treatment. After two months of therapy the score was assessed. If the score were CBS II the same therapy was continued till remission occurs. However if the score was CBS III, IV second line drugs bromocriptine and tamoxifen were used randomly.

Bromocriptine in dose of 1.25-2.5 mg/day
Tamoxifen 20 mg/day

Response was assessed using the same score after 2 month of therapy.

Those cases not responding to the above were again treated with Danazol/ EPO, response assessed after two months using same score.

RESULTS: A total of 104 patients complaining of severe breast pain were examined during the span of 12 months (Nov 2011 – OCT 2012). Out of these 80 cases were deemed fit for the study as they fulfilled the criteria mentioned in material and methods.

TABLE-I AGE

AGE GROUP	NUMBER	PERCENTAGE (%)
15-25	15	18.5%
26-35	30	37.5%
36-45	31	38.75%
46-55	4	5%
TOTAL	80	100%

TABLE –II DISTRIBUTION OF CASES.

TYPE	NUMBER	PERCENTAGE (%)
Cyclical mastalgia	51	63.75%
Non cyclical mastalgia	24	30%
Extramammary pain	5	6.25%
TOTAL	80	100%

TABLE- III RESULT OF Ist LINE THERAPY FOR CYCLICAL MASTALGIA-

DRUG	**NUMBER**	**RESPONSE**	**NO RESPONCE**
EPO	26	9(34.6%)	17(65.38%)
DANAZOL	25	17(68%)	8(32%)
Total	**51**	**26**	**25**

TABLE –IV RESULT OF Ist LINE THERAPY FOR NON CYCLICAL MASTALGIA

DRUG	**NUMBER**	**RESPONSE**	**NO RESPONCE**
EPO	12	2(16.66%)	10(83.33%)
Danazol	12	4(33.33%)	8(66.66%)
	24	6(25%)	18(75%)

TABLE-V RESULT OF IInd LINE THERAPY FOR CYCLICAL MASTALGIA

DRUG	**NUMBER**	**RESPONSE**	**NO RESPONCE**
Tamoxifen	15	10(66.66%)	5(33.33%)
Bromocriptine	10	4(40%)	6(60%)
	25	14	11

TABLE-VI RESULT OF IInd LINE THERAPY FOR NON CYCLICAL MASTALGIA

DRUG	**NUMBER**	**RESPONSE**	**NO RESPONCE**
Tamoxifen	9	4(44.44%)	5(55.53%)
Bromocriptine	9	1(11.11%)	8(88.88%)
Total	**18**	**5**	**13**

TABLE- VII RESULT OF CROSSOVER THIRD LINE THERAPY

DRUG	NUMBER	RESPONSE	NO RESPONCE
Danazole used in patients not responding to EPO	6	4	2
EPO used in patients not responding to Danazole	5	1	4
Non cyclical Danazol	10	4	6
EPO	3	0	3

DISCUSSION: The exact cause of mastalgia is still unknown. Severe mastalgia can however disrupt normal life by interfering with sleep, relationship with husband and children and may also affect occupation of working women. In our study we have tried to treat patient with cyclical mastalgia and true mammary non cyclical mastalgia patients using, Evening primrose oil (EPO) and danazol as line drugs. Those not responding were treated with Tamoxifen or Bromocriptine randomly (IInd) line therapy. Danazol and EPO were used again by crossover as IIIrd line therapy. We found that 38.75% of patients complaining of mastalgia were uncommon in age less than 25 years (18.75%) and above 45(5%). Mean age of mastalgia was 33-40 years. In his study of 232 patients at Welsh national school of medicine **Preece et al**[5] found that most frequent age group complaining of mastalgia was 31-45 years (45) closely followed by 41-45 years (40), and 36-40(32). In another study by **Wisbey et al**[6] Welsh national school of medicine at Cardiff (1983) it was seen that in patients of cyclical mastalgia mean age was 35.7(±8.7),where as in patients with non cyclical mastalgia mean age was (42.5±9.8) years. Extra mammary mastalgia was seen in age group (40±10.2) years. In our study we found 88.25% of cases were married out of which 8.30% were nullipara, 29.57% were unipara and 52.11% were multipara, 91% of the parous women breast fed their children. These findings disapprove the earlier belief that the mastalgia was more common in "frustrated unhappy nullipara" as said by **Jeffcoate et al** [7].

Our study recommended the old finding that mastalgia was more common on left side **Preece et al** [5], 40% of cases had pain in left breast, 30% had pain in right side and 30% had pain bilaterally. However, there was no significant difference in side of pain in patients of cyclical mastalgia. Most of the patients in this study were non vegetarians. This complements the earlier belief that mastalgia is seen more commonly in people with deranged lipid profile due to inadequate intake of essential fatty acids. **Gateley et al**[8]**.** In this study we found fibroadenosis (57.5%) as the commonest cause of mastalgia. Fibroadenosis along with fibroadenoma was present in 16.25% cases. Fibroadenosis with hyperplasia and cystic changes were present in 15% cases. This substantiates that mastalgia can be present in normal breast tissue and the whole process is an aberration of normal development and involution (AND) rather than a disease process. **Shukla HS et al** [9] in his study found that patients showing fibrocystic disease and fibrosis showed poor response to therapy.

Pye et al [10] studied effect of EPO, Danazol and Bromocriptine in a randomised trial in patients with mastalgia. They observed grade I or II response in 77% cases with cyclical mastalgia (Danazol 70%, EPO 45% and 47% response to Bromocriptine placebo response was just 19%). In our study a grade I or II response was seen with 50.98% patients using Ist line

therapy, danazol in 17/25(68%) of patients with cyclical mastalgia, however, 32% patients did not respond to treatment using 200mg/day dose. Most of the patients who responded were with two months. Only 34.6% patients responded to EPO using 3gms/day dose for 2 month, 65.38% did not respond. However, 49.02% patients required IInd line drugs. In a study **Mansel et al[11]** found that response rate to danazol is not significantly altered when used in dose of 200mg/day or 400-600mg/day. It has been unanimously agreed that dose of 200mg/day is adequate.

CONCLUSION: Mastalgia is a common symptom reported in surgical OPD. The highest incidence of mastalgia was found in patients of 36-45 age groups. A most 76% patients were in third to fifth decade of their life. Cyclical mastalgia was the commonest type constituting 63.75% followed by non cyclical intramammary pain which was present in 30% cases. Extra mammary pain was seen in 6.25% cases. Mastalgia was more common in married women probably reflecting early age of marriage in our setup. Mastalgia is more commonly seen in left than right breast (40% vs 28.75%).Mastalgia is more commonly seen in premenopausal women than postmenopausal women (95% vs. 5%). Most of the women had normal menstrual history. Mastalgia was more commonly observed in multiparous and uniparous women (52.11% & 29.57%). Mastalgia was more commonly seen in non vegetarians (63.7% vs 36.25%). FNAC reports proved that fibroadenosis was the cause of pain in 88.75% cases and fibroadenoma alone was in 5% cases.

EPO when used as Ist line therapy in cyclical mastalgia, dose of EPO 3gm/day for 2 months is effective in attaining useful response in 4.6%. Danazol when used in dose of 200mg/day for 2 months is effective attaining useful response in 68% cases. However in non cyclical mastalgia the results were 16.66% and 33.33% for EPO and danazol respectively. After use of third drug (crossover of groups) a response of 45.45% was seen in patients with cyclical mastalgia and 30.76% in non cyclical mastalgia. 15/80 patients were resistant to hormonal manipulation.

We conclude that Danazol seems to be best available drug for both cyclical and non cyclical mastalgia. However, Tamoxifen approaches the same efficacy in both groups; in fact it has a better response in non cyclical mastalgia group. EPO is better drug for young and for women who do not want their period to be disturbed.

BIBLIOGRAPHY:

1. Morrow M The evaluation of common breast problems. Am Fam Physician. 2000 Apr 15; 61(8):2371-8, 2385.
2. Ader DN, Shriver CD. Cyclical mastalgia: prevalence and impact in an outpatient breast clinic sample. J Am Coll Surg. 1997 Nov; 185(5):466-70.
3. Preece PE, Mansel RE, Bolton PM, Hughes LM, Baum M, Gravelle IH. Clinical syndromes of mastalgia. Lancet. 1976 Sep 25; 2(7987):670-3.
4. Fentiman IS, Caleffi M, Hamed H, Chaudary MA Dosage and duration of tamoxifen treatment for mastalgia: a controlled trial. Br J Surg. 1988 Sep; 75(9):845-6.
5. Preece PE, Masel RE, Hughes LE. Mastalgia Psychoneurosis or organic disease? Br, Med.; 1:29-30, 1976.
6. Wisbey JR, Kumar S, Mansel RE, Peece PE, Pye JK, Hughes LE. Natural history of breast pain. Lancet. 1983 Sep 17; 2(8351):672-4.

7. Jeffcoate N, Principles of Gynaecology, London Butterworths, 550,1975
8. Gateley CA, Maddox PR, Pritchard GA, Sheridan W, Harrison BJ, Pye JK, Webster DJ, Hughes LE, Mansel RE.Plasma fatty acid profiles in benign breast disorders. Br J Surg. 1992 May; 79(5):407-9.
9. Shukla HS, Bhargava P.: Successful treatment of mastodynia BJS; 83:220, 1971.
10. Pye JK, Mansel RE, Hughes LE. Clinical experience of drug treatments for mastalgia. Lancet. 1985 Aug 17; 2(8451):373-7.
11. Mansel RE, Wisbey JR, Hughes LE. Controlled trial of the antigonadotropin danazol in painful nodular benign breast disease. Lancet. 1982 Apr 24; 1(8278):928-30.

INCIDENCE OF NON-CANDIDA ALBICANS IN PATIENTS WITH URINARY TRACT INFECTION WITH SPECIAL REFERENCE TO SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY

Ragini Ananth Kashid, Sandhya Belawadi, Gayatri Devi, Indumati

1. Assistant Professor, MD, Department of Microbiology, M. S. Ramaiah Medical College, Mathikere, Bangalore.
2. Professor and HOD, MD, Department of Microbiology, M. S. Ramaiah Medical College, Mathikere, Bangalore.
3. Professor, MD, Department of Microbiology, M. S. Ramaiah Medical College, Mathikere, Bangalore.
4. Consultant Microbiologist, MD, Metropolis Microbiology Lab, M. S. Ramaiah Medical College, Mathikere, Bangalore.

CORRESPONDING AUTHOR
Dr. Ragini Ananth Kashid, M.B.B.S. M.D. (Microbiology)
Flat No. A-601, Vasundhara Ashwini Apartments,
No. 14, Ring Road, Banashankari II stage
Bangalore 560070,
E-mail: ragini_rajgopal@hotmail.com,
Ph: 0091 96203 80002.

ABSTRACT: BACKGROUND AND OBJECTIVES: Fungal urinary tract infections have become frequent, as a result of increased use of broad spectrum antibiotics, corticosteroids, immunosuppressive drugs and bladder catheters in acute care settings. The associated risk factors which are seen in cases of candiduria are: antibiotic therapy, female gender, urinary catheterization, surgical procedure and extended hospitalization. Candiduria has become a potential source of morbidity and mortality if untreated. We undertook a prospective study to note the incidence of non-Candida albicans in patients with urinary tract infection with special reference to speciation, antifungal susceptibility and the associated risk factors. **METHODS:** Candida species isolated from urine samples of patients with urinary tract infection were subjected to speciation using standard yeast identification protocol and CHROM agar. Antifungal Susceptibility testing was done by the disc diffusion method to amphotericin B and fluconazole. Clinical details and risk factors of the patients were noted down. **RESULTS:** Among the 60 culture positive cases, six Candida species which were isolated are : C.tropicalis (66.66%), C.albicans (13.33%), C.parapsilosis (8.33%), C.glabrata (6.66%), C.kefyr (3.33%) and C.guilliermondii (1.66%) The susceptibility pattern showed, that of the 60 isolates, 40% were resistant to fluconazole. No resistance was seen to amphotericin B. **CONCLUSION:** Isolation of non-Candida albicans species was more than Candida albicans. Candida tropicalis was the predominant isolate. The following risk factors were noted: 43.33 % of the patients had diabetes mellitus, 30%had history of prolonged antibiotics (cephalosporin and aminoglycosides), 16.66% had underlying renal pathology, 3.33% had post –renal transplant status, 1.66% were on steroids, 1.66%had pregnancy and 3.33% had no identifiable risk factors.20% patients had an indwelling catheter in them. The antifungal resistance was more in the non-Candida albicans species than in Candida albicans. Hence there is a need for speciation, susceptibility testing of Candida species.

KEY WORDS: Candiduria, antifungal susceptibility testing, Candida speciation, CHROM agar, Non–Candida albicans, risk factors.

INTRODUCTION: The significance of the presence of fungi in urine is not clearly understood. Fungal growth may represent contamination, bladder colonization, and primary infection or disseminated candidiasis.[1] Fungal urinary tract infections have become frequent as a result of increased use of broad spectrum antibiotics, corticosteroids, immunosuppressive drugs and bladder catheters in acute care settings. [2]The associated risk factors which are seen in cases of candiduria are: antibiotic therapy, female gender, urinary catheterization, surgical procedures and extended hospitalization. [3, 4, 5] Candiduria has become a potential source of morbidity and mortality if untreated.[6] We undertook a prospective study to note the incidence of non-Candida albicans in patients with urinary tract infection with special reference to speciation, antifungal susceptibility and the associated risk factors.

MATERIALS AND METHODS: A prospective study, for a period of one year, was conducted in the Department of Microbiology, in which all patients who presented with signs and symptoms of urinary tract infection (bladder discomfort, low grade fever, and leucocytosis) were included in the study. The urine samples from these patients were collected with standard aseptic precautions. Information was collected, on the method in which the urine sample was collected(clean catch, suprapubic, urinary catheter).Data such as age, sex, prolonged antibiotic therapy, antifungal therapy and other possible risk factors were noted. The urine samples were processed within 2-4 hours. Candiduria was diagnosed by urine culture. The urine samples were spread by calibrated loop (0.01 ml) as per standard protocol for urine culture. Plates were incubated aerobically at 37 ^{0} C and read at 24 hours. Candida species isolated on culture plates, with a colony count >1000 colonies /ml, were considered indicate an active UTI. [6]

The Candida isolates thus isolated, were identified to species level ,based on Grams Stain, KOH mount, India ink preparation , Culture on Sabouraud's Dextrose Agar, Germ Tube test, Urea hydrolysis, Cornmeal agar morphology (Dalmau technique), Sugar Fermentation Test, Sugar assimilation test (Auxanographic Plate Method) [8,9] and CHROM Agar morphology.[10, 11]

ANTIFUNGAL SUSCEPTIBILITY TEST :(Disc Diffusion test by Shadomy Ingroff method) was done, using Fluconazole (10 µg) and Amphotericin –B (100 units) discs. The inoculum was standardized to 0.5 Mc Farland units. Candida albicans ATCC 90028 was used as control. [12, 13, 14] The antifungal discs and the dehydrated culture media were procured from Hi- Media, Mumbai.

STATISTICAL ANALYSIS: Descriptive statistics were used to summarize continuous measures. The data was evaluated using 2x2 contingency tables using chi –square test. P values <0.05 were considered to be significant.

RESULTS: In a period of one year, a total of 4380 urine specimens were processed in the Microbiology Laboratory, of which 60 urine samples yielded Candida species. Of these 60 isolates, 48 isolates had been isolated where the specimen was collected by midstream clean catch urine specimen and 12 by indwelling catheter. In this study, there was no urine sample collected by suprapubic puncture method.

The isolation rate of Candida species from urine samples was 1.37 %

65% (39/60) Candida isolates were isolated from males and 35 %(21/60) from females. Among the 60 Candida isolates the species wise distribution was as follows: 66.66 %(40/60) were C.tropicalis, 13.33 %(8/60) were C. albicans, 8.33 %(5/60) were C. parapsilosis,

6.66% (4/60) C. glabrata, 3.33% (2/60) C. kefyr and 1.66% (1/60) were C. guilliermondii. Refer Table 1.The isolation rate of non- Candida albicans species from urine samples was 1.18 %

The following risk factors were noted: 43.33 % (26/60) of the patients had diabetes mellitus, 30%(18/60) had history of prolonged antibiotics (cephalosporin and aminoglycosides), 16.66%(10/60) had underlying renal pathology, 3.33%(2/60) had post – renal transplant status, 1.66% (1/60) were on steroids, 1.66%(1/60) had pregnancy and 3.33%(2/60) had no identifiable risk factors.20% (12/60) patients had an indwelling catheter in them. Refer Table 2.

In the group of patients that had Diabetes mellitus as a risk factor, 61.53 %(16/26) of the isolates were C. tropicalis, 11.53% (3/26) were C. albicans, 11.53% (3/26) were C. glabrata, 11.53 %(3/26) C. parapsilosis and 3.84% (1/26) of C. kefyr.

In the group that had prolonged medication as a risk factor, 77.77 % (14/18) were C. tropicalis, 11.11% (2/18) each were C. albicans and C. parapsilosis.

Among the 10 culture positive cases, of candiduria in patients with underlying renal pathology (other than complications of diabetic nephropathy) 70 %(7/10) were C. tropicalis 10 %(1/10) each of C. albicans, C. glabrata and C. kefyr.

Among the 2 culture positive cases from post renal transplant patients, all 2 (100%) were C. tropicalis.

ANTIFUNGAL SUSCEPTIBILITY TESTING: When tested for Fluconazole, it was observed that of the 8 isolates of C. albicans, 3(37.5%) were resistant. Of the 52, non-Candida albicans isolates, 21 (40.38%) were resistant to Fluconazole. No resistance was seen to Amphotericin B (Table 3).

DISCUSSION: Candiduria is the presence of yeast cells in urine and is an increasingly common finding in hospital patients. [15]Candida spp. is often isolated from urine specimens and has been estimated to account for as many as 21% of urinary yeast isolates. [16] Candiduria is a marker for haematogenous seeding to the kidneys. Candiduria reflects colonization or infection of the lower urinary tract or collecting system of the kidneys. [15] In one study conducted by Lenner T, it was noted that 90% of patients with disseminated candidiasis who died, had renal involvement at autopsy.[17] Candida species is the **fifth** most common nosocomial urinary pathogen in India. [18] In another study by Mirdha B.R.et al 1998 the age range for funguria was 1-75 years. [2]

In a study on candiduria conducted by Artiago Kobayashi, 2004, Brazil, it was noted that 57.8% of female patients had candiduria. [1] In contrast two Indian studies one by Mirdha B.R. et al. and in the Chakrabarthi A.et al., observed that 74.9% and 74.7% subjects with candiduria were males. [2, 19] In our study 65% cases of candiduria were males (This could be an incidental finding in our study, as the number of males are more in the study).

Urinary tract candidiasis and candiduria is seen in association with disseminated candidiasis, diabetes mellitus, pregnancy, administration of antibiotics and unclean catheters. [15, 20] The risk to develop candiduria increases by 12 fold after urinary catheterization, 6 fold after the use of broad spectrum antibiotics and urinary tract abnormalities, 4 fold following abdominal surgeries, 2 fold in the presence of diabetes mellitus and 1 fold in association with corticosteroid administration. However, no significant difference was found between the compared groups in terms of female sex and age. [21]

Catheterization can cause infection by introducing organisms during catheterization process or by allowing migration of the organisms into the bladder along the surface of the

catheter from the external periurethral surfaces.[22]

Diabetics are at an increase risk of fungal UTI. Diabetes mellitus may predispose patients to fungal candiduria by predisposing them to Candida colonization of vulvovestibular area (in women) by enhancing urinary fungal growth in the presence of glycosuria by lowering host resistance to invasion by Candida species as a consequence of impaired phagocytic activity and promoting stasis of urine in a neurogenic bladder. Guze and Harley observed that more than half the patients with funguria had diabetes mellitus. [17] In the present study, 43.33% had diabetes mellitus as the risk factor.

Candiduria has become a potential source of morbidity and mortality if left untreated. It has been associated with 20% mortality in patients with urological pathology who require surgery. [2] The present study had 10 cases of candiduria with underlying renal pathology other than diabetic nephropathy and its complications. Of these 10 cases, 6 (60%) cases of benign prostatic hypertrophy, 1 each (10%) of fungal cystitis, pelvi- uretero junction block, renal failure and posterior urethral valve.

Chakrabarthi A.et al (1999) studying nosocomial candidaemia identified immunosuppressive therapy in organ transplant recipients as a risk factor for acquiring infection with Candida species.[22] In 2 cases of post renal transplant patients, who were on immunosuppressive therapy , Candida tropicalis was isolated from urine.

Prasad et al. state that isolation of non- Candida albicans spp. was higher (52.4%) as compared to C. albicans (47.6%).[18] Chakrabarthi A et al found C. tropicalis was the commonest species isolated from urine (58.7%) followed by C. albicans. [19] In our study too, C. tropicalis was isolated more frequently than C. albicans.

ANTIFUNGAL SUSCEPTIBILITY TESTING: We adopted the Disc diffusion method as it is simple and easy to perform. But to confirm resistance of any strain, broth dilution test should be used and disc diffusion method can be used for preliminary screening.

The antifungal susceptibility pattern showed that 40 % of the Candida isolates were resistant to Fluconazole. No resistance was seen for Amphotericin B.

From the data of the antifungal susceptibility testing, it was observed that resistance to Fluconazole was more in the non-Candida albicans group ($p<0.05$) when compared to C. albicans. (Refer Table 3 and Results section).

This study mainly intended to define the spectrum of the various non-Candida albicans species and their antifungal susceptibility pattern, in cases of urinary tract infection. Mortality with candiduria is high in debilitated patients and with those with advanced age. In view of antifungal resistance being reported, identification of Candida species and their antifungal susceptibility pattern, is necessary to decide on the on the choice of antifungal drugs.

Table 1: Distribution of Candida specie

Candida species	Number n=60	Percentage%
C. tropicalis	40	**66.66**
C. albicans	8	**13.33**
C. parapsilosis	5	**08.33**
C. glabrata	4	**06.66**
C. kefyr	2	**03.33**
C. guilliermondii	1	**01.66**

Table 2: Associated risk factors

Risk factor	Number =n/60	Percentage %
Diabetes mellitus	26	43.33
Broad spectrum antibiotics	18	30.00
Underlying renal pathology	10	16.66
Post-renal transplant status	02	03.33
Steroids	01	01.66
Pregnancy	01	01.66
No identifiable risk factor	02	03.33

Table 3: Antifungal susceptibility test

Species	Fluconazole Sensitive	Fluconazole Intermediate	Fluconazole Resistant	Amphotericin B Sensitive	Amphotericin B Intermediate	Amphotericin B Resistant
C.tropicalis(40)	23	0	17	40	0	0
C. albicans(8)	5	0	3	8	0	0
C.parapsilosis(5)	4	0	1	5	0	0
C. glabrata (4)	3	0	1	4	0	0
C. kefyr(2)	0	0	2	2	0	0
C.guilliermondii(1)	1	0	0	1	0	0

REFERENCES:

1. Kobayashi A, Fernandes L, Miranda C, Sousa D, Silva R. Candiduria in hospital patients: A study prospective. Mycopathologia 2004; 158: 49-52.
2. Mirdha BR, Sethi S, Banerjee U. Prevalence of fungal species in patients with funguria. Indian J of Med Res. 1998; 107:90-93.
3. Sobel JD. Management of asymptomatic candiduria. Int J Antimicrob Agents. 1999; 11: 285-88.
4. Lundstorm T, Sobel J. Nosocomial candiduria: A review. Clin Infect Dis 2001; 32: 1602-07.
5. Sobel JD, Vaquez JA. Fungal infections of the urinary tract. World J Urol. 1999; 17: 410 – 14.
6. Ang BSP, Talenti A, King B, Steekelberg JM, Wilson WR. Candidaemia from a urinary tract source: Microbiological aspects and clinical significance. Clin Infect Dis. 1993; 17 (4): 626-66.
7. Bukhary ZA. Candiduria: A review of clinical significance and Management. Saudi J Kidney Dis Transpl. 2008; 19: 350-60.
8. National workshop in Medical Mycology.29th Annual Congress of Indian Association of Medical Microbiologists. MICROCON 2005. Medical Mycology Laboratory Procedures. Sri Ramachandra Medical College and Research Institute, Porur, Chennai. Oct 2005.

9. Workshop on fungal infections of eye, ear and skin: A laboratory perspective, Department of Microbiology, St. Johns Medical College, Bangalore. 2005; 1-15.
10. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol. Aug 1994; 32(8): 1923-29.
11. Horvath L, Hospenthal, Murray KC, Dooley PD. Direct isolation of Candida species from blood cultures on the chromogenic medium CHROMagar Candida. J of Clin Microbiol. 2003; 41: 2629-32.
12. National Committee for Clinical Laboratory Standards. 2004. Method for antifungal disk diffusion susceptibility for yeasts. Approved guideline M44-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. Chakrabarthi A, Ghosh A, Kanta A, Kumar P. In vitro antifungal susceptibility of Candida. Indian J of Med Res. 1995; 102:13-19.
14. Chakrabarthi A, Mohan B, Shrivastava SK, Marak RSK, Ghosh A, Ray P. Change in the distribution and antifungal susceptibility of Candida species isolated from candidaemia cases in a tertiary care centre during 1996-2000. Indian J of Med Res. 2002; 116:5-12.
15. Chander Jagdish. Candidiasis. Textbook of Medical Mycology, 3rd ed: New Delhi: Mehta Publishers; 2002. p. 266-90.
16. Sumangala BM. Candidiasis. J of the Academy of Clin Microbiologists 2004; 6(15): 67-69.
17. Vazquez JA, Sobel SD. Mucosal candidiasis. Inf Dis Clinics North America 2002; 793-820.
18. Prasad KN, et al. Role of yeasts as nosocomial pathogens and their susceptibility to Fluconazole and Amphotericin. Ind J of Med Res. 1999; 110:11-17.
19. Chakrabarthi A, Reddy TCS, Singhi S. Does Candiduria predict candidaemia? Ind J of Med Res. 1997; 106; 513-16.
20. Rippon JW. Medical Mycology.3rd ed. Philadelphia: W.B. Saunders
21. Bukhary ZA. Candiduria: A review of Clinical significance and management. Saudi J Kidney Dis Transpl. 2008; 19: 350 – 60.
22. Chakrabarthi, Singh K, Das S. Changing face of nosocomial candidaemia. Ind J of Med Microbiol. 1999; 17(78): 160-66.

IDENTIFICATION OF UROVIRULENT MARKERS IN UROPATHOGENIC ESCHERICHIA COLI.

N. Padmaja, Anand Acharya, P. Nageswar Rao

1. Assistant Professor, Department of Microbiology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.
2. Associate Professor, Department of Pharmacology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.
3. Professor & Head, Department of Microbiology, Konaseema Institute of Medical Science, Amalapuram, Andhra Pradesh.

CORRESPONDING AUTHOR
Dr. Anand Acharya,
KIMS Hospital, Amalapuram,
Andhra Pradesh.
E-mail: anand_kims@yahoo.co.in,
Ph: 0091 09963598050.

ABSTRACT: The present study was conducted in the Department of Microbiology, Konaseema Institute of Medical Sciences, Amalapuram, East Godavari District from August 2011 to January 2012. Fifty Escherichia coli (E.coli) strains isolated from urine samples of different clinical entities and 25 feacal isolates were studied for the detection of virulence markers of E.coli. There are 27 uropathogenic E.coli (UPEC) isolates from 50 E.coli & 5 UPEC from 25 controls. Among isolates tested the most common virulent marker is haemolysin 21 (42%), followed by Mannose resistant haemagglutination 16 (32%), cell surface hydrophobicity 13 (26%). In this, there are 14 cases with only one virulence marker, 8 with 2 marker combinations and 15 cases with combination of 3 markers.

KEY WORDS: Uropathogenic Escherichia coli, Urovirulent Markers, Urinary Tract Infection (UTI)

INTRODUCTION: UTI is one of the most important causes of morbidity and mortality. E.coli is the most frequent urinary pathogen isolated from 50 % - 90 % of all uncomplicated urinary tract infections. It is now recognized that there are subsets of faecal E.coli which can colonize periurethral area, enter urinary tract and cause symptomatic diseases. These are currently defined as Uropathogenic E.coli. It has been traditionally described that certain serotypes of E.coli were consistently associated with uropathogenicity and were designated as uropathogenic E.coli. These isolates express chromosomally encoded virulence markers. The virulence factors include different adhesins, haemolysin production, haemagglutination, cell surface hydrophobicity. The present study was designated to determine the urovirulence factors of E.coli isolated from the patients of UTI and to study their antimicrobial susceptibility pattern.

MATERIALS AND METHODS: The study was conducted in the Department of Microbiology, KIMS, Amalapuram, East Godawari from August 2011 to January 2012. 50 E.coli strains isolated from urine samples & 25 faecal isolates were studied for 1. α Haemolysin on 5% sheep blood agar. 2. Mannose Resistant Haemagglutination. 3. Cell surface hydrophobicity. 4. Antibiotic susceptibility testing by Kirby - Bauyer disc diffusion method.

INCLUSION CRITERIA: Adult patients with UTI attending various clinical departments of KIMS and adult healthy individuals for stool samples are taken.

SAMPLES: 1. Clean catch midstream urine samples from the patients (50). 2. Stool samples of adult healthy individuals (25)

COLLECTION AND CULTURE OF SAMPLE: Sample is transported to Microbiology lab with in half an hour and processed by 1. Wet film preparation. 2. Culturing on Mac Conkey's agar, Blood agar & CLED agar medium & incubated aerobically at 37^0 C for 24 hrs. 3. Growth on plates and significant bacterial count. i.e. more than 10^5 colonies / ml. urine and tested for further identification of E.coli by various biochemical reactions. Such E.coli were screened for virulence markers.

HAEMOLYSIN: For detecting haemolysin, 5% sheep B.A is used and a zone of lysis around each colony is observed after overnight incubation at 37^0C, if haemolysin is produced.

MANNOSE SENSITIVE HAEMAGGLUTINATION: HA is detected by clumping of erythrocytes by bacterial fimbriae in the presence of D-Mannose. The test was carried out as per the direct bacterial HA test slide method and mannose sensitive and resistant HA tests. HA was considered when it occurred in the presence of 2 % D- Mannose and Mannose sensitive, when it was inhibited by D-Mannose.

CELL SURFACE HYDROPHOBICITY: This was done by Salt Aggregation test. Strains are considered hydrophobic, if they are aggregated in concentration of < 1.4 M. Ammonium sulphate.

ANTIBIOTIC SUSCEPTIBILITY TESTING: This was performed on all isolates of E.coli by Kirby-Bauer`s disc diffusion method on Muller Hinton agar.

RESULTS: Of the 50 patients, 0 - 15 age group comprised of 11 persons, 16 - 40 age group comprised of 18 persons and > 40 years group comprised of 21 persons. Females (30) are more than male (20) patients. Among various clinical entities, 30 cases presented with lower UTI (60%), 14 cases with asymptomatic bacteriuria (28%) and 6 with pyelonephritis (12%).

Among 50 cases tested 27 (54%) were positive for virulence markers and out of 25 controls, 5 (20%) were positive for virulence markers. Among the isolates tested, the most common virulent marker is Haemolysin 21 (42%) followed by Mannose Resistant Haemagglutination (MRHA) 16 (32%) and Cell Surface Hydrophobicity (CSH) 13 (26%). In control group, the occurrence of Haemolysin was 1 (20%), MRHA 3 (60%), CSH 1 (20%). (Note: Percentage calculated as per total 50 isolates). There are 14 cases positive for 1 marker, 8 cases positive for 2 markers and 5 cases positive for 3 markers.

41% isolates were sensitive to Amikacin, 78% to Nitrofurantoin, 72% sensitive to Cefotaxime, 52% to Cephalexin, 46% to Co-trimoxazole. High resistance is seen for Norfloxacin 80% followed by Ampicillin 73%, Cefuroxime 68%. (Note: Percentage drug sensitivity and resistance pattern calculated for total number of 50 strains isolated).

No. Of Positive Virulence Markers - Table I

Composition	No. of Tested	Positive virulence markers	%
Cases	50	27	54%
Control	25	5	20%

Antibiotic Sensitivity Test - Table II

S.No.	Drug	Sensitivity	%	Resistant	%
1	Amikacin	41	82%	9	18%
2	Nitrofurantoin	39	78%	11	22%
3	Cefotaxime	36	72%	14	28%
4	Cephalexin	26	52%	21	48%
5	Co-trimoxazole	23	46%	27	54%
6	Cefuroxime	16	32%	34	68%
7	Ampicillin	13	26%	37	74%
8	Norfloxacin	10	20%	40	80%

DISCUSSION: Considering the high degree of morbidity in urinary tract infection the uropathogenic E.coli is receiving the more attention. The present study was undertaken to study the virulence factors namely Haemolysin, MRHA and CSH. 50 E.coli isolates from urine of cases with clinically diagnosed UTI are taken as study group and 25 E.coli from faeces of healthy persons as control group. The high incidence of UTI was observed above 40 years age group followed by 16-40 years age group. This is because; the elderly patients are likely to be predisposed to conditions like 1. Urinary tract obstruction. 2. Poor bladder emptying 3. Diabetes and 4. Prostate enlargement in elderly males. These factors favour colonization of bacteria and play an important role in UTI.

High incidence of UTI is observed in females (30) than in males (20), which is due to 1. Short urethra and 2. Close proximity of urethra to perianal region.

Regarding AST, most of the isolates are sensitive to Amikacin (82%) and Nitrofurantoin (78%). Whereas most of the isolates are resistant to Norflox, Ampicillin, Cefuroxime and Cephalexin. This shows that Aminoglycosides and Nitrofurantoin are useful for most of UTI and high level of resistance indicates the lack of rational drug use. In view of emerging resistance antimicrobial therapy should be started after culture sensitivity report. This will prevent the development of resistance of bacteria towards important antibiotic and we will have choice of antimicrobial agent for complicated urinary tract infection.

REFERENCES:

1. Orskov, F.1984, Genus I.Escherichia castellani and Chalmers 1919. In: Krieg, N.and Holt, J.G. (eds), Bergey`s Manual of systemic bacteriology. London: Williams & Wilkins, 420-23.
2. Smith HW. The haemolysins of Eschericha coli. J. Pathol Bacteriology 1963; 85: 197-211.
3. Kaijser B.Immunology of Escherichia coli. K. Antigen and its relations to UTI. J. Infectious diseases 1973; 127:672-77.
4. Quackenbush RL, Falkow S. Relationship between colicin V activity and virulence in E.coli. Infect Immune 1979;24:562-64.
5. Hageberg L, Jodal U, Korhonen Tk, Lidin – Janson G, Lindberg U, Svanborg Eden C. Adhension, haemagglutination and virulence of E.coli causing Urinary track infections. Infect Immune 1981;31:564-70.
6. Lindahl M, Faris A, Wadstrom T, Hjerten S. A new test based on salting out to measure relative surface hydrophobicity of bacterial cells. Biochim Biophys Acta 1981;677:471-76
7. Orskov I, Orskov F, Birch-Andersen A, Kananmori M, Svanborg-Eden C, O, K, H and fimbrial antigens in E.coli serotypes associated with pyelonephritis and cystitis. Scand J Infectious diseases 1982; Suppl 33:18-25.
8. Cavalieri SJ, Bohach GA, Snyder IS. E.coli alpha haemolysin: Characteristics and probable role in pathogenicity. Microbiol Rev 1984;48:326-43.
9. Holmes, B and Gross, R.J. 1990. Coliform bacteria: various other members of the Enterobacteriaceae. In: Parker, M.T. and Collier, L.H. (eds), Topley & Willson`s Principles of bacteriology, Virology and Immunity, 8th edn. London: Edward Arnold, 415-41.
10. Siegfried L, Marta K, HanaP, Molokacova M and Filika J. Virulence-associated factors in E.coli strains isolated from children with urinary tract infections. Journal of Medical Microbiology 1994;41: 127-32.
11. Johnson J: Microbial virulence determinats and pathogenesis of Urinary tract infection. Infect Dis Clin North Am 2003; 17:261-78.

A STUDY OF POSTOPERATIVE WOUND INFECTION AMONG POST SURGICAL PATIENTS AT CALICUT MEDICAL COLLEGE, KERALA, INDIA

Prasanna Gupta[1].

1. Assistant Professor, Department of Microbiology, Konaseema Institute of Medical Sciences, Amalapuram, Andhra Pradesh, India.

CORRESPONDING AUTHOR
Dr. Prasanna Gupta[1], Dept. of Microbiology,
Konaseema Institute of Medical Sciences,
Amalapuram- 533201, Andhra Pradesh, India,
E-mail: pramin1414@yahoo.co.in,
Ph: 0091 9630238572.

ABSTRACT: BACKGROUND: Postoperative wound infections or surgical site infections (SSIs) are associated with increased morbidity and mortality as they can cause delay in recovery, increase length of stay and increased health care costs. This study was conducted to find out the prevalence and sensitivity pattern of surgical site infections among post surgical cases at Calicut Medical College. **METHODOLOGY:** The study was conducted for a period of one year from July 2007 to June 2008. Two swabs were collected from each site. One swab was used for direct smear examination after Gram staining and second swab was subjected to culture and antibiotic sensitivity testing by standard microbiological techniques. **RESULTS:** The total numbers of surgeries done during the one year period in three surgical units were 1902. The number of clinically suspected cases was 102 (5.36%). The study included 27 'clean', 32 'clean-contaminated' , 13 'contaminated' and 30 'dirty' cases. Culture proven cases were 36 out of 102. Lowest infection rate was seen in clean (18.5%) surgery followed by clean-contaminated (37.5%), contaminated (38.5%) and dirty surgeries (47%). MRSA (Methicillin resistant Staphylococcus aureus) (83%) and multidrug resistant gram negative bacilli were predominant isolates. Alarming rate of resistance to ciprofloxacin, cefazolin, ceftriaxone and ampicillin were observed. **CONCLUSION**: The commonly used antibiotics for empiric treatment of post operative wound infections may not be effective. Development of a suitable antibiotic policy and surveillance is essential to reduce the postoperative wound infection rates.
KEY WORDS: surgical site infections; antibiotic susceptibility; India

INTRODUCTION: Postoperative wound infections or surgical site infections (SSIs) are associated with increased morbidity and mortality as they can cause delay in recovery, increase length of stay, increase health care costs by delaying discharge with associated increase in the need for investigations, treatment and nursing care.

Low rate of SSIs are directly related to education, awareness of the cause of infection and the introduction of practices that reduce risk.[1] According to WHO report 2002, prevalence rate of health care associated infection (HCAI) is 7.7% - 9% in developed countries and 10-11.8% in developing countries. The common HCAI as per CDC update 2007 is UTI (32%) followed by postoperative wound infections (22%), nosocomial pneumonia (15%) and nosocomial septicemia (14%).[2] Causative organisms of post operative wound infections are Staphylococcus aureus and Streptococcus pyogenes in clean surgery. E. coli, Klebsiella species, Pseudomonas species, Acinetobacter species, Staphylococcus aureus and Streptococcus species

are usually found in abdominal and gynaecological surgeries. E. coli, Proteus species, Klebsiella species, Pseudomonas species and Acinetobacter species are associated with urological surgeries. Orthopedic surgeries commonly have infections with Staphylococcus aureus and Gram negative bacilli according to the data from the National Nosocomial infections surveillance system of the centres for disease control and prevention 2007(CDC)[3].

So we planned to study the prevalence, bacteriological profile and susceptibility pattern of SSI associated pathogens in our hospital.

MATERIALS AND METHODS: The study was conducted in the Department of Microbiology, Medical College, Calicut for a period of one year from July 2007 to June 2008. Patients from three surgical units S1, S3 and S5 were subjected to the study. The total number of elective and emergency surgeries done during the one year period in the above three units was 1902, which included 1074 elective (major) and 828 emergency cases. One hundred and two cases of clinically suspected postoperative wound infection (Fifty nine elective and fourty three emergency) from the above cases were studied in detail. The study included twenty seven 'clean', thirty two 'clean-contaminated' , thirteen 'contaminated' and thirty 'dirty' cases. Samples were collected from patients using two sterile cotton swabs. One swab was used for direct smear examination after Gram staining. The second swab was subjected to culture and antibiotic sensitivity testing of the isolates by Stokes method for Staphylococcus species and Kirby Bauer method for Gram negative bacilli. The following antibiotic discs were used for sensitivity testing of Gram positive cocci: Penicillin (10 units), Erythromycin (15mcg), Gentamycin (10 mcg), Vancomycin(30 mcg), Cefazolin(30 mcg). Oxacillin screen agar was used for Staphylococcus species to rule out MRSA. For Gram negative bacilli Ampicillin (10 mcg), Gentamycin (10mcg), Cefazolin (30mcg), Ceftriaxone (30mcg), Amikacin (30mcg) and Ciprofloxacin (5mcg) were used for sensitivity testing. For Pseudomonas species Ceftazidime (30mcg) was also besides above mentioned antibiotics.

RESULTS: The overall prevalence of postoperative wound infections was 5.36% (102). Among 102 clinically suspected cases studied, bacteriologically proven surgical site infection was identified in 36 patients. The prevalence of culture positive infections was 35% (36/102) (Table1). Lowest culture positivity rate was seen in clean surgery (18.5%) followed by clean-contaminated (37.5%), contaminated (38.5%) and dirty surgeries (47%).

Table 1: Prevalence of infections in various types of surgeries (n=102)

	Category of surgery	**No. of clinically suspected cases**	**No. of cases with proven infection**	**Prevalence of infection (%)**
A	Clean	27	5	18.5
B	Clean-contaminated	32	12	37.5
C	Contaminated	13	5	38.5
D	Dirty	30	14	47

Staphylococcus aureus was isolated from 18 cases, Escherichia coli from 15 cases and Enterobacter species from 2 cases. Klebsiella pneumoniae, Acinetobacter spp. and Serratia marcescens was isolated from one case each. Pseudomonas aeruginosa was isolated from 4 cases (Table 2).

Table 2: Organisms isolated from infected wounds (n=42)

Organisms	No. of isolates	% of isolation
Staphylococcus aureus	18	43
Escherichia Coli	15	36
Pseudomonas aeruginosa	4	9.5
Klebsiella Oxytoca	1	2.3
Enterobacter kobei	1	2.3
Enterobacter intermedius	1	2.3
Acinetobacter baumannii	1	2.3
Serratia marcescens	1	2.3

Out of 18 isolates of Staphylococcus aureus none were sensitive to penicillin and erythromycin. All isolates were sensitive to vancomycin. Fifteen isolates of S. aureus were found to be MRSA (Methicillin resistant Staphylococcus aureus). Of the total 24 gram negative bacterial isolates none were sensitive to Ampicillin. Interestingly 22 of these gram negative isolates were multi drug resistant (92%). Amongst Gram negative isolates fifteen were Escherichia coli. All the strains were resistant to ampicillin, cefazolin, ceftriaxone and ciprofloxacin. Amikacin had a sensitivity of 73.3% (Table 3). Figure 1 and 2 depicts prevalence of post operative wound infections.

Table 3: Susceptibility pattern of the isolates in percentage (n=42)

ANTIBIOTIC	S. aureus (18)	E.Coli (15)	Enterobacter spp. (2)	Pseudomonas aeruginosa (4)	Klebsiella spp. (1)	Serratia marcescens (1)	Acinetobacter spp. (1)
Penicillin	Nil	NT	NT	NT	NT	NT	NT
Erythromycin	Nil	NT	NT	NT	NT	NT	NT
Gentamicin	22.2	13.3	Nil	25	100	Nil	100
Ampicillin	NT	Nil	Nil	NT	Nil	Nil	Nil
Vancomycin	100	NT	NT	NT	NT	NT	NT
Cefazolin	22.2	Nil	Nil	Nil	Nil	Nil	100
Ceftazidime	NT	NT	NT	25	NT	NT	NT
Oxacillin	27.7	NT	NT	NT	NT	NT	NT
Piperacillin	NT	NT	NT	25	NT	NT	NT
Amikacin	NT	73.3	100	25	100	Nil	100
Ciprofloxacin	NT	Nil	50	25	Nil	Nil	100
Ceftriaxone	NT	Nil	Nil	NT	Nil	Nil	100

Abbreviations: NT - Not tested

Table 4: Preoperative hospital stay and infection rate (n=102)

Period of preoperative hospital stay	No.of cases	No.of infected cases	% of infection
1-3 days	61	18	29.5
4-7 days	16	5	31.2
7-14 days	15	7	43.7
>14 days	10	6	60
Total	102	36	35

Table 5: Age of the patient & infection rate (n=102)

Age group	No.of patients	No.of infection cases	Percentage
15-25	20	4	20
26-35	14	4	28.5
36-45	13	4	30.7
46-55	20	7	35
56-65	19	8	42.1
66-75	10	5	50
76-85	6	4	67
Total	102	36	35

Table 6: Sex wise distribution of the patients with wound infection (n=102)

No. of patients	Sex	No. of infection cases	Percentage
70	Male	25	36
32	Female	11	34.3
102	Total	36	35

Fig.1: Prevalence of postoperative wound infection in the study group

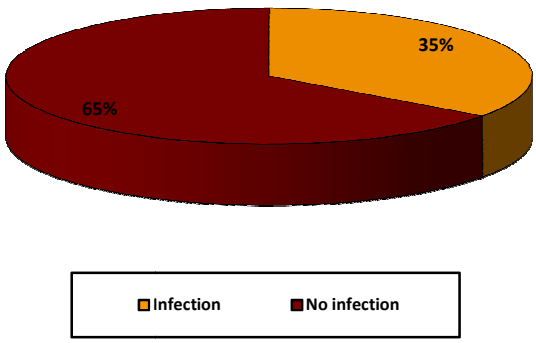


Fig: 2 prevalence of postoperative wound infection in different types of surgery

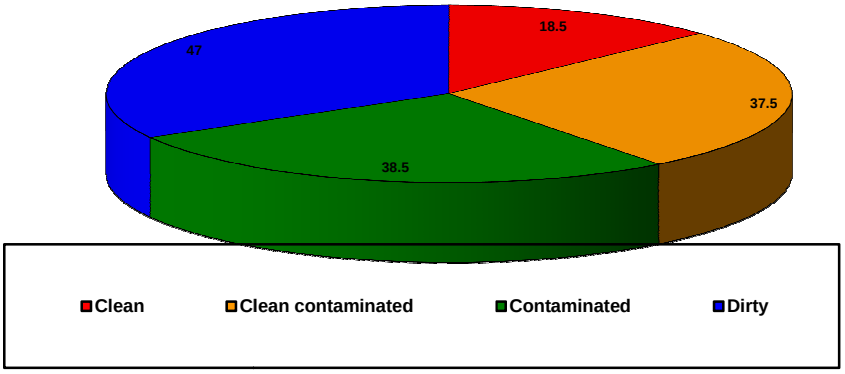
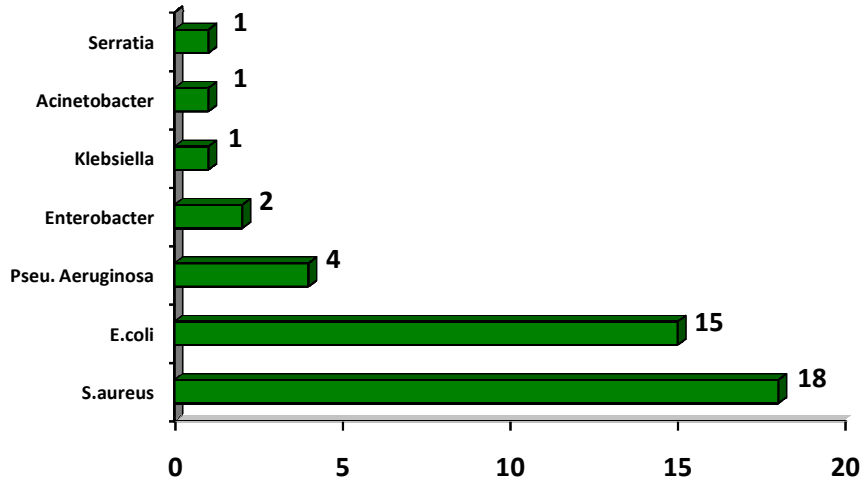


Fig.3: Organisms isolated from infected wounds



DISCUSSION: Charles D Ericsson also reports a rate of 1% for clean surgeries and 5 to 10% for 'clean-contaminated' cases.[4] An overall infection rate of 4.53% is reported by Yalcin et al in his study conducted at Cumhuriyet University Medicine Faculty Hospital in Turkey between January 1992 and December 1993.[5] According to a study the incidence of SSIs with regard to abdominal surgical sites and operating conditions for clean wounds is 1.5-3.7%, clean – contaminated is 3-4%, contaminated is 8.5% and dirty-infected wounds is 28-40%.[6]

The reported overall infection rates of postoperative wound infections from various centers show wide variation.[7-9] A prolonged preoperative hospital stay has been identified as an important risk factor in infection by many authors like Shanson, Timothy D Jacob and Yalcin et al. It promotes acquisition of multidrug resistant hospital strains.[5, 10, 11.] The results of this study

shows a high infection rate in association with a prolonged pre-operative stay in the hospital. (Table-4)

The infection rate of this study also shows an increase with age as observed in many other studies also (Table 5).[10, 12, 13] Male sex was identified as one of the risk factors of postoperative wound infection by Velasco E et al 1998 based on the study on 1205 patients over a period of 1 year at National Cancer Institute Hospital Brazil.[14] In the present study male patients had a slightly higher infection rate (36%) compared to females (34.3%)(Table 6).

In this study (eighteen) 43% of the isolates was Staphylococcus aureus, of which fifteen (83%) were MRSA. Escherichia coli accounted for fifteen cases (36%). This was followed by Pseudomonas aeruginosa four (9.5%), Enterobacter species two (4.6%), Klebsiella pneumoniae one (2.3%), Acinetobacter spp. one (2.3%) (Table 2 and Figure 3). Review of bacteriology of surgical wounds in different features reveal that the results are in accordance with the observations of the present study.[10, 15-20] Table 3 explains the susceptibility profile of the isolates. A prospective study of surgical site infections in a teaching hospital in Goa reported 79% of isolates were Gram negative and almost 64% demonstrated a high degree of antimicrobial resistance.[20] High degree of drug resistance was observed against ciprofloxacin, cefazolin, ceftriaxone and ampicillin.

CONCLUSION: The alarming rate of resistance to ciprofloxacin, cefazolin, ceftriaxone and ampicillin for major postoperative wound infections precludes the use of these commonly used antibiotics for empiric treatment of post operative wound infections. The type of surgeries, length of preoperative stay had an important role in determining the pattern of wound infection. Development of a suitable antibiotic policy is essential to reduce the postoperative wound infection rates. Establishment of proper surveillance programme is also essential.

ACKNOWLEDGEMENTS: Funding: None. I would like to express my sincere gratitude to Dr. Beena Philomina J., Associate Professor Department of Microbiology Calicut Medical College, Kerala for her contribution and support during this work and I express my sincere thanks to Dr. Raghuram V. Associate Professor, Community Medicine, KIMS Amalapuram, for his valuable suggestions during preparation of this manuscript.

REFERENCES:

1. Mangram AJ, Horan TC, Pearson ML et al. Guideline for prevention of surgical site infection. Infect control Hosp Epidemiology 1999; 20: 247-78.
2. Ramani Bai JT. Hospital infection – Present scenario. Proceedings of the fourth triennial conference of the academy of clinical Microbiologists and pre-conference seminar on changing trends in hospital infections.August 22nd & 23rd 2008 (18-23)
3. National nosocomial infections surveillance system, Centers for disease control and prevention (CDC) Health care associated infection (HCAI) updated 2007.
4. Ericsson CD and Rowlands BJ. Surgical infection: Principles and management of antibiotic usage. Physiologic basis of modern surgical care. 1988; 113-35.
5. Yalcin AN and Bakir M. Postoperative wound infections. Journal of Hospital Infection 1995; Apr 29(4): 305-9.
6. Abdominal surgical site infections : incidence and risk factors at an Iranian teaching hospital . Seyd Mansour Razavi, Mohammad Ibrahimpoor , Ahmad Sabouri Kashani and

Ali Jafarian BMC Surgery 2005 , 5:2 online at www. Biomed central .com/1471-2482/5/2

7. Kotisso B and Aseffa A. Surgical wound infection in a teaching hospital in Ethiokia. East African Medical Journal. 1998 Jul; 75(7): 402-5.
8. Salemi C, Anderson D and Flores D. Surgical site infection risk index rates. Infect control Hosp. Epidemiol 1997 Apr; 18(4): 246-7.
9. Antos KR, Fonseca LS, Bravo Neto GP and Gotijo Filho PP. Surgical site infection: rates, etiology and resistance patterns of antimicrobials among strains isolated at Rio De Janeiro University Hospital. Infection 1997 Jul-Aug; 25(4): 217-20.
10. Jacob DT, Richard L and Simmons. Preoperative antimicrobial prophylaxis. Master of surgery. 1998; 3rd edition, Vol.1; 134-45.
11. Shanson DC. Hospital Infection. Microbiology in clinical practice 1999; 3rd Edition: 429-58.
12. Gregory WJ and MeNabb PC. Pseudomonas cepacia. Infection control 1986 May; 7(5): 281-4.
13. Shirahatti RG, Joshi RM, Vishwanath YK, Shinkren et al. Effect of preoperative skin preparation on postoperative wound infection. J Postgrad Med 1993 Jul-Sept; 39(3): 134-6.
14. Velasco E, Thuler LC, Martins CA et al. Risk index for prediction of surgical site infection after oncology operations. Am J Infect Control 1998 Jun; 26(3): 217-23.
15. Topley and Wilson's microbiology and microbial infection. Hospital - acquired infection. 1998; Vol.3: 9th edn. 187-219.
16. Forrest APM, Carter DC and Cleod. Infections and Antibiotics. Principles and practice of surgery. 1995. 3rd edn, 67-83.
17. Saha SC, Zaman MA, Khan MR and Ali SM. Common aerobic bacteria in postoperative wound infection and their sensitivity pattern. Bangladesh Med Res Counc Bull 1995 Apr; 21(1): 32-7.
18. Urschel JD. Necrotizing soft tissue infections. Post graduate Medical J 1999 Nov; 75(889): 645-9.
19. Giacometti A and Cirionio. Epidemiology and Microbiology of surgical wound infections. J of Clin Microbiology 2000 Feb; 38(2): 918-22.
20. Umesh S. Kamat, A.M.A.Fereirra, M.S.Kulkarni, D.D.Motghare. A prospective study of surgical site infections in a teaching hospital in Goa. Indian J. Surg.(May – june2008)70:120-124

CHARACTERIZATION AND SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES FROM HIV - SEROPOSITIVE PATIENTS IN A TERTIARY CARE HOSPITAL

Apurba Sankar Sastry, Sandhya Bhat K, Anand Sankar Sastry, Kumudavathi M S,

1. Assistant Professor, MD, DNB, Department of Microbiology, Meenakshi Medical College, Kanchipuram, Tamilnadu.
2. Assistant Professor, MD, DNB, Department of Microbiology, Meenakshi Medical College, Kanchipuram, Tamilnadu
3. Senior Resident, MD, Department of Medicine, MKCG Medical college, Brahmapur, Orissa.
4. Tutor, D (Bact), Department of Microbiology, Meenakshi Medical College, Kanchipuram, Tamilnadu.

CORRESPONDING AUTHOR

Dr. Apurba Sankar Sastry, Assistant Professor,
Department of Microbiology,
Meenakshi medical college and Research Institute,
Kanchipuram, Near Chennai, India
E-mail: drapurbasastry@gmail.com,
Ph: 0091 444327314.

ABSTRACT: BACKGROUND: Difference in expression of virulence factors and in antifungal susceptibility among different Candida species has raised the need for species-level identification. Opportunistic infection is usually caused by Candida albicans, however, over the last decade reports of non-albicans Candida causing this infection is increasing. **AIM:** The present study was undertaken to assess the role of various Candida species as opportunistic pathogens in Human Immunodeficiency Virus (HIV) seropositive patients and testing their antifungal susceptibility pattern. **METHODS AND MATERIAL**: 100 clinical samples were obtained from HIV seropositive patients and 50 from HIV seronegative patients and were speciated by the conventional methods and also by HiCrome Candida Differential Agar (CHROM agar). Susceptibility testing of the isolates for Fluconazole and Itraconazole was performed by Macro broth dilution technique. **RESULTS:** Candida albicans was the commonest species isolated followed by C.dubliniensis, C.parapsilosis, C.tropicalis, C.krusei and C.glabrata. Antifungal susceptibility testing from HIV seronegative patients showed all Candida albicans and Candida tropicalis isolates were sensitive to both Fluconazole and Itraconazole. Candida krusei (1 isolate), Candida glabrata (1 isolate), Candida dubliniensis (3 isolate) showed resistance to Fluconazole and Candida parapsilosis (1 isolate) showed dose-dependent susceptibility with MIC = 16 µg/ml. Itraconazole resistance was observed for Candida krusei (1 isolate), Candida glabrata (1 isolate), with an MIC range of ≥1 mg/L. All other isolates, even which were resistant to Fluconazole were sensitive to Itraconazole. All Candida isolates from HIV seronegative patients were sensitive to both Fluconazole and Itraconazole. **CONCLUSION:** Increasing trend of Non albicans Candida species causing various invasive infections and their prevalence in a particular geographical area warrant the need of speciation of Candida species. The long-term use of azoles as prophylaxis for Invasive candidiasis can result in the selection of Candida isolates that are more resistant to azole therapy. The non albicans Candida species like C.glabrata and C. krusei are more resistant to Fluconazole and Itraconazole compared to C. albicans. Hence the antifungal susceptibility pattern plays an important role for successful treatment of Candidiasis. The use of Macrobroth dilution method using BHI broth technique will help in detecting the antifungal susceptibility pattern in a less equipped laboratory.

KEYWORDS: Antifungal susceptibility, Human Immunodeficiency Virus (HIV), ChromAgar

Full text: Isolation and antifungal susceptibility testing of Candida isolates from various clinical samples in Human Immunodeficiency Virus (HIV) Seropositive Patients and HIV Seronegative Patients.

INTRODUCTION: Candida species are well known as a member of the normal flora as well as opportunistic pathogens. Over the past few decades, the number of opportunistic infections caused by Candida spp has been increased tremendously. The indiscriminate long-term use of antibiotics, indwelling intravascular catheters, immunosuppresives, cytotoxic therapies, immune defects and more recently AIDS patients are among the significant risk factors associated with Candidiasis [1, 2, 3]. In the last decade however, the incidence of infections due to Candida albicans have decreased and been replaced by non-albicans Candida spp like Candida tropicalis, Candida parapsilosis, Candida glabrata and Candida krusei with more reduced susceptibility to antifungal agents[4,5].

The potential clinical importance of species level identification important as various Candida isolates differ in the expression of putative virulence factors and antifungal susceptibility [2, 6]. The increased isolation rates of non-albicans Candida species and a gradual shift in the antifungal susceptibility profile to azole antifungal agents, especially in immunocompromised patients, have warranted the need to diagnose the pathogen quickly as well as monitor the laboratory data for possible emergence of resistance [7, 8,9] .

The available methods for antifungal susceptibility testing are Micro and Macro Broth Dilution method, Agar Dilution method, Semisolid Agar Dilution method and E-test. Increasing reports of non-albicans Candida species with drug resistance causing infections and increasing number of HIV seropositive patients motivated us to undertake the study to differentiate various Candida spp to find out the most prevalent species and to perform antifungal susceptibility testing to know the difference in the susceptibility pattern between HIV seropositive and seronegative patients by Macrobroth dilution method using Brain Heart Infusion broth (BHI) for Fluconazole and Itraconazole.

MATERIAL AND METHODS: A total of 150 clinical samples (oral swab, blood, vaginal swab, urine) were obtained from HIV seropositive (100) and HIV seronegative (50) patients (clinically suspected cases of candidiasis) attending to outpatient department or admitted in the hospital at Meenakshi medical college, Kanchipuram from May 2009 to November 2010, after taking informed written consent. None of our patients were on antifungal drugs during sample collection. With universal precautions, samples were collected and transported to the laboratory without delay and were processed.

Samples were inoculated on Sabouraud's dextrose agar (SDA). Additionally, samples were also inoculated on HiCrome Candida Differential Agar (CHROM agar) (Hi Media Pvt Ltd, Mumbai) for early presumptive identification of various Candida species, based on colored colony morphology (table-1). SDA slants and CHROM agar plates were incubated at 37 ^{0} C for 48 hr. Isolates were further characterized by performing the following conventional techniques- Germ tube production, chlamydospore formation on corn meal agar, sugar assimilation profile and differential growth on SDA at 37 ^{0} C and 42 ^{0} C [8,10-11]. Gram staining of all the isolates was done from SDA and microscopic morphology was noted (figure-1).

Antifungal susceptibility testing of all the isolates for Fluconazole and Itraconazole was performed by Macro broth dilution technique according Clinical and Laboratory Standards Institute (CLSI) guidelines. For each isolate Minimum Inhibitory Concentration (MIC) for Fluconazole was calculated as the lowest concentration at which 80% inhibition of growth occurred relative to that of drug free control, and MIC for Itraconazole was calculated as the lowest concentration at which 50% reduction of turbidity was produced, as compared with that For the drug-free control 12-13.

ANALYSIS OF RESULTS: CLSI breakpoints were used for Fluconazole and Itraconazole. Isolates were considered susceptible for Fluconazole if their MIC was ≤8 µg/mL, susceptible-dose dependent (S-DD) if their MIC was 16-32 µg/mL, and resistant if their MIC was ≥64 µg/mL and for Itraconazole susceptible if their MIC was≤0.125, susceptible-dose dependent (S-DD) if their MIC was >0.125 to <1 and resistant if their MIC was ≥1 mg/L determined after 48 h of incubation [12-15].

RESULTS: Out of 100 HIV seropositive patients, Candida species were isolated from 48 samples while 52 samples showed no growth. Out of 48 samples with growth, one Candida species was isolated in 44 samples and mixture of 2 Candida species in 4 samples. (Table-2)Out of 52 isolates, 40 were isolated from oral thrush, 6 from urine, 3 from blood, and 3 from vaginal swab.

Candida albicans was the commonest species isolated (28 strains) followed by 13 strains of C. dubliniensis, 5 strains of C.parapsilosis, 3 strains of C.tropicalis,2 strains of C.krusei and 1 strain of C.glabrata were identified (fig-2).

Out of 50 HIV seronegative patients, Candida species were isolated from 24 samples while 26 samples showed no growth. Out of 24 samples with growth, one Candida species was isolated in 20 samples and mixture of 2 Candida species in 4 samples. Out of 28 isolates, 24 were isolated from oral thrush, from urine and 4 from vaginal swab. (Table-2)

Candida albicans was the commonest species isolated (18 strains) followed by 6 strains of C. dubliniensis, 3 strains of C.parapsilosis, 1 strain of C.tropicalis were identified.

Out of 40 Candida isolates from oral thrush, 24 isolates were C.albicans, 13 isolates were C.dublinienis, 2 isolates were C.tropicalis and 1 isolate was C.parapsilosis.

Antifungal susceptibility testing of Candida isolates from HIV seropositive patients showed all Candida albicans and Candida tropicalis isolates were sensitive to both Fluconazole and Itraconazole (100%) and Candida krusei (1 isolate), Candida glabrata (1 isolate), Candida dubliniensis (3 isolates) showed resistance to Fluconazole with MICs of 64 µg/ml at 48 hr, and Candida parapsilosis (1 isolate) showed dose-dependent susceptibility with MIC = 16 µg/ml(figure3).

Itraconazole resistance was observed for Candida krusei (1 isolate), Candida glabrata (1 isolate), with an MIC range of ≥1 mg/L. All other isolates, even which were resistant to Fluconazole were sensitive to Itraconazole. Out of 13 isolates of Candida dubliniensis, three showed resistance (23%) to Fluconazole but all were sensitive (100%) to Itraconazole (figure-4). All these resistant strains were isolated from oral thrush. One out of 5 isolates of Candida parapsilosis showed dose dependant susceptibility to Fluconazole, which was also isolated from oral thrush.

Antifungal susceptibility testing of Candida isolates from HIV seronegative patients showed all Candida albicans and non albicans Candida isolates were sensitive to both Fluconazole and Itraconazole (100%) and no antifungal drug resistance observed.

DISCUSSION: C.albicans and non-albicans Candida are opportunistic yeasts, causing severe disease in immunocompromised individuals, like in AIDS patients [17]. Oropharyngeal Candidiasis is one of the most common opportunistic infection in patients with AIDS. Infact oropharyngeal Candidiasis is known to be an AIDS indicator disease [4]. In the present study out of 52 Candida isolates, C.albicans (53.8%) was the predominant isolate followed by Candida dubliniensis (25%), which was similar to the study done by Lopez-Dupla et al in 1992 who reported 44.4% isolation of C.albicans and Wabale et al in 2010 (73.3%) from HIV seropositive patients [4,18].

In the present study out of 52 Candida isolates from HIV seropositive patients, 40 Candida species were isolated from oral thrush. Out of 40 Candida isolates from oral thrush, 24 isolates were C.albicans, 13 isolates were C.dublinienis, 2 isolates were C.parapsilosis and 1 isolate was C.tropicalis.

Non albicans Candida as the causative agent of oral thrush have been reported from various places ranges from 6.55%-70%. In our study the isolation rate of non albicans Candida species from HIV seropositive patients from oral thrush was 16/40 i.e.40%. Majority of non albicans Candida isolated were C.dubliniensis (13/16). Sullivan et al reported 64 C.dubliniensis from 55 Irish and Australian HIV seropositive patients [19].

The increasing emergence of "non-albicans Candida" thus seems to be associated with HIV pandemic. Since C. dubliniensis closely resembles C.albicans phenotypically it is possible that it is being missed in most of laboratories were only germ tube is solely used of the identification of C.albicans. Emergence of C. dubliniensis infection in HIV seropositive patients is a matter of concern due to the emergence of resistance to commonly used azole antifungals [4].

In our study, all the resistant Candida spp. was isolated from oral thrush. In contrast to our study, resistance was observed for Candida spp. isolated from blood in study by Canan et al in 2010[15]. This could be because of low number of blood isolates in our study.

The use of automated systems such as API20 is unaffordable due to its high cost. Conventional sugar assimilation testing methods can be used for identification, but it takes long time and procedure is cumbersome. Molecular based techniques such as PCR and DNA fingerprinting are very useful in speciation but are not readily available in routine microbiology laboratories [20].

The colonies of the Candida species can be distinguished following primary isolation from clinical specimens by using CHROM agar medium, which is very much cost effective. CHROM agar shows sufficient sensitivity to grow the most important yeasts. It can serve as primary isolation and differentiation medium for clinical specimens likely to contain yeasts. A major advantage is the ability to detection of mixed cultures of yeast in clinical specimens. Hence CHROM agar Candida would be a useful, cost effective tool for rapid identification of various Candida species directly from clinical samples [2, 5, 9, 21].

In the present study, CLSI reference Macrobroth dilution method (M27-A3) was used to determine the susceptibility of 52 Candida isolates to Fluconazole and Itraconazole. The ability to determine MIC results within 24hour is potentially advantageous for early clinical application of antifungal susceptibility results. The present study also performed 24 hour and 48 assessments, and only a few isolates required 48 hour of incubation for optimal growth [13]. Antifungal susceptibility testing revealed resistance to Fluconazole among C. dubliniensis isolates, where as all C.albicans isolates were sensitive. 3 (23%) isolates of C. dubliniensis were resistant to Fluconazole with MIC range of 64 µg/ml, which matches with observation made by Chunchanur SK et al (22.7%) [8]. However, isolates of C. dubliniensis resistant to Fluconazole may still be sensitive to other azole compounds such as Itraconazole and Voriconazole [22], which

matches with our study results. Recently there has been an increased incidence of treatment failures in Candidiasis patients receiving prolonged Fluconazole [23]. Replacement of C. albicans by C. dubliniensis is known to occur in patients treated with Fluconazole. The antifungal pressure exerted by this drug influences the oral microbial ecology in these patients, as species that are better able to adapt to antifungal pressure persist over those that are suppressed by the treatment [8, 24].

The second most common non-albicans Candida species overall isolated from all the samples in our study was C. parapsilosis 5/24(20.8%) which was similar to other reports documented elsewhere [18, 26].

The morbidity and risk associated with Candidiasis, along with increased incidence of treatment refractory Candidiasis as well as the high incidence of AIDS, makes it important that species identification of Candida isolates should be done along with antifungal susceptibility testing in most of the tertiary care laboratories [25]. The use of Macrobroth dilution method using BHI broth, for antifungal susceptibility testing will help in detecting the antifungal susceptibility pattern in a resource constrained laboratory.

CONCLUSION: Though Candida albicans continues to the common & important pathogen among the Candida spp, there is a drastic increase in the incidence of other species like Candida krusei, Candida tropicalis, Candida glabrata and Candida parapsilosis. Hence identification of Candida to the species level has become compulsory to assist the selection of appropriate antifungal agents in treatment of invasive candidiasis because most of the Non albicans Candida especially Candida glabrata and Candida krusei exhibit reduced Fluconazole susceptibility. The long-term use of azoles in the prophylaxis of systemic mycoses can result in the selection of Candida isolates that are more resistant to azole therapy. Hence rapid species identification and susceptibility testing is of great need for treating physician. Proper identification of Candida species, their prevalence in a particular geographical area and studying their antifungal susceptibility pattern is important for successful treatment of Candidiasis. The use of Macrobroth dilution method using BHI broth technique will help in detecting the antifungal susceptibility pattern in a less equipped laboratory. Although the number of Candida species and antifungal agents used in the present study were limited, the results provide a preliminary idea about the isolates susceptibility patterns. Even though our hospital is a tertiary care centre, none of the Candida albicans isolates showed the resistance pattern to Fluconazole and Itraconazole. Hence clinicians can use these two azole antifungals to treat the patients with candidiasis in our hospital setting. However, our study has few limitations, as we have obtained very low no. of isolates from blood, urine and vaginal swab compared to oral swabs; we were not able to draw any clear significance from theses samples.

Candida species	**Colony colour**	**No. of isolates from HIV seropositive patients (n-52)**	**No. of isolates from HIV seronegative patients (n-28)**
Candida albicans	Light-green	28	24
Candida dubliniensis	Dark-green	13	-
Candida parapsilosis	Cream to pale pink	5	3
Candida	Blue with pink	3	1

tropicalis	halo		
Candida krusei	Pink	1	-
Candida glabrata	Pink	1	-

Table-1-Guideline for speciation of various Candida isolates CHROM agar medium [16]and the results of various Candida species isolated

HIV serological status	Total number of patients	No growth observed	Growth observed	Single Candida spp. isolated	Mixture of 2 Candida spp. isolated	Total number of Candida spp .isolated
HIV seropositive	100	52	48	44	4 (4×2=8)	44+8=52
HIV seronegative	50	26	24	20	4(4×2=8)	20+8=28

Table 2: Results of total number of Candida spp. isolated from HIV seropositive and seronegative patients.

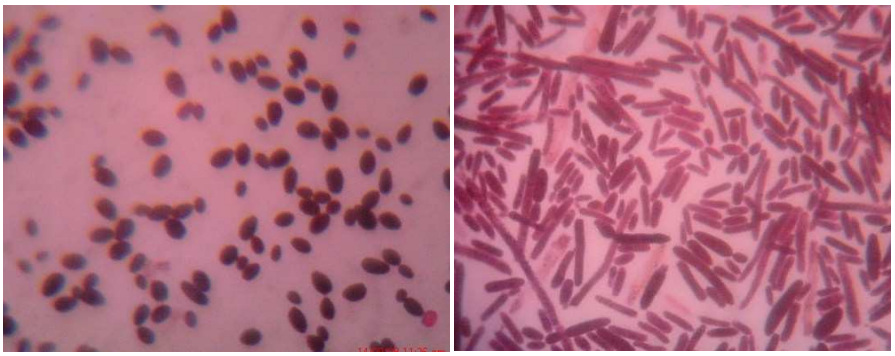


Figure- 1a **Figure-1b**

Figure-1 : Gram staining from SDA (1a- Candida albicans, 1b- Candida krusei)

Figure-2 -different chromogenic reactions of various Candida species isolated.

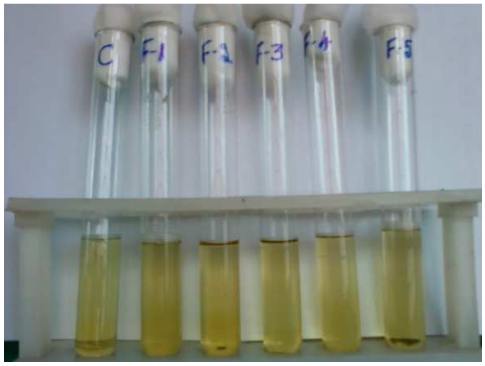


Figure-3 (Macrobroth dilution of antifungal (Fluconazole) susceptibility testing (break point method), showing- C- control without drug, F1-F5- various Candida isolates), F1 and F2 showing turbidity (resistant to Fluconazole), F3-F5- optically clear (susceptible to Fluconazole),

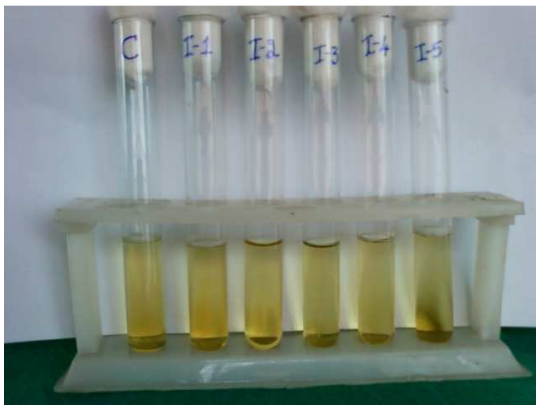


Figure-4 Macrobroth dilution of antifungal (Itraconazole) susceptibility testing (break point method) ,showing- C- control without drug, I1-I5- various Candida isolates, I1 showing turbidity (resistant to Itraconazole), I2-I5- optically clear (susceptible to Itraconazole)

REFERENCE LISTS:

1. Branchini ML, Pfaller MA, Rhine-Chalberg J , Frempong T, Isenberg HD. Genotypic variation and slime production among blood and catheter isolates of Candida parapsilosis. J Clin Microbiol 1994;32:452-456.
2. Raut SH, Varaiya A. Differentiationof Candida dubliniensis on Chrom agar and Pal,s agar. Indian Journal of Medical Microbiology;(2009)27(1):55-8.
3. Khan ZK, Gyanchandani A. Candidiasis-A Review. PINSA B64 No.1(1998): 1-34
4. Wabale V, Kagal A, Bharadwaj R. Characterization of Candida species from oral thrush in Human Immunodeficiency Virus (HIV) Seropositive and Seronegative Patients. Bombay Hospital Journal 2008;50(2):212-7.
5. Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, Gonzalez-Gomez N, Ponton J, et al. Evaluation of the New Chromogenic Medium Candida ID2 for Isolation and Identification

of Candida albicans and other Medically Important Candida species. Journal of Clinical Microbiology Sep2006;44(9):3340-5.

6. Murray MP, Zinchuk R Larone DH. CHOMagar Candida as the sole primary medium for isolation of yeasts and as a source medium for rapid-assimilation of trehalose test. J Clin Microbiol 2005; 43:1210-2.
7. Tortorano AM, Viviani MA, Barchiesi F, Arzeni D, Rigoni AL, Cogliati M, et al. Comparison of Three Methods for Testing Azole Susceptibilities of Candida albicans Strains Isolated Sequentially from Oral Cavities of AIDS Patients. Journal of Clinical Microbiology, 1998;36(6):1578-83.
8. Chunchanur SK, Nadgir SD, Halesh LH, Patil BS, Kausar Y, Chandrasekhar MR. Detection and antifungal susceptibility testing of oral Candida dubliniensis from human immunodeficiency virus-infected patients. Indian J Pathol Microbiol 2009;52(4):501-4.
9. Freicker-Hidalgol H, Orenga2 S, Lebeau1 B, Pelloux 1 H, Brenier-Pinchart 1 MP, Ambroise-Thomas1 P et al. Evaluation of Candida ID, a New Chromogenic Medium for Fungal Isolation and Preliminary Identification of Some Yeast Species. J Clin Microbiol.2001 April:39(4):1647-9.
10. Rippon JW. Candidiasis and the pathogenic yeasts. In: Martin Wonseiwicz editors. Medical Mycology. Philadelphia: W.B. Saunders; 1988. p. 531- 81
11. Khan ZU, Ahmad S, Mokaddas E, Al-Sweih N, Chandy R. Sunflower seed husk agar: A new medium for the differentiation of Candida dubliniensis from Candida albicans. Indian J Med Microbiol 2005;23:182-5.
12. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts;approved standard. Third Edition: Approved Standard M27-A3. Wayne, PA, USA: CLSI; 2008.
13. Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA, et al. Antifungal Susceptibility Testing: Practical Aspects and Current Challenges. Clin Microbiol Rev. 2001 October; 14(4): 643–58.
14. Rex JH, Walsh TJ, Sobel JD, et al. Practice Guidelines for the treatment of candidiasis. J Infect Dis. 2000; 30: 662-678.
15. Evci C, Ener B, Goral G, Akcaglar S. Comparative evaluation of the antifungal susceptibility of Candida isolates from blood specimens: results of a study in a tertiary care hospital in Bursa, Turkey. Turk J Med Sci 2010;40(1):141-9.
16. Latha R, Sasikala R, Muruganandam N, Venkatesh BR. Study on the shifting patterns of Non Candida albicans in lower respiratory tract infections and evaluation of the CHROMagar in Identification of the Candida Species. J. Microbiol. Biotech. Res 2011;1(3):113-9.
17. Willinger B, Manafi M. Evaluation of CHROMagar Candida for rapid screening of clinical specimens for Candida species.Mycoses1998;42:61-5.
18. Lopez-Dupla M, Sarz PM, Garcia VP, Ortega EV, Uriol PL, et al. Clinical, endoscopic immunologic and therapeutic aspects of oropharyngeal and esophageal candidiasis in HIV infected patients: A survey of 114 cases. Am J Gastroenterol 1992;87(12):1771-5.
19. Sullivan DJ, Westerneng TJ, Haynes KA, et al. Candida dubliniensis sp. now.:Phenotypic and molecular characterization of a novel species assosciated with oral candidiasisin HIV-infected individuals. Microbiol 1995;141:1507-21.

20. Alves SH ,de Loreto ES, Linares CE, Silverira CP, Scheid LA, Pereira DI, et al. Comparison among Tomato juice agar with other three media for differentiation of C.dubliniensis from C.albicans. Rev Inst Med trop S Paulo 2006;48: 119-21.-
21. Odds FC, Bernaerts R. CHROMagar Candida, a new Differential Isolation Medium for presumptive Identification of Clinically Important Candida Species. Journal of Clinical Microbiology, Aug. 1994;32(8):1923-9.
22. Kirkpatrick WR, Revankar SG, Mcatee RK, Lopez-Ribot JL, Fothergill AW, McCarthy Di, et al. Detection of Candida dubliniensis in oropharyngeal samples from human immunodeficiency virus- infected patients in North America by primary chromagar Candida screening and susceptibility testing of isolates. J Clin Microbiol 1998;36:3007-12.
23. Albertson GD, Nimi M, Cannon RD, Jenkinson HF. Multiple Efflux Mechanisms Are Involved in Candida albicans-Fluconazole Resistance. Antimicrobial Agents and Chemotherapy, Dec.1996;40(12):2835-41.
24. Hamza OJ, Matee MI, Moshi MJ, Simon EN, Mugusi F, Mikx FH, et al. Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol 2008;8: 135.
25. Pfaller MA. Nosocomial Candidiasis: Emerging Species, Reservoir, and Modes of Transmission. Clinical Infectious Diseases 1996;22(2):89-94.
26. Walmsley S, Kings, Mc Geer A, Ye Y, Richardson,S. Oropharyngeal Candidiasis in patients with Human Immunodeficiency virus: Correlation of clinical outcome with in vitro resistance, serum azole levels and immunosuppression. Clin Infect Dis 2001: 32 : 1554–61.

CANAL CONFIGURATION IN THE ROOTS OF MAXILLARY SECOND PREMOLAR: A CLINICAL STUDY

M. R. Ganesh.

1. Reader, M. D. S. Department of Conservative & Endodontic, Adiparashakthi Dental College. Melmaruvathur, Chennai.

CORRESPONDING AUTHOR
Dr. M. R. Ganesh,
C. S. T. Compound at the back of G. P. O,
Mumbai-1, Maharashtra, India,
E-mail: watvrukshpippal909@gmail.com,
Ph: 0091 8600117037.

ABSTRACT: Modified access cavity preparation and X ray- interpretation were used clinically to find out the additional canals in the root of maxillary second premolars. In 85% of the case two canals were located and treated, of these 30% had two separate apical foramen and 50.66% had two canal, joined at the apical third in 5% of the cases, two roots with two different canals were located and in 15% of the cases, one canal was located.
KEY WORDS: Access Preparation, Apical Foramen.

INTRODUCTION: In the modern era of endodontics where clinicians have completely understood the root canal anatomy and configuration.[1,2], variation in the morphology of the dental pulp is caused by genetic and environmental influences. The canal configuration has clinical as well as biological significance. It is well known fact that root canal microbial flora is most complex. [3] Very few studies have related to the presence of an additional canal in the maxillary second premolars. The main aim of the present clinical study was to find out the canal configuration in the maxillary second premolars which were undergoing enododontic treatment.

MATERIAL AND METHODS: 100 patients with no age and sex bar were selected for this study. Periapical radiograph of the tooth was well examined. In carious tooth, the caries was excavated with the help of excavator and round bur at slow speed hand piece. Access through the occlusal surface was taken with round diamond point. Access cavity was ovoid in a buccopalatal direction. Then with long tapered diamond point; the initial penetration of the pulp chamber was done towards the axis of the palatal canal. The root of the pulp chamber was completely removed using the cutting action of the bur, only on the withdrawal stroke to avoid the possibility of damage to the floor of the pulp chamber. The pulp chamber was made wide buccopalatally. As the root canal openings lies well below the cervical level and may not be seen easily, the floor of the pulp chamber extended well apically below the cervical level. First palatal canal was located palatally and then the buccal canal was located in the centre of the pulp chamber. Pulp tissue was extirpated and the initial instrumentation was done. With root canal instruments in two canals, X-ray was taken. Due to overlapping of bucfal and palatal canals, radiographs were repeated with different angulation.

Root canals were biomechanically prepared and obturated when they were absolutely dry and the teeth were asymptomatic.

RESULTS: Out of 100 maxillary second premolars; only 10 premolars had single canal. 5 premolars had type IV canals with two separate roots. 40 premolars had type IV canals and 45 premolars had type II and type III canals.

There was no significant difference in the results on male and female or the left and right sided premolars.

Table showing number of teeth, type of canal and its percentage

No. of teeth	Type of canal	Percentage
45	II & III	50.66%
40	IV	30.00%
10	Inhibition	15%
5	IV	5%

DISCUSSION: Many root possess additional canal and variety of canal configuration. The maxillary second premolar tends to be single rooted tooth. The type I canal form is prevalent; however over 24.5% of these may present type II and type III canal and further 24.5% may have type IV and type IV form with two canals at the apex.

In this study, in many teeth, initially only palatal canal was located. Then by extending pulp chamber buccally and more cervically buccal canal was also located. The percentage of type II and type III canals was very high (i.e. 51.66%) as compared to type I canal that was only 10%. Percentage of type I canal was very low when compared with standard text book on Endodontics which report 50 to 64%. Percentage of type IV canal was acceptable 30% compared to text books where it is reported to be 24.5%. One canal at the apex was also acceptable (60%) compared to the text books reporting 74.5%. Two roots with two different canals was found in 4.5 which is rarely mentioned in the text books.

In 37.11% two separate apical foramina were located and in 60.5% one foramen was located.

CONCLUSION: The root canal system is complex and the canals may divide and rejoin each other and possess different forms which are considerably more involved.

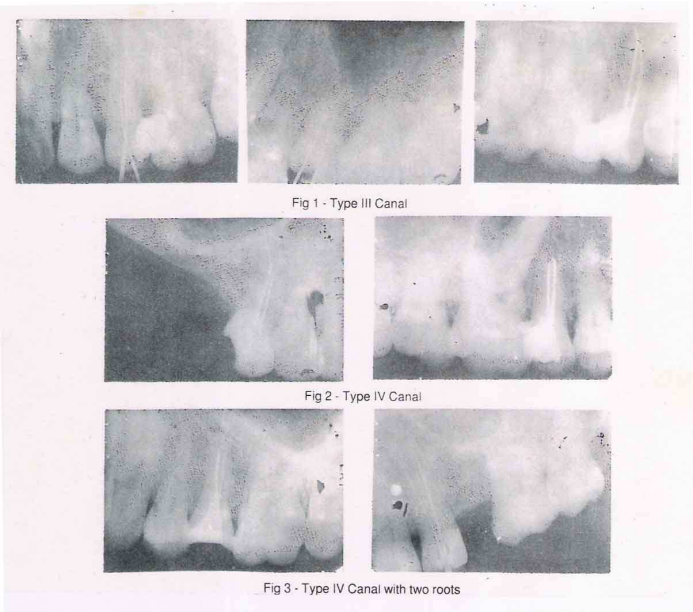


Fig 1 - Type III Canal

Fig 2 - Type IV Canal

Fig 3 - Type IV Canal with two roots

REFERENCES:

1. Siqueira JF, Mechanical reduction of bacterial population in the root canal by in strumentation technique. JOE 1999;25.
2. Siqueira JF, Chemomechanical reduction of the bacterial population in root canal after instrumentation and irrigation with 1%, 2.5% and 5.25% sodium hypochlorite solution, JOE 2000;26.
3. Sundqvist, Ecology of root canal flora. JOE 1992.
4. Cohen S. Pathways of the pulp. 1980, p. 97.
5. Harty F J. Endodontics in Clinical Practice 1990, p. 35.
6. Fogel H.M. Canal configuration in the mesiobuccal root of the maxillary first molar: J Endod. 1994; 20:135-137.
7. Wein, Basis of successful endodontic therapy, Textbook of endodontics.

COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFECT OF CALCIUM HYDROXIDE PASTES USING FOUR DIFFERENT VEHICLES

M. R. Ganesh.

1. Reader, M. D. S, Department of Conservative & Endodontic, Adiparashakthi Dental College. Melmaruvathur, Chennai.

CORRESPONDING AUTHOR
Dr. M. R. Ganesh,
C. S. T. Compound at the back of G. P. O,
Mumbai-1, Maharashtra, India,
E-mail: watvrukshpippal909@gmail.com,
Ph: 0091 8600117037.

ABSTRACT: AIM: The present study evaluated the antibacterial effect of calcium hydroxide pastes using four different vehicles namely propylene glycol, glycerin, distilled water and camphorated phenol. **MATERIAL AND METHOD:** Calcium hydroxide pastes prepared with two commonly used vehicles namely distilled water and camphorated phenol. The antibacterial activity of these pastes were tested against five anaerobic organism that can commonly occur in the infected root canal. The pastes were also prepared using two chemicals such as propylene glycol and glycerin. **RESULTS AND CONCLUSION:** The result of this study show that calcium hydroxide paste made with propylene glycol gives enhanced antibacterial action and sustains it for longer period of time. Propylene glycol being biological acceptable vehicle, it can be recommended for routine use as a vehicle over glycerin.

KEY WORDS: Calcium hydroxide, Propylene Glycol, Glycerin, Camphorated monochloro phenol.

INTRODUCTION: Calcium hydroxide has been widely used in endodontics as an intracanal medicament. Cleaning and shaping is one of the most important step of successful endodontic therapy. The endodontist today, concentrates more upon instrumentation, irrigation through cleaning and shaping for the elimination of responsible microbial flore from the infected root canal [6,7,9]. Disinfection of the infected root canal is established by the comprehensive effect of instrumentation irrigation and intra canal medication contributes to maximum therapeutic success. Calcium hydroxide is generally mixed with vehicles namely distilled water, CMCP, glycerin and also propylene glycol to dissociated into calcium and hydroxyl ion thereby creating alkaline environment in its vicinity, not allowing the growth of micro-organisms [3,4] Dissociation of calcium hydroxide into non aqueous vehicles is not clearly understood and the biological consequences of endodontic application of calcium hydroxide mixed with a non aquous vehicles are not known. However there are very few study related to propylene Glycol that show as better vehicle exhibits no tissue irritating effect, alcoholic in nature and remains in paste from for longer time. Glycerin which has been used as intracanal lubricant and calcium hydroxide glycerin paste was found to have superior sealing property in the roots. The antibacterial activity of a paste with propylene glycol and glycerin against anaerobic organisms has not been clearly understood and reported till date. The purpose of this study is to comparatively evaluate of antibacterial effect of calcium hydroxide using four different vehicles against the five anaerobic organisms that can commonly seen in the infected root canals.

MATERIAL AND METHOD: Considering the microbial flora of infected root canal, five micro organisms were obtained. This study evaluates the antibacterial effect of calcium hydroxide against anaerobic organisms commonly found in infected root canal.

1) Prevotella intermedia 2) Prophyromonas gingivalis 3) Fusobacterium Nubleatum 4) Peptostroptococcus micros 5) Streptococcus milleri.

Calcium hydroxide paste was divided into four different group.

Group I : Calcium hydroxide with distilled water (CH-DW)
Group II : Calcium hydroxide with Propylene glycol (CH-PG)
Group III : Calcium hydroxide with glycerin (CH-GY)
Group IV : Calcium hydroxide with CMCP (CH-CMCP)

One gram of Calcium hydroxide powder mixed with one cc of appropriate liquid on a glass slab and paste were prepared with the help of sterile instrument under sterile condition.

The culture media used for the study were brain hart infusion and blood agar. Four wells of 5mm diameter were cut on each solid media using sterile metallic punch fitted with bulbed teat. A standard loop with on internal diameter of 4mm which could deliver 0.01ml of the suspension of culture of test organism, then spread across the plate using sterile cotton swab. The wells were then filled with the four different preparation of calcium hydroxide and culture plates were then incubated at 37^0C. The zone of inhibition was measured after 48, 96 and 166 hours and results observed.

RESULTS: All the different preparation of Calcium hydroxide showed positive zone of inhibition against the microorganism tests. Calcium hydroxide CMCP pastes showed significant higher zone of inhibition as compared to other groups. There was little difference between the antibacterial activity of calcium hydroxide Propylene glycol pastes and calcium hydroxide glycerin pastes. These pastes showed higher zones of inhibition than calcium hydroxide distilled water pastes. The inhibitory effect of calcium hydroxide Propylene glycol pastes and calcium hydroxide glycerin pastes enhanced with days of incubation with days of incubation that was not significant. The CH-CMCP pastes showed highest antibacterial activity against the micro-organisms as compared to other pastes. P. intermedia and P. gingivalis exhibited large zones of inhibition. Fusobacteria and streptococcus milleri showed small growth of inhibition. It was observed in the present study that among all the calcium hydroxide preparation. Only CH-PG pastes remain in the paste from for a longer period of time and exert significant antibacterial action. The average diameter of the zones of inhibition for experimental group and other group were similar.

Zone of inhibition

Organism Observation	**Period**	**Zone of inhibition (mms)**			
		Group I	**Group II**	**Group III**	**Group IV**
P. Intermedia	Day 2	2.0	1.8	1.5	1.4
	Day 4	2.0	2.1	1.2	1.3
	Day 7	1.9	1.9	1.2	1.6
P.Gingivalis	Day 2	2.9	1.7	3.7	2.4

	Day 4	2.6	1.6	4.0	2.4
	Day 7	3.1	1.7	4.1	2.3
F. Nucleatum	Day 2	1.2	1.5	0.9	2.8
	Day 4	1.2	1.8	1.3	3.0
	Day 7	1.2	1.7	0.7	2.7
P.Micros	Day 2	0.4	1.0	1.4	3.0
	Day 4	0.5	1.4	1.1	2.6
	Day 7	0.5	1.7	1.3	2.9
S. Milleri	Day 2	2.1	2.0	1,3	2.4
	Day 4	2.3	1.7	1.2	2.5
	Day 7	1.8	1.6	1.4	2.7

Group I Calcium hydroxide + Distilled water
Group II Calcium hydroxide + Propylene Glycol
Group III Calcium hydroxide + Glycerin
Group IV Calcium hydroxide + Camphorated mono chloro phenol

DISCUSSION: Infected root canal exhibit a mixed microbial flora with anaerobic organism that is one of the major causes of pulpal infection. In modern endodantic, disinfection of root canal is established by combined effect of instrumentation & irrigation that has been further reduced the role of intracanal medicament just to limit the microbial growth in between the visit. Calcium hydroxide is more commonly used intracanal medicament that nearly fulfills the ideal requirement and when mixed with vehicles to from a paste, placed in contact with root canal space and periapical tissues to exert its antibacterial effect. The ditch place diffusion through agar method was used to chick the antibacterial property of an against. Four wells of 5mm diameter were cut on each solid agar media under sterile condition. The wells were then filed with different prep ration of calcium hydroxide and agar plates were then incubated at 37^0C and the zone of inhibition observed. [13] Distilled water does not possesses appreciable antibacterial property. The result showed that CH-DW pastes produced zone of inhibition that was significantly smaller, than other groups. The results of the study is similar to the studies done in the past. [2] Propylene glycol and glycerin possess significant antibacterial property and their pastes with calcium hydroxide showed antibacterial effect against organisms tested. The result showed that they produced large zone of inhibition as compared to CH-DW pastes.

They dissociates calcium hydroxide into calcium and hydroxyl ions due to the absorbtion of water by the hygroscopic nature of propylene glycol and glycerin gradually which can be contributed to the gradual ionic release.[12] Hence the calcium hydroxide propylene glycol paste and calcium hydroxide glycerin pastes remain in the paste from for longer period of time.

As compared to propylene glycol, glycerin is found to be more toxic and tissue irritant topically. It require further investigation related to biocompatibility before recommendation it as a vehicle for calcium hydroxide.

It was observed in the present study that among all the calcium hydroxide paste, CH-CMCP pasts exhibited relatively high inhibition zone. The results are similar with the finding of past studies.[8] calcium hydroxide combined with CMCP result in the formation of insoluble weak salt which may interfere with hydroxyl dissociation and reduce the long term activity.[1] CMCP is volatile liquid and rapidly lost from the paste.[15] It is irritant to the periapical tissue. It was also observed in the study that only CH-PG and CH-GY pastes had better handling properties, long

term activity as compared to the other pastes. They also have better flow and good sealing properties in the root canal space.[11] The CH-DW pastes and CH-CMCP pastes has a tendency to set fast and poor handling properties.

Considering the result of the study and review of literature, it can be said that the routine use of propylene glycol in place of CMCP can be recommended as vehicle. For calcium hydroxide in view of efficacy, better handling characteristic and non irritating properly to the periapical tissue, Glycerin can be use as alternative vehicle to propylene glycol.

CONCLUSION: The results of this study are suggestive of:

1) CH-CMCP paste gave the best results (maximum zone of inhibition) but its clinical use is objectionable due to its tissue irritating property and poor handling characteristics.
2) The routine use of propylene glycol in place of CMCP can be recommended as vehicle for calcium hydroxide in view of its efficacy better handling and non tissue irritating properties.
3) The use of glycerin as an alternative vehicle for calcium hydroxide.
4) Zone of inhibition is not a true indicative of the antibacterial activity. However, the result of the study were used to compare the antibacterial effect of calcium hydroxide using four different vehicles. Further investigation is required in vivo to test the antibacterial activity of the different calcium hydroxide pastes described in our study.

REFERENCES:

1. Donald, A. Gorden TM, Del Rio CE. The effect of three vehicles on the pH of calcium hydroxide. Oral Surg 1982; 54 : 560-565.
2. Difore PM. Peters DD, Jean AS, Lewis M. The antibacterial effect of calcium hydroxide apexification pastes on Streptococcus Sanguit. Oral Surg 1983; 55 : 91094.
3. Estrela C. Invito determination of direct antimicrobial effect of calcium hydroxide JOE, 198; 24.
4. Estrela C. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Brazil Dent J. 1995; 6-85-19.
5. Fabricus et.al, Predominant indigenous oral bacteria isolated from infected root canal after varied times of closure, Scand J Dent Rs. 1982; 90-134.
6. Frederick R, Antibacterial efficacy of calcium hydroxide, iodine, potassium iodide, betadine scrub with and without surfactant against E. faecalis invitro. Oral Surg Oral Med Oral Pathol Oral Radio, Endodo 2004; 98.
7. Gomes, Invitro antimicrobial activity of several concentration of sodium hypochlorite and chlorhexidine gluconate in the elimination of e. facalis. JOE 2003; 34.
8. Harrison IW, and Madonia JV. Antimicrobial effectieness of Para chlora phenol. Oral Surg 1970; 30 : 267-275.
9. Marcia Valera, Effect odf sodium hypochlorie and intracnal medicaments on Candia albicans in root canals, JOE 2000; 27.
10. Martindale. The extra pharmacopoeia 30th ed. London : The pharmaceutical press, 1993; 1496-1407.
11. Rivera EM, William K. Placement of calcium hydroxide in stimulated canals, comparison of glycerin versus water. J Endod 1994 ; 20 : 445-448.

12. Sylvia TS, Francis Research, Bhat KS. Effect of four vehicles on the pH of calcium hydroxide and the release of Ca++ion. Oral Surg. 1995; 80 : 459-464.
13. Sundquist G. Ecology of Root canal flora. J Endod 1992; 18 : 427.
14. Treanor HF. Bactericidal efficiency of intra canal medications. Oral Surg 1972; 33 : 791.
15. Thomas PS, Bhat KS, Kotian MK : Antibacterial property of dilute Formo Acresol, Eugenol and Propylene Glycol. Oral Surg. 1980; 49 : 166-170.

TESSIER CLEFT NO. 30; PRESENTING WITH MEDIAN CLEFT OF THE LOWER LIP AND MANDIBLE, ANKYLOGLOSSIA AND MIDLINE CERVICAL WEB CAUSING NECK CONTRACTURE

Utpal Kumar Bordoloi.

1. Associate Professor, Department of Plastic Surgery, Assam Medical College, Dibrugarh, Assam and Consultant Plastic Surgeon, Srishti Hospitals and Research Center, Dibrugarh.

CORRESPONDING AUTHOR
Dr. Utpal Kumar Bordoloi.
Consultant Plastic Surgeon,
Srishti Hospitals and Research Center,
Dibrugarh, Assam.
E-mail: drutpalbordoloismailbox@rediffmail.com,
Ph: 0091 9435030597.

ABSTRACT: Tessier cleft no. 30 is a rare congenital deformity and presents with varying degree of severity from isolated median cleft of the lower lip to cleft of the manubrium sterni involving the mandible, tongue, floor of the mouth, hyod bone, thyroid cartilage and strap muscles of the neck. the cleft is frequently associated with ankyloglossia and median web in the neck extending from chin, causing neck contracture. I report a case of median cleft of lower lip, severe ankyloglossia, cleft of mandibular symphysis and a fibrotic bend extending from the chin to the suprasternal notch with a web, causing contracture of the neck; in a thirteen year old girl. The deformity was corrected in single stage, consisting of release of the tongue from floor of the mouth and lower alveolus, fixation of the mandibular cleft with titanium miniplate, repair of the cleft of the lower lip, excision of the fibrotic bend and correction of neck contracture with multiple z-plasties.
KEY WORDS: Median Cleft of lower lip; median cleft of mandible; Tessier cleft no. 30.

INTRODUCTION: I report a case of Tessier cleft no. 30; a rare congenital deformity; presenting with median cleft of the lower lip, cleft of the mandibular symphysis, severe ankyloglossia and a fibrotic bend extending from the chin to the suprasternal notch with a median web of skin causing contracture of the neck. The deformity was corrected by a single stage surgical procedure and the post operative period was uneventful.

CASE HISTORY: RRP, a thirteen year old female from Dhemaji district in north-eastern part of Assam, attended with median cleft of the lower lip and contracture of the neck due to a bend and a web of skin in the neck. On examination, her tongue was attached to the floor of the mouth and lower alveolus causing difficulty in speech. The median fibrous bend and the associated web of skin extended from the chin and the hyoid bone to suprasternal notch (Figure-1,2). Mobility could be elicited between the both halves of the mandible. Her dental occlusion was normal and all four lower incisors were present. No other congenital abnormality could be detected clinically and her general health was good. X-ray showed a complete cleft of the symphysis of the mandible (Figure-3). The surgical procedure was performed under general anaesthesia. The tongue was released from the lower alveolus and the floor of the mouth by releasing a thick and flat fibrous bend (Figure -4), which was continuous with the bend in the neck. The tongue as such was normal. The surgical defect in the floor of the mouth and the tongue could be repaired directly. The mandible was fixed with titanium mini plate after

freshening of the edges. The cleft of the lower lip was repaired in three layers. Finally, the fibrous bend was excised by splitting the skin web. Multiple Z-Plasties were performed to correct the neck contracture. The post operative period was uneventful (Figure-5, 6).

DISCUSSION: Tessier cleft no. 30 coincides with the caudal extension of the cleft no. 0[16]. Couronne in 1869 first described the median cleft of the lower jaw and there are very few case reports in literature since then[16]. It is caused by failure of midline union of the first pair of branchial arches. Associated failure of fusion of lower pairs of branchial arches account for often reported caudal extension of the deformity[14,16]. Severity of the deformity varies. Thus, there may be a midline notch in the lower lip to complete midline cleft of the lower lip1; associated with or without notching of the mandible2 to complete cleft of the symphysis of the mandible[5,,8], bifid or absence of the tongue[5,9], absence of the hyoid bone, hypoplasia of the thyroid cartilage, hypoplasia of the strap muscles of the neck, bifid or absent manubrium sterni[7] and bifid sternum with ventriculo-septal defect[2]. Various authors report ankyloglossia where the tongue is attached to the floor of the mouth and lower alveolus. A fibrotic bend in the neck with web of skin causing contracture of the neck[3,5,12] is also reported in several cases. Other associated anomalies include cleft lip, cleft palate, Pierre-Robin anomaly, hemifacial microsomia, dermoid cyst of neck[13], ectopic salivary gland[2], haemangioma of lower lip[4], Syndactyly, brachydactyly, clubfoot and congenital anomalies of the heart and the great vessels[2].

The present reported case presented with median cleft of the lower lip (involving the full height of the lower lip), complete cleft of the mandibular symphysis, ankyloglossia, and midline cervical web causing neck contracture. There were no other associated congenital anomalies. Family and maternal history was not suggestive.

Like any facial cleft surgery, correction of soft tissue is the primary treatment. Mandibular surgery can be deferred till the age of ten years unless there is Emergency like breathing difficulty or feeding problems[10,2]. Surgical procedures on mandible include fixation of both mobile bone segments with stainless steel wire[6], Vitallium or Titanium plate with or without bone graft[5]. One stage reconstruction using rib graft has been reported in literature[17]. In my case the gap between the mandibular halves appeared in jaw open position only. The both halves of the mandible could be fixed with titanium mini plate after freshening of the edges. Bone graft was not required and dental occlusion was normal.

REFERENCES:

1. Tahmedullah, Muhammed Bilal, Naseem ul Haq, Fabio Abenavoli. Reconstruction of rare craniofacial clefts: An early experience. JPMI,2011;25:163-7.
2. Roshani E. Rana, Vinita A. Puri, Rahul K Thakkur, A. S. Baliarsing. Median cleft of mandible and lower lip with ankyloglossia and ectopic minor salivary gland on tongue. Indian J Plast Surg, January-June, 2004; 37.
3. Joshi, G. Chimanchode, M. Jagannathan. Congenital midline cervical web causing neck contracture as an isolated deformity. Eur J Plas Surg,2003;112:935.
4. Morioka, Daichi M.D.; Simic, Radoje M.D.; Vlahovic, Aleksandar M.D.;Kravljanac, Djordje M.D. Tessier 30 Median Mandibular Cleft Associated With Lower Lip Haemangioma. Plast & Reconst Surg, sept 2003; 112:935.

5. Ishii M, Ishii Y, Moiyama T, Enomoto S, Ono T, Ohyma K. Seventeen year follow-up of a patient with median cleft of lower lip, mandible and tongue with flexion contracture:a case report. Cleft Palate Craniofacial J, 2002; 39:555-9.
6. Seyhan T, Kylyur H. Median cleft of lower lip: report of two new cases and review literature. Ann Otol Rhinol Laryngol,2002;111:217-21.
7. Anoop Verma and Nirved Jain. Tessier Cleft No. 30 (Median Cleft from Lower Lip to Manubrium). Indian J Paediatrics,2001;68:1163-64.
8. Tiwari VK. Median cleft of lower lip and mandible: A case report. Indian J of Plast Surg,2000;33:98-100.
9. S A Subramani,Gurumurty,B Sasashiva Murty. Tongue cleft and other associated unusual congenital malformations in two successive generations. Letter to editor. Indian j Plastic Surgery,2000;33:103.
10. Amstrong AP, Waterhouse N. Tessier 30 Median Mandibular Cleft: A case report and literature review. Br J plast surg, 1996;49:536-8.
11. M T Longaker, J W Siebert. Microsurgical correction of facial contour in congenital craniofacial malformations: the marriage of hard and soft tissue.Plastic and reconstructive surgery,1996;98:942-50.
12. Kececi Y, Gencosmanoglu R, gorken C, Cagdas A. Facial cleft no. 30. J Craniofac Surg, 1994; 5:263-4.
13. Surendren N, Varghese B. Midline cleft of lower lip with cleft of mandible and midline dermoid in the neck. J Pediatric Surg,1991;26:1387-8.
14. Oostrom CA, Vermeij-Keers, Gilbert PM, Van Der Meulen JC. Median cleft of lower lip and mandible: Case reports, a new embryogenic hypothesis and subdivision. Plast Reconstr Surg,1996;97:312-20.
15. Sheman JE, Goulian D. The successful one stage surgical management of a midline cleft of lower lip, mandible and tongue. Plas Reconstr Surg, 1980;66: 756-9.
16. Henry K. Kawamoto, Jr. Rare craniofacial clefts. Plastic Surgery. Joseph G. McCarthy. WB Saunders Company.1990; 2927-41.
17. Millard DR Jr, wolfe SA, Berkowitz S. Median cleft of lower lip and mandible: Correction of the mandibular defect. Br J Plast Surg, 1979;32:345-7.

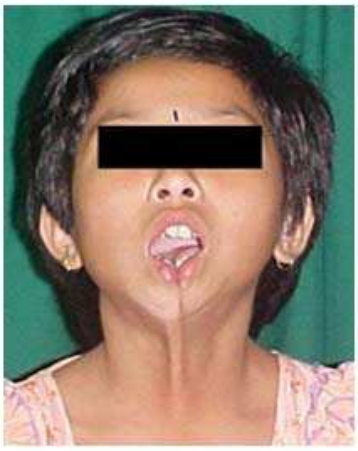


Figure 1: The cleft of the lower lip and the cervical web.

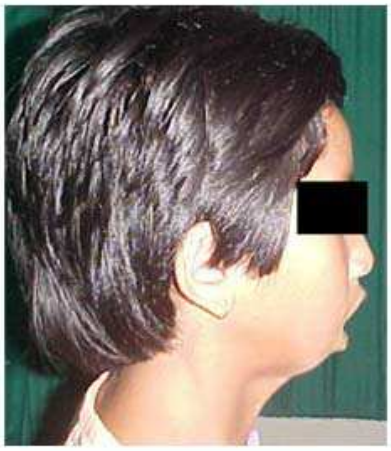


Figure 2: The lateral view clearly shows the cervical web.

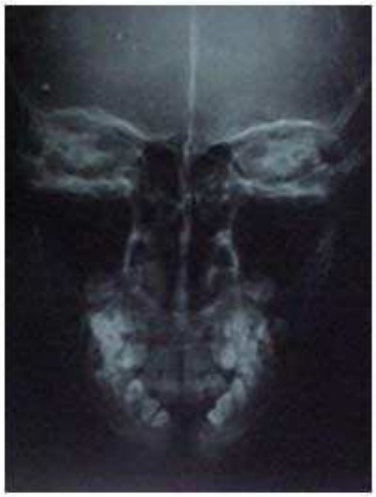


Figure 3: X-ray showing the mandibular cleft.

Figure 4: Intra operative photograph after release of the tongue.

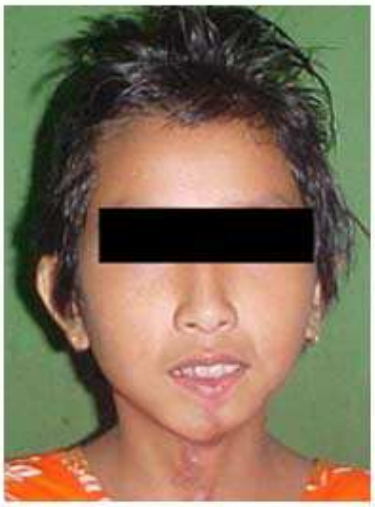


Figure 5: post operative frontal view.

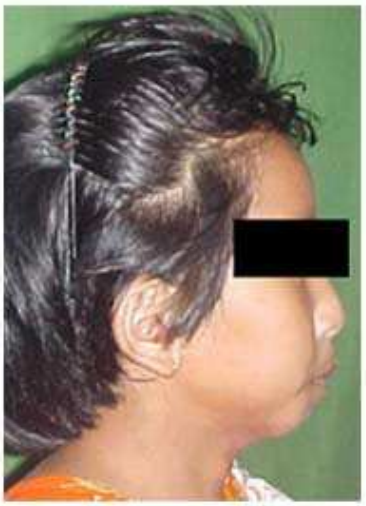


Figure 6: Post operative lateral view.

FULL MOUTH REHABILITATION IN A CASE OF SEVERELY ATTRIDED DENTITION- A CASE REPORT

Chauhan Chirag, Shah Vrinda, Shah Darshana, Doshi Paras.

1. Professor, Department of Prosthodontics, Ahmedabad Dental College and Hospital, Gujrat.
2. Resident, Department of Prosthodontics, Ahmedabad Dental College and Hospital, Gujrat.
3. Professor and Head, Department of Prosthodontics, Ahmedabad Dental College and Hospital, , Gujrat.
4. Reader, Department of Prosthodontics, Ahmedabad Dental College and Hospital, Gujrat.

CORRESPONDING AUTHOR
Dr. Vrinda Shah,
Department of Prosthodontics and Crown and Bridge,
Ahmedabad Dental College and Hospital, Gujarat, India,
E-mail: drvrinda89@gmail.com,
Ph: 0091 9824587809.

ABSTRACT: PATIENT: A 48 year old patient reported with complaint of food lodgement in wearfacets because of severe worn out teeth. Pankeymann- Schuyler philosophy was followed for the rehabilitation as it is a well organized procedure, where anterior guidance is first established followed by restoration of the posterior teeth. **DISCUSSION AND CONCLUSION**: The goal of full mouth rehabilitation should be the restoration and maintenance of the health of the entire oral mechanism. It demands rehabilitation within the physiological and functional harmony of the stomatognathic system. Careful evaluation of the etiology, history and factors relative to occlusal vertical dimension are essential to appropriate treatment planning. The complexity in treating full mouth rehabilitation cases is not only because of its long treatment time but also at times the lack of clarity in the treatment objective.
KEY WORDS: Full Mouth Rehabilitation, Vertical Dimension, Anterior guidance.

INTRODUCTION: Full mouth rehabilitation is a challenging treatment modality that improves the appearance of the patient and corrects imperfections in the occlusion. Vertical dimension, centric relation, speech and muscle tone are essential fundamentals of full mouth rehabilitation. There is a need to analyze each aspect carefully with regard to existing natural dentition and its relationship with the stomatognathic system. Full mouth rehabilitation tends to create smile that is not only esthetic but also functionally comfortable.[1]

A case has to be treated not only by correcting worn out, broken or discolored teeth but also requires treating the oral cavity holistically. Every patient with extreme tooth wear has unique treatment needs.[2] The steps in treatment of these patients include a comprehensive examination, diagnostic mounting, careful planning and sequencing of various steps, discussion with the patient of the different treatment alternatives and careful execution of the treatment plan.[3]

OUTLINE OF THE CASE: A 48 year old man with good general health reported to the department of Prosthodontics crown and bridge dentistry, Ahmedabad dental college, Gujarat with a chief complaint of food lodgement and worn out teeth. No significant systemic history was reported. Patient was having habit of betel nut chewing since 15 years.

Clinical and radiographic examination revealed generalized attrition with reduced vertical dimension of occlusion and Loss of anterior guidance. (Fig. 1) Full mouth rehabilitation of the mouth was planned to restore the function, esthetics and comfort of the patient. The patient was informed regarding the diagnosis, treatment planning, and expenses. Consent from the patient was taken before the initiation of the procedure.

TREATMENT SEQUENCE:

I. **OCCLUSAL SPLINT FABRICATION**: As there was loss of Vertical dimension, restoration of the Vertical dimension was first considered. Diagnostic impression was taken with irreversible hydrocolloid. Maxillary cast was mounted on Hanau Wide vue (ARCON) articulator, using Facebow transfer and mandibular cast was mounted using centric relation record. All the deflective contacts were checked and removed from the cast as well as from mouth. Occlusal splint was fabricated by using self cure acrylic resin (DPI RR Cold cure, India). Vertical dimension was raised by 2 mm in the occlusal splint (Fig. 2) The patient was asked to wear the occlusal splint for the maximum possible time in a day except while eating and in the night for a period of 6 weeks and was asked to report after 6 weeks. After one week, the patient reported that he did not have any difficulty adapting to the new position. The patient was asked to visit every alternate week for six weeks. The acceptance of restored vertical dimension by the patient initiated the further treatment.

II. **PROVISIONALIZATION**: Tooth preparation for metal ceramic restoration was done with minimal occlusal reduction. (Fig. 3) First, all the anterior teeth were prepared. Impressions of the prepared teeth were made with irreversible hydrocolloid in stock trays (Fig. 4). The maxillary occlusal splint was modified by removing the anterior section and posterior portion was used as a centric relation record. Then impression compound jig was made using posterior portion in place and jig was used to get new centric record using bite registration material (virtual refill cadbite registration material- ivoclar vivadent).Maxillary and Mandibular casts were formed, mounted on Hanau articulator. Provisional anterior restorations were prepared with heat-polymerized hard acrylic resin (DPI-Heat Cure India). Anterior determinant of vertical dimension was checked with anterior provisional in oral cavity. Speaking line, smile line and lower lip line was assessed for optimum visibility of upper and lower anteriors. In addition labiolingual and superior-inferior positioning of anterior teeth was checked using labiodental sound (F& V) and Silverman's closest speaking space. Occlusal plane was established by using Broadrick flag [4] (Fig.5). Then posterior teeth were prepared and Provisional restorations were cemented (Fig.6). Lower posterior teeth were restored in harmony with anterior guidance followed by the restoration of the upper posterior teeth. The patient was followed up for another six weeks to further assess adaptation to the proposed vertical dimension before the permanent restorations.

III. **FINAL RESTORATION**: After six week final impressions were made using polysiloxane impression material (Photosil DPI) (Fig.7). Impressions were poured with die stone (Type IV Kalrock, Kalabai Dental Pvt Ltd. Mumbai, India). With anterior provisional in placed, centric and protrusive records were made with bite registration material (virtual refill cadbite registration material- ivoclar vivadent) and facebow transfer and mounting on Hanau wide vue articulator was done. Articulator was programmed using the protrusive record. Wax patterns were fabricated. Casting was done in base metal alloy. The metal frame-works were tried and

adjusted for fit intraorally (Fig.8). The porcelain build-up was carried out and the bisque bake try-in was taken. The glazed restorations were examined and finally luted with glass ionomer cement (Ketac Cem- 3M ESPE) (Fig. 9). Group function scheme for occlusion was followed (Fig.10). Oral hygiene instructions were given. The patient was followed up after one week. The patient was pleased with esthetics, function, and comfort of the prostheses.(Fig.11)

DISCUSSION: Rebuilding of severely attrided dentition has been a challenge to a dentist's skill and capabilities. The concept of complete mouth rehabilitation is dependent basically upon three proved and accepted principles. These are; the existence of a physiological rest position of the mandible which is constant, the recognition of a variable vertical dimension of occlusion and the acceptance of a dynamic, functional centric occlusion.

Many clinical studies indicate that, vertical dimension of occlusion is maintained even with rapid wear. As the occlusal surface wears, compensatory alveolar process elongates by progressive remodeling of the alveolar bone.[5] As a result there is no loss of vertical dimension unless tooth loss occurs. However, occlusal wear may occur more rapidly than continuous eruption depending on the etiology of the wear. Therefore, it is critical to verify loss of occlusal vertical dimension prior to restoration at an increased vertical dimension. So combination of methods like phonetics, facial appearance and measuring the interocclusal distance are used to verify the lost vertical dimension. Occlusal splint is used as a means to raise the vertical dimension of occlusion for 6 weeks. Basic function of a splint is referred to as muscle deprogrammer and it helps the condyle in returning to their centric relation position.

The three prime requirements of full mouth rehabilitation are healthy TMJ, harmonious anterior guidance and noninterfering posteriors. These three factors are interrelated and any disharmony between these will affect the stomatognathic system.

The anterior teeth are usually restored first so as to achieve functional and esthetically viable anterior guidance. Anterior guidance is the dynamic relationship of the lower anterior teeth against the upper anterior teeth through all ranges of function. Anterior guidance plays a very important role in full mouth rehabilitation following centric relation.[6] Anterior guidance forms the anterior control to provide posterior disclusion. The job of anterior guidance is to protect the posterior teeth from lateral or protrusive stresses. The facebow transfer is a must to relate the anterior guidance with the opening and closing axis. It is required to reproduce the arc of closure from the patient to the articulator.

The three main things to be taken care of, while replacing posterior teeth, are achieving posterior disclusion, establishing the plane of occlusion and deciding the type of occlusal scheme. Disclusion refers to separation of opposing teeth during eccentric movements of mandible, as reported by Christensen.[7] Posterior occlusion should have equal simultaneous contacts so that it does not interfere with either the TMJs in the back or the anterior guidance in the front. Occlusal interference can be detrimental to the health of the patient. Deflective occlusal interference can cause painful symptoms in the muscle, teeth or other oro-facial structures. A proper plane of occlusion must permit disclusion of all the teeth on the balancing side when the mandible is moved laterally. The reconstruction of vertical dimension of occlusion should be done at the centric relation and it should be acceptable for the patient at the neuro-muscular level [8].

The patient had severely worn down mandibular anteriors and wear facets on the canine. Hence group function occlusion was followed to avoid functional overload on canines, which can be detrimental to the overall oral health of the patient. Group function refers to the

distribution of lateral forces to a group of teeth rather than assigning all forces to one particular tooth. Lateral pressure is distributed to all working side teeth in order to prevent overloading of the canine. Little or no modification was done on the occlusal surface for this patient to preserve the tooth structure for better structural durability.

The provisional restorations play a critical role in the successful treatment of the full mouth rehabilitation patient. The provisional restorations should be esthetic and also fulfill the functions so that the effect can be followed in the temporary before making the final restoration.

CONCLUSION: Full mouth rehabilitation is a treatment modality which not only focuses on the esthetics and functional aspect of the dentition but also improves upon the health of the whole stomatognathic system. A detailed diagnosis and treatment planning is necessary to achieve predictable success. The restoration of normal healthy function of the masticating apparatus is the ultimate aim of full mouth rehabilitation. Full mouth rehabilitation by Pankeymann-Schuyler philosophy is successful approach. Patient was satisfied with esthetic and masticatory efficiency.

REFERENCES:

1. Dawson PE. Functional occlusion from TMJ to smile design. Mosby St. Louis, Elsevier. 2007:18-26, 27-32, 75-83, 429-52
2. Binkley TK, Binkley CJ. A practical approach to full mouth rehabilitation. J Prosthet Dent 1987;57(3): 261-6.
3. Rivera-Moreles WC, Mohl ND. Restoration of vertical dimension of occlusion in the severely worn dentition. Dent Clin North Am 1992;36(3):651-64
4. Lynch CD, McConnell RJ. Prosthodontic management of the curve of Spee: use of the Broadrick flag. J Prosthet Dent 2002;87(6):593-7
5. Dawson PE . Vertical Dimension. In: Peter E.Dawson. Evaluation, diagnosis and treatment of occlusal problems. 1989, 2 Ed. Cv Mosby Company, St. Louis Baltimore, Toronto. 56-71
6. Dawson PE. Anterior Guidance. In: Peter E.Dawson. Evaluation, diagnosis and treatment of occlusal problems. 2nd Ed. Cv Mosby Company, St. Louis Baltimore,Toronto. 1989;274-297
7. Pokorny PH, Wiens JP, Litvak H. Occlusion for fixed prosthodontics: a historical perspective of gnathological influence. J Prosthet Dent 2008; 99(9):299-313.
8. Bloom DR, Padayachy JN. Increasing occlusal vertical dimension- Why, When and How. Br Dent J 2006;200(4):199-203

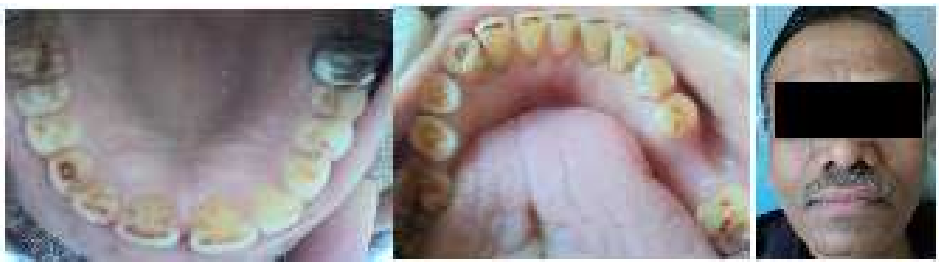


Fig.1: Pre-treatment

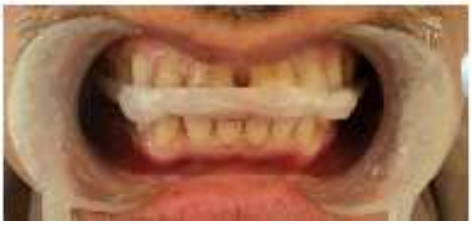


Fig.2: Occlusal Splint

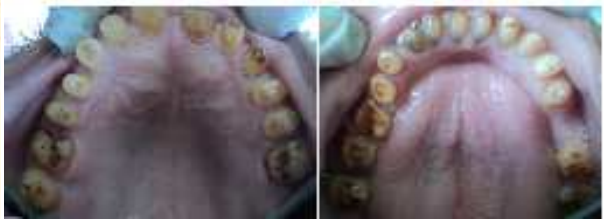


Fig.3: Tooth Preparation

Fig.4 Impression with irreversible hydrocolloid

Fig.5: Broadrick Flag

Fig.6: Final Impression

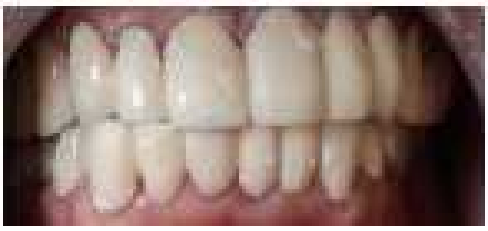
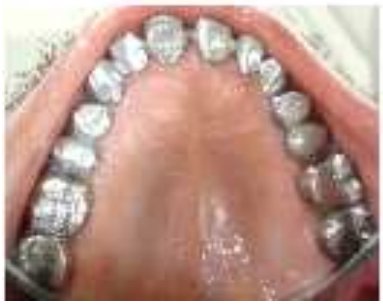


Fig.7: Provisional Restoration



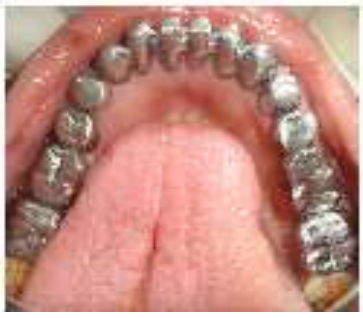


Fig.8: Metal Trial

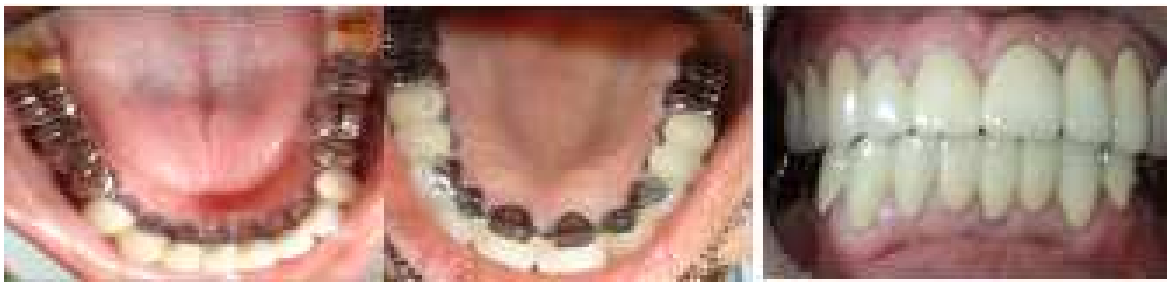


Fig.9: Final Restoration

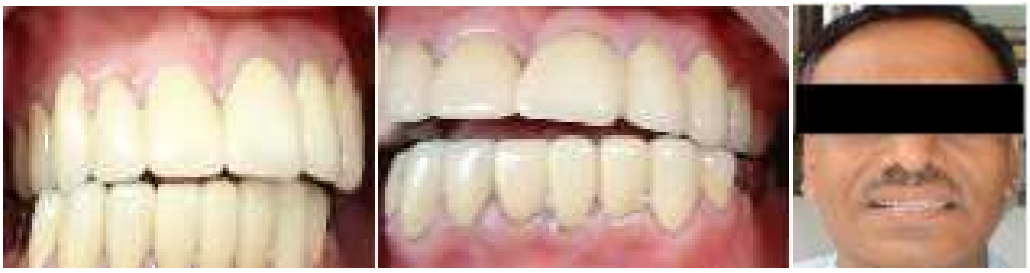


Fig.10: Group Function Fig.11: Post treatment

A STUDY OF HISTO PATHOLOGICAL CHANGES OF PLACENTA IN SEVERE ANAEMIA

Adil S. A. K. Nausheen Rumana

1. Assistant Professor, Department of Pathology, Mysore Medical College and Research Institute, Mysore.
2. Associate Professor, Department of Physiology, Mysore Medical College and Research Institute, Mysore.

CORRESPONDING AUTHOR
Dr. S. A. K. Adil, Assistant Professor,
#259, 5th Main, Bannimantap, 'C' Layout,
Mysore –570015.
E-mail: saka291@yahoo.co.in,
Ph: 0091 9019691259.

ABSTRACT: BACKGROUND: Maternal disorders will influence the placenta, as it forms a functional unit between the mother and the fetus. Severe anaemia has many effects on important organs. In pregnancy it is associated with late abortions, prematurity, low birth weight and stillbirths[1, 2, 3, 4, 5.] Microscopic abnormalities of placenta may provide crucial information. Hence, the present study was under taken to analyse, the spectrum of histopathological changes in placenta, in severe anaemia during pregnancy. **METHODS:** One hundred and twelve placentas were studied of which, 56 belonged to mothers with severe anaemia and 56 belonged to mothers with normal haemoglobin. Histological sections made from placental tissue bits, taken from the central placenta were studied. Four hundred villi were counted, and different histopathological features were quantified. The mean number of villi showing syncytial knots, vasculo- syncytial membrane, fibrinoid necrosis and villous stromal fibrosis was determined. The comparison of the means was done using students unpaired't' test. Test of proportion was applied wherever necessary. **RESULTS:** Significant microscopic changes were observed in placentae of anaemia group as compared to controls. Villi in placentas from mothers with severe anaemia showed excessive syncytial knots ($p<0.001$), increased fibrinoid necrosis of the villi ($p<0.001$), increased villous stromal fibrosis ($p<0.001$) compared to villi of placentas from mothers with normal haemoglobin. The difference of proportion of villi showing vasculo-syncytial membranes between the two group was statistically not significant ($p>0.05$).
CONCLUSION: Severe anaemia in pregnancy alters the placental morphology and adequate treatment of anaemia may therefore be critical to normal placental function.
KEY WORDS: Placenta, villi, severe anaemia.

INTRODUCTION: The fetus, placenta, and the mother constitute the triad of contributors to pregnancy outcome. Examination of the placenta in cases of poor pregnancy outcome and maternal disorders, provides, vital information to both obstetricians and neonatologist. Severe anaemia is a potentially hazardous hematological disorder that may occur in pregnancy. It affects many important organs of the body.[6]There is paucity of literature on histopathology of placenta in severe anaemia during pregnancy. Few previous studies on histopathological changes in placenta have shown varying results. These conflicting results may be due to the fact that the design of these studies varied. Hence, the present study was under taken to analyse the spectrum of histopathological changes in placenta in severe anaemia during pregnancy.

MATERIAL AND METHODS: This present prospective study on placentae obtained from mothers delivering at district hospital, Belgaum, from April 2004 to March 2005, was undertaken in the Department of Pathology, Jawaharlal Nehru Medical College, Belgaum. Written consent was taken from the mothers after explaining the study details and mother's proforma was maintained. A total of 112 placentae were studied, of which, 56(50%) placentae were from mothers with severe anaemia (Hb < 7g/dl) who formed the anaemia group and 56(50%) placentae were from normal term mothers (Hb ≥11g/dl) who formed the control group.

INCLUSION CRITERIA:

CONTROL GROUP:

1) Singleton uncomplicated term pregnancy 2) Baby weight > 2.5 Kg 3)Gestational period >37- 41weeks 4)Blood pressure < 140/90mm of Hg throughout pregnancy 5) Haemoglobin value is 11g/dl and above.

ANAEMIA GROUP:

1) Singleton pregnancy 2) Gestational period >37-41 weeks 3) Blood pressure < 140/90mm of Hg throughout pregnancy 4) Haemoglobin value is <7g/dl.

EXCLUSION CRITERIA:

1. Associated obstetric complications of pregnancy.
2. Associated medical disorders of pregnancy.
3. Twin pregnancies.

Haemoglobin estimation was done by cyanmethaemoglobin method. The severity of anaemia among the mothers was judged by the criteria suggested by WHO.[7, 8.]

Gross lesions of the placenta were examined and the placenta was kept for fixation in 10% formal saline for 24-48 hours. After fixation, the placenta was cut into vertical strips (bread loaf manner) of 0.5 cms thickness each. Four slices were taken from the central area of each placenta and sections were cut from the central zone of each slice as recommended by Fox H.[9]

The tissue bits were then processed through series of dehydration steps and embedded in wax blocks. Tissue sections of 5 µm thickness were cut from each block and stained by conventional haematoxylin and eosin stain and according to findings, special staining techniques like P.A.S (Periodic Acid Schiff), Van Geison, Von Kossa and Masson's trichrome were employed. The different histological findings were observed against the background of normal standards of histology of placenta as described by Fox H.[9]

Different histological features were quantified. In each placenta four hundred villi were counted and the incidence of various histological features was determined, according to the method proposed by Fox H.[9] The mean number of villi showing syncytial knots, vasculo-syncytial membrane, fibrinoid necrosis and villous stromal fibrosis was then determined. The comparison of the means was done using students unpaired 't' test. Test of proportion was applied wherever necessary.

RESULTS: Syncytial knots were present on >30 percent of the villi in 34/56(60.71%) placentae of the anaemia group and 11/56(19.64%) placentae of the control group. The difference of proportion of placentae showing high villous syncytial knot counts (>30% of the villi) between the two groups was statistically significant ($p<0.001$) (Table1).
This shows that higher proportion of placentae in anaemia group show syncytial knots on more number of villi.

Vasculo-syncytial membranes were present in >20 percent of the villi in 7/56 (12.5%) placentae of the control group and 2/56(3.57%) placentae of the anaemia group. The difference of proportion of placentae showing vasculo-syncytial membranes in >20 percent of the villi between the two group was statistically not significant ($p>0.05$) and none of the placentae in both the groups showed deficiency of membranes (i.e. <5% of the villi) or had high vasculo-syncytial membrane count (i.e. >30% of the villi) (Table2).

Fibrinoid necrosis was seen in >3 percent of the villi in 6/56 (10.71%) placentae of the control group and 30/56(53.57%) placentae of the anaemia group. The difference of proportion of placentae showing fibrinoid necrosis in >3 percent of the villi between the two groups was statistically very significant ($p<0.001$).This shows that fibrinoid necrosis is commonly seen in placentae of anaemia cases (Table 3).

Increase in villous stromal fibrosis was seen in 4/56 (7.14%) placentae of the control group and 24/56(42.85%) placentae of the anaemia group. The difference of proportion of placentae showing increase in stromal fibrosis in >3 percent of the villi between the two groups was statistically very significant ($p<0.001$). This shows that increase in villous stromal fibrosis is commonly seen in placentae of anaemia cases (Table 4).

The mean number of villi showing syncytial knots was19.7±7.55 in control group and that in anaemia group was31.01±3.29, the mean number of villi with vasculo-syncytial membrane in control group was15.63±4.45 and that in anaemia group was12.05±4.83, the mean number of villi showing fibrinoid necrosis was1.64±1.43 in control group and 3.26±2.29 in anaemia group, the mean number of villi showing increase in stromal fibrosis was1.45±1.39 in control group and that in anemia group was 4.08±2.07.

The difference in the mean number of villi showing syncytial knots ($p<0.001$), vasculo-syncytial membrane ($p<0.01$), fibrinoid necrosis ($p<0.01$) and increase stromal fibrosis ($p<0.001$) between the two groups was statistically significant between the two groups. (Table 5).

DISCUSSION: In severe anaemia, there is decrease in oxygen carrying capacity of the blood which affects tissue perfusion and therefore, vital organs like heart, kidney, liver, lungs and brain would show signs of reduced work function. The growth and survival of the fetus in utero is directly related to formation, development and maturation of the placenta. Severe anaemia during pregnancy has a significant impact on mother and fetus. It is a cause of pre-placental hypoxia, which may give origin to fetal hypoxia resulting in serious problems for the developing fetus.

Histopathological examinations of the placenta can reveal changes which may be associated with maternal disorders and also result in feto-maternal complications. Placental studies are quantitative rather than qualitative as some changes take place in placenta before it separates from the uterus. The changes are considered pathological when the extent of involvement is greater than normal[9]. The present study was carried out to compare various parameters of placental histology and their significance.

Syncytial knots are composed of aggregates of small, closely packed densely staining nuclei protruding from the villous surface into the intervillous space. In the literature, it is hypothesized that villous hypovascularity leads to formation of syncytial knots, indicating sequestration of aged nuclei is being accelerated or augmented so as to use optimally the amount of trophoblast available for transfer purpose.[9] Increased syncytial knots in placentae in anaemia suggested that an attempt was being made to form new villi so as to increase an effective surface area for exchange.[10]

Syncytial knots in more than 30% of the villi are considered excessive.[11]In the present study, high villous syncytial count (i.e. over 30% of the villi showing syncytial knots) were seen in 34/56 placentae of anaemia group as compared to 11/56 placentae of control group. A study reported low incidence of excessive syncytial knots in anaemia group.[12]

But, other studies have reported high villous syncytial counts.[13, 14.] Few studies did not find any difference in the incidence of syncytial knots in anaemia and control group.[15, 16.]

Vasculo-syncytial membranes are focally differentiated areas of syncytiotrophoblast which are specifically concerned with materno-fetal transfer. It consists of attenuated anuclear syncytiotrophoblast stretched over, and in close apposition to, a sinusoidally dilated vessel.[9] Vasculo-syncytial membrane counts measure the approximation of the villi to optimal maturity and hence give a good indication of the ability of the placenta to supply oxygen to the foetus.[9]

Placentae in which between 6 and 30 percent of the villi show Vasculo-syncytial membranes are said to have a normal count.[17.] In the present study, all the placentae in both groups had Vasculo- syncytial membrane count within the normal range. None of the placentae showed deficiency of membranes (<5% of the villi) nor had high Vasculo-syncytial membrane count (>30% of the villi). The mean number of villi with vasculo-syncytial membrane with per 100 villi was 12.08 in anaemia group compared to 15.62 in control group the difference between the two groups was statistically significant ($p<0.01$) (table 5)But there was no deficiency of the membranes in the anaemia group and the control group. It may therefore just suggest placental maturity in both the groups. In contrast, an earlier study documented increase in the vasculo-syncytial membrane counts in anaemia group.**[10]**

Fibrinoid necrosis is seen as a nodular mass of homogeneous acidophilic material in the villi. Placentae in which fibrinoid necrosis involving upto three percent of placental villi is considered as normal.[18]

Fibrinoid necrosis may be a degenerative change in villous trophoblast.[18] Recent literature suggests it to be an immunological reaction within the trophoblastic tissue.[9]

In the present study, 30/56 (53.57%) placentae of anaemia group showed more villi with fibrinoid necrosis count as compared to 6/56 (10.71%) placentae of control group. The mean number of villi showing fibrinoid necrosis per 100 villi was 3.26 in anaemia group and 1.64 in the control group. The difference between the two groups was statistically significant ($p<0.001$) this finding was in conformity with earlier studies.[13, 14, 16.] In contrast, a study has reported decreased incidence of villi with fibrinoid necrosis, in severe anaemia subgroup.[12]

In term placenta normally ≤ 3 percent of villi may show increase in stromal fibrosis.[19] Increase villous stromal fibrosis seen in the anaemic placentae may be the result of relative hypoxia in the peripheral part of the placental lobule.

In the present study, 24/56 (42.85%) placentae of anaemia group revealed increase in the incidence of villi with stromal fibrosis as compared to 4/56 (7.14%) placentae of control group. The difference between the two groups was statistically very significant ($p<.001$). The mean number of fibrotic villi per 100 villi was 4.08 in anaemia group and 1.44 in the control

group. [10] Earlier studies have also reported increase in villous stromal fibrosis in the anaemia cases. [12, 13, 15, 16.]

CONCLUSION:

The present study which was undertaken to evaluate the possible histopathological changes and quantitate them showed significant microscopic changes in placentae of anaemia group as compared to controls.

1. Placenta in severe anaemia showed excessive syncytial knots, increased fibrinoid necrosis of the villi, increased villous stromal fibrosis.
2. Quantitative determination of placental changes is essential in study of placenta as normal pregnancies can also show significant placental changes.
3. Severe anaemia in pregnancy alters the placental morphology and adequate treatment of anaemia may therefore be critical to normal placental function.

ACKNOWLEDGEMENT: Dr. P. V. Patil **MD, PhD, DHA, FIC Path.** Professor and H.O.D, Department of Pathology, J. N. Medical College, Belgaum for guiding me in the study.

REFERENCES:

1. Klebanoff MA, Shrono PH, Selby JU, Trachtenberg AI, Gaubard BI. Anemia and spontaneous preterm birth. Am J Obstet Gynaecol 1991; 164: 59-63.
2. Liebermani E, Ryan KJ, Monson RR. Association of maternal hematocrit with premature labour. Am J Obstet. Gynaecol. 1988; 159:107.
3. Murphy JE, O' Riordan J, Newcombe RG, Coles EC. Relation of haemoglobin levels in first and second trimester to outcome of pregnancy. Lancet 1986; 1:992.
4. Agarwal KN. Functional consequences of nutritional anaemia. Proc Nutr Soc Ind. 1991; 37: 127-132.
5. Pandya P, Hazra MN.Fetomaternal outcome in patients of severe anaemia in pregnancy.J Obstet Gynaec India1993;43(1):5-10
6. Firkin F, Chesterman C, Penington D, Rush B.The red cell and anaemia.In:de Gruchy's clinical haematology in medical practice.5th edn.Calcutta: Oxford university press; 1990.26-27.
7. World Health Organization. Prevention and management of severe anaemia in pregnancy. Geneva WHO; 1993.
8. World Health Organization. Preventing and controlling Iron deficiency anaemia through primary health care. WHO publications 1989.2
9. Fox H. Pathology of placenta. 2nd edn Philadelphia: W.B. Saunders Co.Ltd; 1997.
10. Dhall U. Histological changes in placenta in anaemia: A Quantitative study. J Anat Soc India 1994; 43(1):21-26
11. Fox H. The significance of villous syncytial knots in the human placenta. J Obstet Gynaec Brit Cwlth. 1965; 72:347-355.
12. Rangnekar AG, Darbari R. Placental changes in pregnancy anaemia. Study of one hundred cases. J Obstet Gynaecol India 1993; 43; 473-478.
13. Sabharwal BD, Malhotra V, Sofat R, Duggal A. Histopathology of placenta in pregnancy anaemia. J Obstet Gynaecol India 1987; 37:773-776.

14. Gyatri V, Jain S,Kalra R,Karla VB,Sareen PM,Lodha KS et al.Histopathology of placenta in pregnancy anaemia.J Obstet Gynaecol India 1983;33:187-189.
15. Agboola A. Placental changes in patients with a low haemotocrit. The British Journal of Obstetrics and Gynaecology. 1975; 82:225-227.
16. Khanna S, Chand S, Singla PN, Agarwal KN. Morphological study of placenta in pregnancy anaemia. J Obstet Gynaecol India 1979;22:7-12.
17. Fox. H. The incidence and significance of vasculo-syncytial membranes in the human placenta. J Obstet Gynaec Brit Cwlth. 1967;74:28-33.
18. Fox H. Fibrinoid necrosis of placental villi. J Obstet Gynaec Brit Cwlth. 1968; 75:448-452.
19. Fox H. Fibrosis of placental villi. J Pathology and bacteriology. 1968; 95:573-579

Table 1: Incidence of increased syncytial knots:

Group	Percentage of villi with syncytial knots	Number of placentae	Percentage	z value	p value
Control	**≤ 30%**	**45**	**80.35**	**4.4330**	**<0.001**
	>30%	**11**	**19.64**		
Anemia	**≤ 30%**	**22**	**39.28**		
	> 30%	**34**	**60.71**		

Table 2: Incidence of Vasculo-syncytial membranes:

Group	Percentage of villi with vasculo syncytial membrane	Number of placentae	Percentage	z value	p value
Control	**≤ 20**	**49**	**87.5**	**1.7380**	**>0.05**
	>20	**7**	**12.5**		
Anaemia	**≤20**	**54**	**96.42**		
	>20	**2**	**3.57**		

Table 3: Incidence of increased fibrinoid necrosis (Intravillous fibrinoid):

Group	Percentage of villi showing fibrinoid necroses	Number of placentae	Percentage	z value	p value
Control	**≤ 3**	**50**	**89.28**	**4.8558**	**<0.001**
	>3	**06**	**10.71**		
Anaemia	**≤3**	**26**	**46.42**		
	>3	**30**	**53.57**		

Table 4: Incidence of increased fibrotic villi (Increased villous stromal fibrosis):

Groups	Percentage of fibrotic villi	Number of placentae	Percentage	z value	P value
Control	**≤ 3**	**52**	**92.85**	**4.3644**	**<0.001**
	>3	**4**	**7.14**		
Anaemia	**≤3**	**32**	**57.14**		
	>3	**24**	**42.85**		

Table 5: Mean number of histological parameters (per 100 villi):

Histological parameter	Control group (n=56)	Anaemia group (n=56)	p value
Syncytial knots	**19.7±7.55**	**31.01±3.29**	**<0.001**
Vasculo-syncytial membranes	**15.63±4.45**	**12.05±4.83**	**>0.01**
Fibrinoid necrosis	**1.64±1.43**	**3.26±2.29**	**<0.01**
Fibrotic villi	**1.45±1.39**	**4.08±2.07**	**<0.001**

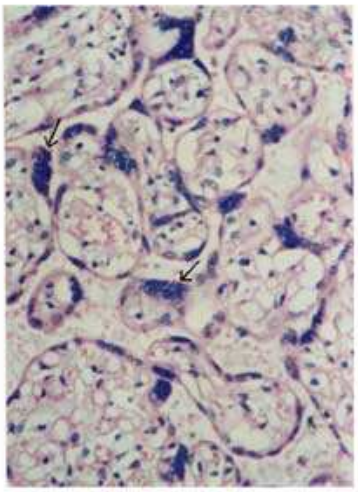


Fig 1-Villi showing excessive Syncytial knots (H &E, X 10)

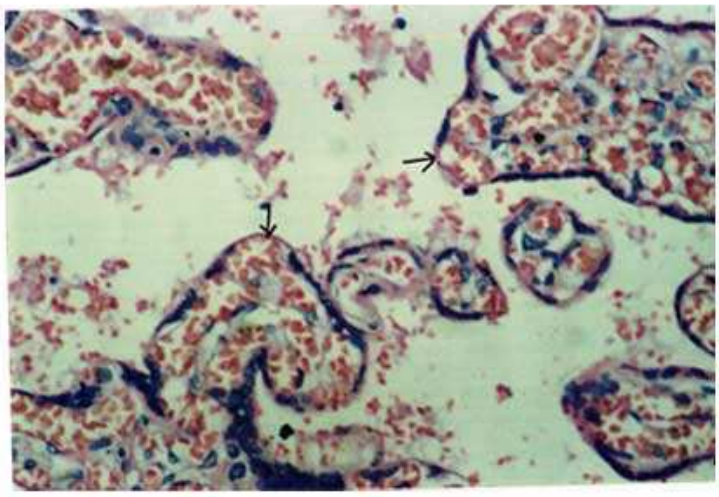


Fig 2-Villi showing Vasculo-syncytial membranes (H & E, X40)

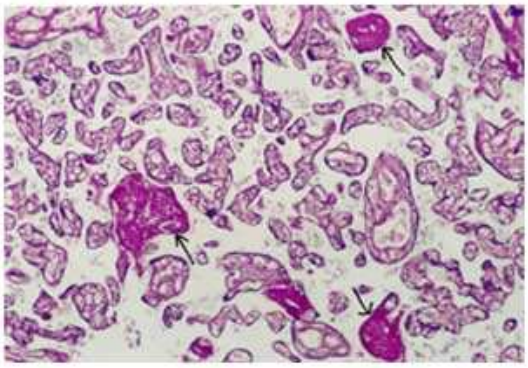


Fig 3-Villi showing Fibrinoid necrosis (Intravillous fibrinoid) (P.A.S, X40)

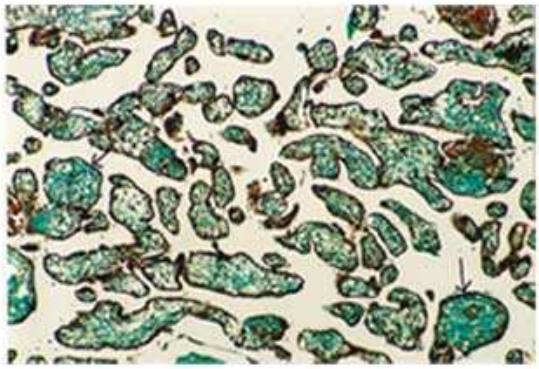


Fig 4-Villi showing Increased Stromal fibrosis (M.T.S, X10)

LASERS IN PROSTHODONTICS- A REVIEW.

Sandesh Gosawi, Sanajay Kumar, Rohit Lakhyani, Shraddanand Bacha, Shivaraj Wangadargi

1. Professor & HOD, Department of Prosthodontics, College of Dental Sciences, Amargadh, Gujarat, India.
2. Senior Lecturer, Department of Prosthodontics, College of Dental Sciences, Amargadh, Gujarat, India.
3. Senior Lecturer, Department of Prosthodontics, College of Dental Sciences, Amargadh, Gujarat, India.
4. Senior Lecturer, Department of Prosthodontics, College of Dental Sciences, Amargadh, Gujarat, India.
5. Reader, Department of Prosthodontics, College of Dental Sciences, Amargadh, Gujarat, India.

CORRESPONDING AUTHOR

Dr. Sanajay Kumar, Sr. Lecturer,
Department of Prosthodontics,
College of Dental Sciences, Amargadh,
Gujarat State, India.
E-mail: drsanajay@in.com,
Ph: 0091 07359270096.

ABSTRACT: Since the development of the ruby laser by Maiman in 1960, a variety of studies on the potential applications of lasers in dentistry have been conducted. Many applications like computer aided design and rapid prototyping technology, and study of occlusion in complete dentures using three-dimensional laser scanner have been developed. Its applications range from fixed Prosthodontics to treatment of dentinal hypersensitivity to surface treatment of base metal alloys. Today it even extends to the fields of dental implantology and maxillofacial Prosthodontics. This article reviews and summarises various studies of the laser applications in Prosthodontics.

KEY WORDS: LASER, complete denture, CAD/CAM, Impression, Dentinal hypersensitivity, Alloys, Crown, Preparation, Welding, Dental implants, maxillofacial prosthesis.

INTRODUCTION: Light is an integral part of our life. The early 20th century saw one of the greatest inventions in science & technology, in that **LASERS** (Light Amplification by Stimulated Emission of Radiation) which later went on to became a gift to health sciences. A laser is an instrument that produces a very narrow, intense beam of light energy (electromagnetic radiation) through a process called stimulated emission. Albert Einstein is usually credited for the development of the laser theory. He was the first one to coin the term "Stimulated Emission" in his publication "Zur Quantentheorie der Strahlung", published in 1917 in the "Physikalische Zeitschrift"[1].

The use of lasers for treatment has become a common phenomenon in the medical field. Theodore Harold Maiman is generally given credit for building the first working ruby laser and operating it for the first time on May 16, 1960 at the Hughes Research Laboratory in Malibu, California. MASER a microwave amplifier by Charles H.Townes, P.Gordon et al became the basic principle for laser pumping. This set the stage for a "snowball effect" which would lead to the development of many laser systems, which we utilize in healthcare today. The application of a laser to dental tissue was reported by Stern and Sognnaes and Goldman et al. in 1964, describing the effects of ruby laser on enamel and dentine with a disappointing result. However, with the recent advances and developments of wide range of laser wavelengths and different delivery systems, researchers suggest that lasers could be applied for the dental treatments.[1]

Currently, numerous laser systems are available for dental use. Neodymium-doped: Yittrium-Aluminium-Garnet (Nd: YAG), carbon dioxide (CO2) and semiconductor diode lasers

have already been approved by the United States Food and Drug Administration for soft tissue treatment in oral cavity. The Erbium doped: Yttrium-Aluminium-Garnet (Er: YAG) laser was approved in 1997 for hard tissue treatment in dentistry [2].

BACKGROUND KNOWLEDGE: The basic components of a laser are straightforward and are always similar regardless of the type of equipment. They include an active lasing medium within an optical cavity (resonator) and a pumping source (energy source). The optical cavity consists of two mirrors placed on either side of the laser medium. Due to this arrangement, photons resulting from the stimulated emission will form a continuous avalanche process. As long as the pumping energy maintains the population inversion in the active medium, more stimulated photons are created thus producing energy. The energy is absorbed and emitted in the resonator and with the aid of mirrors, is reflected and resonates within this chamber, and ultimately produces laser light. Because one of the mirrors is partially transmissive, some of the laser energy escapes at one end of the device into a delivery system .Consequently; a laser is just a source to generate a high energetic beam of light, which is monochromatic, collimated and coherent. In medical is the photo thermal effect in the range of m sec to sec of irradiation time. The light energy is converted into thermal energy, which is locally cooled by water that irrigates the irradiated and surrounding tissue. As the temperature increases at the surgical site, the tissues can be warmed up to (37-50°C), coagulated (60-70°C), welded (70-90°C), and vaporized (100-150°C). If the laser energy continues to be absorbed by the tissue, carbonization occurs (>200°C) and with it the possibility of significant tissue damage. Consequently, both target and surrounding tissues can be subjected to these harmful effects.[3,4]

LASERS USED IN PROSTHODONTICS:

I) COMPLETE DENTURE PROSTHODONTICS:

i) Prototyping and CAD/CAM (Computer Aided Design and Computer Aided Manufacturing) technology.
ii) Analysis of occlusion by CAD/CAM.
iii) Analysis of accuracy of impression by laser scanner.

II) FIXED PARTIAL DENTURE:

i) Tissue management.
ii) To treat dentinal hypersensitivity.
iii) Pretreatment of non-precious alloys.
iv)Crown preparation?

III) REMOVABLE PARTIAL DENTURE:

Laser welding.

IV) IMPLANT DENTISTRY:

i) Soft tissue surgery.
ii) Implant surface debridement.
iii) Implant surface treatment.

V) MAXILLOFACIAL PROSTHODONTICS:

i) Sintering with CAD/CAM technology.

I) COMPLETE DENTURE PROSTHODONTICS:

I) PROTOTYPING AND CAD/CAM TECHNOLOGY: The term rapid prototyping (RP) refers to a class of technologies that can automatically construct physical models from Computer-Aided Design (CAD) data. These "three dimensional printers" allow designers to quickly create tangible prototypes of their designs, rather than just two-dimensional pictures. Such models have numerous data.

In addition to prototypes, RP techniques can also be used to make tooling (referred to as rapid tooling) and even production-quality parts (rapid manufacturing).A software package slices the CAD model in to a number of thin (eg.0.1mm) layers, which are then built up one atop another. Rapid prototyping is an additive process, combining layers of paper, wax, or plastic to create a solid object.

In contrast, most machining processes (milling, drilling, grinding, etc.) are "subtractive" processes that remove material from a solid block. RP's additive nature allows it to create objects with complicated internal features that cannot be manufactured by other means.[5,6]

LASER RAPID FORMING OF A COMPLETE TITANIUM DENTURE BASE PLATE:[7] This technique uses the combination of the CAD/CAM and LRF (Laser Rapid Forming) methods for forming the titanium plate of a complete denture. Laser scanner, reverse engineering software, and standard triangulation language (STL) formatted denture base plate and sliced into a sequence of numerical controlled codes.

The denture plate will be built layer-by-layer, on the LRF system.

After the traditional finishing techniques, this denture plate will be acceptable for use in patients.

II) STUDY OF COMPLETE DENTURE OCCLUSION USING BY THREE-DIMENSIONAL TECHNIQUE:[8] After fabrication of new dentures the occlusion can be examined and studied with the help of laser scanner technique and three-dimensional reconstruction. The relationship between the parameters of balanced occlusion can also be analyzed.

III) ANALYSIS OF ACCURACY OF IMPRESSION BY LASER SCANNER:[9] Several studies have made comparisons in the dimensional accuracy of different elastomeric impression materials. Most have used two dimensional measuring devices, which neglect to account for the dimensional changes that exist along a three-dimensional surface.

The scanning laser three-dimensional (3D) digitizer can delineate x, y, and z coordinates from a specimen without actually contacting the surface. The digitizer automatically tracks coordinates with precision and stores data as the number of points on a surface with a resolution of 130 mm at 100 mm. These exacting features suggest that the laser digitizer might accurately and reliably measure the dimensions of dental impression materials while avoiding subjective errors.

The image is built up and landmarks identified which allow superimposition of the images and so enable the differences between two similar images to be calculated.

The 3D laser captures complex 3D texture-mapped models and they are exported into a 3D (Scan Surf) software application where it is built and triangulated into a 3D meshwork image

of the object. The scanning process is accomplished within a minute whereas the software analysis takes much longer. The software superimposes the two objects by either registering landmarks or by registering as iterative closest point (ICP). This finds an optimal fit between the two surfaces and in effect acts as a reference area. Once superimposed, the difference of the two surfaces is calculated as the shortest distance of each point on one object surface from a second object surface, within a range of 0.5 mm.

Three-dimensional digitizers will eventually become less expensive, require less maintenance, track faster, and be available with more standardized software.

FIXED PARTIAL DENTURE:

I) TISSUE MANAGEMENT:

Crown lengthening:[4] This is a procedure when inadequate crown height is present for crown restoration an adequate crown height is created by removing required gingival soft tissue.

With the help of the lasers soft tissue crown lengthening can be done without raising a flap. By its thermal effect the laser seals vascular and lymphatic vessels at the same time it vaporize the excess gingival tissue.

Since no flap was required for this surgery, sutures were not necessary and the wound healed by secondary intention.

ADVANTAGES:

Increased coagulation that yields a dry surgical field and better visualization.
Tissue surface sterilization and therefore, reduction in bacteremia.
Decreased swelling, edema of target tissue.
Decreased pain, and in some cases no need of anesthesia while surgery.
Faster healing response and increased patient acceptance.
Less chair-side time.

II) TO TREAT DENTINAL HYPERSENSITIVITY:[10,11]

There are many theories to describe the mechanism of hypersensitivity, but all conclude that open dentinal tubules are the path of stimuli to elicit response.

The first laser use for the treatment of dentine hypersensitivity was reported by Matsumoto et al (1985) using Nd: YAG laser. Many of the lasers investigated can induce significant thermal effects, if laser parameters are inadequately controlled, giving rise to concerns regarding thermal damage to temperature sensitive pulpal tissue.

MECHANISM OF ACTION OF LASERS IN TREATING DENTINAL HYPERSENSITIVITY: The precise mechanism of the ablation of hard tissues with the laser remains unclear. One theory suggested that when laser interacts with the hard tissue it is absorbed by the water and hydroxyapatite. The laser heats the water causing it to become steam. This expansion during the change of state of water causes cracking of the tissue. As the steam expands it also forces the cracked material away from the ablation zone. Because this is a very rapid action, it is explosive (micro explosion) in nature. The lasers used for the treatment of the dentinal hypersensitivity are divided in to two groups.

HE-NE LASER: The first use of this laser for the treatment of dentine hypersensitivity was reported by Senda et al. (1985).Irradiation modes were two types: pulsed (5 Hz only) and

continuous wave (CW) mode. Treatment effectively ranged from 5.2 to 100%.The mechanism involved is mostly unknown, but according to physiological experiments it increases the action potential of related nerve fiber. Its action will be long lasting.

GAALAS LASER: The first use of this laser for the treatment of dentine hypersensitivity was reported by Matsumoto et al.(1985).An output power of 30 mW was used for the treatment. Irradiation mode was CW, and irradiation time ranged from 0.5 to 3 min. Treatment effectiveness ranged from 85 to 100%.The mechanism of action involved is blocking the depolarization of C-fiber afferents. (Wakabayashi et al.1992-1993).

ND: YAG LASER: The first use of this laser for the treatment of dentine hypersensitivity was reported by Matsumoto et al. (1985).The output power used may vary but 1-2 W is sufficient most commonly. The use of black ink as absorption enhancer is recommended when using Nd: YAG laser to prevent deep penetration of the laser beam through the enamel and dentin and excessive effects on pulp.[12]

CO_2 LASER: The first use of this laser for the treatment of dentine hypersensitivity was reported by Moritz et al.(1996).Effect of this laser on dentinal hypersensitivity treatment is due to the occlusion or narrowing of dentinal tubules. This laser irradiation may also cause dentinal desiccation, yielding temporary clinical relief of dentinal hypersensitivity (Fayad et al.1996).CO_2 laser with 1 W for 5-10 s duration could treat dentin hypersensitivity without adversely affecting the pulp.[13]

ER: YAG LASER: This laser came up after all the above to avoid some of the disadvantages they have. These lasers are basically advantageous than above in the nature that these produce very less heat while application due to better absorption by water and hydroxyapatite. Desensitizing effects of this laser are effective and long lasting compared to above lasers at 80 mj and 3 Hz output. Its desensitizing affects attributed to the deposition of insoluble salts in the exposed dentinal tubules.[14,15]

ER: CR: YSGG LASER: This is a recent laser tool using in dentistry for variety of purposes. The mechanism of dentin removal as explained above for other erbium laser is by "thermo mechanical process" in which the emission laser light is absorbed by the water within the hydroxyapatite of a dental hard tissue. The water is then heated and evaporated, resulting in a high pressure of steam that causes a micro explosion of tooth tissue below the melting point of tooth tissue (approximately 1,200ºc). Cumhur Sipahi, Nukhet Berk and Julide Ozen 2006 showed that long time-low potency (LT/LP) Er, Cr: YSGG lasers at 0.5W potency for 30s produce required dentinal tubule closure to treat dentinal hypersensitivity.[16,17]

III) CROWN PREPARATION:[18] Crown preparation with lasers a debated topic still. There are no conclusive studies yet showed the use of lasers for crown preparation purposes. But still some commercial companies say that they can be used. The following is the details what these companies say:

Er, Cr: YSGG laser is used most commonly now.

It uses hydrokinetic technology (laser-energized water to cut or ablate soft and hard tissue).

Because of this mechanism local anesthesia is not required in many cases, making this more comfortable procedure for the patient, and of course, saving time and anesthetic use by the patient.

The laser hand piece resembles a high-speed
hand piece but with fiber-optic tips instead of a bur, which directs the laser energy at a focal point approximately 1-2 mm from the tissue surface.

The crown preparation should be started on maximum setting for cutting enamel (6 W,90% air,75% water),started with a defocused mode for 30 seconds to 1 min for anesthesia of tooth.

While placing the gingival margin setting will be reduced 1.25 W, 50% air, 40% water to control the cutting tip, for the purpose of accuracy.

To finish with the interproximal, buccal, lingual/palatal reduction cuts will be performed with the dentin settings 4W, 65% air, 55% water.

The laser has to be reset at 2.25 W, 65% air, 55% water to finish the buccal cusp overlay, and the final Margi nation of the proximal and lingual surfaces.

ADVANTAGES:

For vital crown preparation no need of local anesthesia, as laser causes temporary paresthasia of nerve endings.

Procedure is accurate and faster than the conventional method.

DISADVANTAGES:

Trained dentist required the particular use.

III. REMOVABLE PARTIAL DENTURES:[19]

Laser welding:

One of the modern methods of removable partial dentures defect repairs uses the pulsed laser with relative low average out power. This is known as a precise and rapid joining method, but its success depends on the control of many parameters.

Eg: For Co-Cr alloy frameworks:

The welding parameters were determined for each defect type and working step (fixing, joining, filling, planning).Adequate combination of pulse energy (6-14 J), pulse duration (10-20 ms) and peak power (600- 900 W) depending on the working stage improves the success of the welding procedure.

IV. IMPLANT DENTISTRY:[20,21]

FOR STERILIZATION OF SOCKET: In immediate implant dentistry after extraction of tooth, without any infection, socket can be sterilized immediately without any pain.

IN CASE OF PERI- IMPLANTITIS: Since the laser does not transmit damaging heat, it can be utilized to vaporize any granulation tissue as well as clean the implant surface in peri-implantitis cases. This procedure eliminated the acute state of peri-implantitis, resulting in positive GTR, and allowing the patient extended use of the implant.

TO DEBRIDE THE IMPLANT SURFACE: Miller Robert has shown that treatment of the contaminated implant surface by mechanical and chemotherapeutic means has met with mixed success. Development of a laser system operating at 2780 nm and using an ablative

hydrokinetic process offers the possibility for more efficient decontamination and debridement. Laser ablation using the Er: Cr: YSGG laser is highly efficient at removing potential contaminants on the roughened implant surface while demonstrating no effects on the titanium substrate.

V.IN MAXILLOFACIAL PROSTHESIS:[22] New advances in rapid prototyping technologies have demonstrated significant advantages compared to more conventional techniques for fabricating facial prosthesis. The use of selective laser sintering technology is an alternative approach for fabricating a wax pattern of maxillofacial prosthesis. This new approach can generate the wax pattern directly and reduce labor-intensive laboratory procedures.

SLS (SELECTIVE LASER SINTERING): The SLS (Selective Laser Sintering) is a method of computer aided designing using mainly the laser. In this method models are generated directly from 3-D computer data then converted to STL files, which are then sliced in to thin layers (typically about 0.1 mm/0.004 inches) using the associated computer software. The laser sintering machine produces the models on a removable platform by applying incremental layers of the pattern material. For each layer, the machine lays down a film of powdered material with an accurate required thickness, again a fresh film of powder is laid down, and the next layer is melted with exposure to the laser source. This process continues, layer by layer, until the pattern is completed.

ADVANTAGES:

Manufacturer time is reduced.

More precision can be achieved.

REFERENCES

1. T.H.Maiman. 1960.Stimulated optical radiation in ruby. NATURE; Aug 6.
2. Ishikawa I, Aoki A, Takasaki AA. 2004. Potential applications of Erbium:YAG laser in periodontics. J Periodontal Res. Aug;39(4):275-85.
3. A.J.Welch, Jorge H.Torres, Wai-Fung Cheong. 1989. Laser physics and Laser-tissue interaction. Texas heart institute journal; 16:141-9.
4. Dr.AndrE Chartand. April 2005.Integrating laser dentistry in to aesthetic dentistry. Cosmetic dentistry, Oral health.
5. Richard J.Thomas US. Dec 26, 2006. Method for automatically creating a denture using laser altimetry to create a digital 3-D oral cavity model and using a digital internet connection to a rapid steriolethographic modeling machine. United States patent US 7,153,135 B1.
6. Sun YC, Lu PJ, Wang Y et al. 2007.Research and development of computer aided and rapid prototyping technology for complete dentures. Chinese Journal of Stomatology; 42:6:324-9.
7. Wu J, Gao B, Tan H et al. 2008 Aug 21. A feasibility study on laser rapid forming of a complete titanium denture base plate. Lasers Med Sci.
8. E.Y.Lu, F.Q.Zhang, X.J.Chen et al. June 28-July1, 2006. A study of complete denture e occlusion using by three-dimensional technique. IADR exhibition.
9. Sinal Shah, Geeta Sundaram, David Bartlett et al. 2004.The accuracy of a 3D laser scanner using superimposition software to assess the accuracy of impression techniques. Journal of Dentistry; 32:653-658.
10. Kimura Y, Wilder-Smith P, Yonaga K et al. 2000. Treatment of dentine hypersensitivity by lasers: a review Clin Periodontal; 27:715-721.

11. Cumhur Sipahi, Nukhet Berk, Julide Ozen et al. 2006.Tubule-occluding effect of desensitizing laser treatment on prepared dentin surfaces: An environmental SEM study. Int J Prosthodont;19:37-39.
12. Bor-Shiun Lee, Chun-Wei Chang, Weng-Pin Chen et al. 2005. In vitro study of dentin hypersensitivity treated by Nd: YAP laser and bioglass. Dental Materials; 21:511-519.
13. Wan-Hang Lan, Kau-Wu Chen, Jiiang-Huei Jeng et al. 2000.A comparison of the morphological changes after Nd: YAG and CO_2 laser irradiation of dentin surfaces. Journal of Endodontics; 26:8:450-453.
14. Oberhofer O, Sculean A. 2008.Er: YAG laser and desensitizing effects on dentin and dental cervices. J Oral Laser Application; 3.
15. Yuki NISHIMOTO, Masayayuki OTSUKI, Monica YAMUTI et al. 2008. Effect of pulse duration of Er: YAG laser on dentin ablation. Dental Materials Journal; 27:3:433-439.
16. Piyanart Ekworapoj, Sharanbir K.Sidhu, and John F.McCabe. 2007. Effect of different power parameters of Er: Cr: YSGG laser on human dentine. Lasers Med Sci; 22:175-182.
17. Emre Altundasar, Bahar Ozcelik, Zafer C.Cehreli et al. 2006.Ultra morphological and histochemical changes after Er: Cr: YSGG laser irradiation and two different irradiation regimens .JOE; 32:5:465-468.
18. Dr.Rosh nash. Oct 2002.Crown and veneer preparation using the Er, Cr:YSGG Waterlase™ hard and soft tissue laser. Cotemporary esthetics and restorative practice,80-85.
19. Liliana sandu,V.Birdeanu. 2006. Laser welding procedures applied to removable partial denes framework repairs.Europian cells and materials.11 (2):29.
20. Stefan stubinger, Frank homann. 2008. Effect of Er: YAG, CO2 and diode laser irradiation on surface properties of zirconia endosseous dental implants. Laser in surgery and medicine. 40(3):223-228.
21. Miller, Robert J. 2004.Treatment of the contaminated implant surface using the Er, Cr;YSGG laser. Implant dentistry. 13(2):165-170.
22. Guofeng Wu, Bing Zhou. 2008. Selective laser sintering technology for customized fabrication of facial prostheses. J Prosthet Dent; 100:56-60.

Fig I Nd: YAG laser emission.

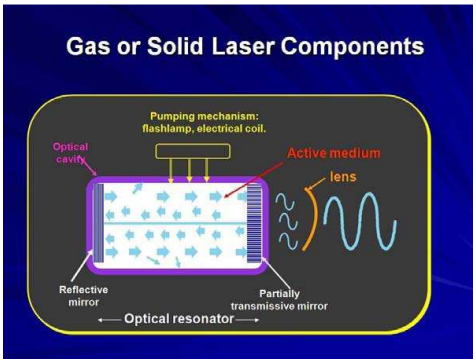


Fig II Images captured by the scanner of the teeth.

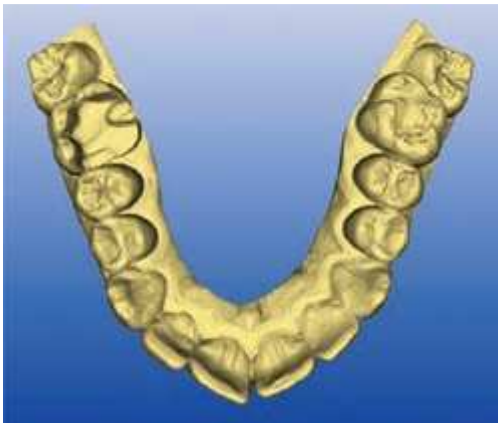
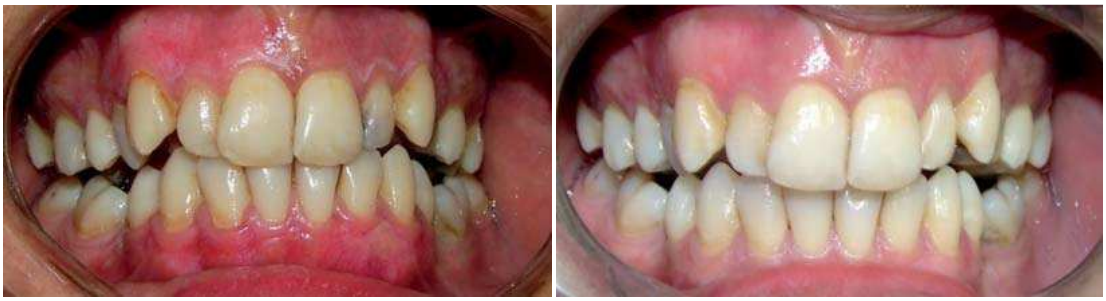


Fig III

a.Pre-operative. **b.Laser tip.**



c. Post-operative

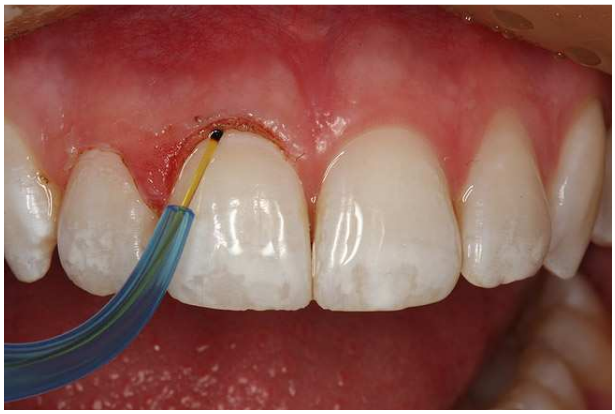
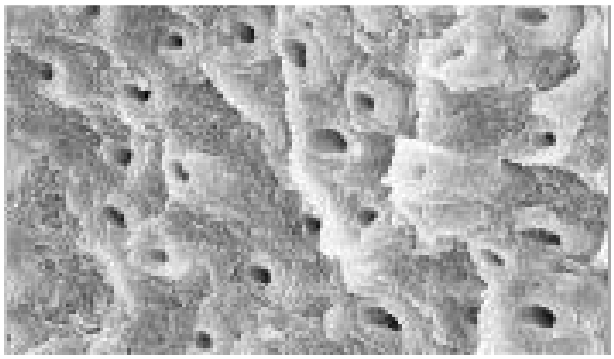


Fig IV a. SEM photograph of dentin Er;YAG laser.

b.Plume from dentin ablation with the ablated with Er; YAG laser.



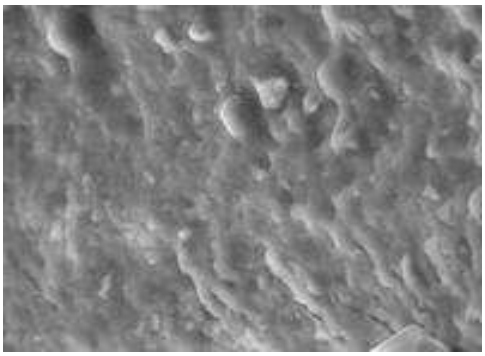


Fig VI

a. Cracked continuous bar & lingual bar.

b. Repaired with laser welding

FIG V TOOTH PREPARATION WITH LASER

STUDY OF ENZYMATIC ANTIOXIDANT MARKER & LIPID PEROXIDATION STATUS IN PATIENTS WITH OSTEOARTHRITIS

Manoj Narayan Paliwal, A. N. Sontakke, Prachi Paliwal

1. Assistant Professor, MD, Department of Biochemistry, R. D. Gardi Medical College, Ujjain.
2. Professor & Head, MD, Department of Biochemistry, MIMER Medical College, Pune.
3. Demonstrator, Department of Biochemistry, R. D. Gardi Medical College, Ujjain.

CORRESPONDING AUTHOR

Dr. Manoj Narayan Paliwal (Biochemistry Dept.)
R. D. Gardi Medical College, Agar Road,
Surasa, Ujjain (M.P.) Pin- 456003,
E-mail: dr.manojpaliwal@yahoo.co.in,
Ph: 0091 09303158744, 0091 9977692204.

ABSTRACT: BACKGROUND/AIMS: Osteoarthritis is an inflammatory & degenerative disorder of joints. The exact pro-oxidant & antioxidant status is not clear in osteoarthritis. Our aims were to estimate levels of the lipid peroxidation (in terms of MDA) & enzymatic antioxidant (in terms of superoxide dismutase) in serum of osteoarthritis patients & compare them with the levels in normal healthy controls. **MATERIAL & METHODS:** A study was performed at the department of biochemistry at Pd. Dr. D. Y. Patil medical college, Pimpri, Pune-18(M.S.) In 30 patients of osteoarthritis serum levels of enzymatic antioxidant Marker (SOD) & lipid peroxidation status (MDA) were estimated by spectrophotometry. Thirty healthy controls were also included in the study & serum levels of same parameters were also measured. **STATISTICAL ANALYSIS:** It was performed by using the student unpaired t test. **RESULT:** A serum level of enzymatic antioxidant marker (SOD) was increased in the patients than in the controls. The serum lipid peroxidation (MDA) level was increased in the patients than in the controls.

KEYWORDS: Osteoarthritis (OA), Superoxide dismutase (SOD), Malondialdehyde (MDA)

INTRODUCTION: Osteoarthritis is a degenerative & inflammatory, joint disease, characterized by progressive erosion of articular cartilage. Osteoarthritis joins heart disease & cancer as one of the dividens of growing older .The age related changes in cartilage include alterations in proteoglycans & collagen .Chondrocyte plays an important role in the process & plays a cellular basis of the disease.[1]

At the sites of inflammation of joint increased free radical activity is associated with activation of neutrophils, phagocytosis by macrophages, which involve respiratory bursts phenomenon& uncoupling of variety of cellular redox systems. [2,3] & lead to ultimately increased peroxidation of unsaturated lipids of the membrane.

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction & cell damage. Antioxidants are compounds that dispose, scavenge, & suppress the formation of free radicals or oppose their actions.[4]

This study evaluates the association between lipid Peroxidation & enzymatic antioxidant marker in osteoarthritis.

To the best of our knowledge, only very few studies have been performed with respect to the estimation of the serum enzymatic antioxidant levels in osteoarthritis & their role in prevention & treatment of osteoarthritis. In the light of this explanation, the present study was

undertaken to find out the levels of the enzymatic antioxidant (SOD) in the serum of osteoarthritis.

MATERIAL & METHOD: This study was conducted in the department of biochemistry, Pd. Dr. D. Y. Patil medical college, Pimpri. Pune-18.

The present study consists of thirty clinically diagnosed cases of osteoarthritis ranging in the age from 30 to 60 years. The control group consists of age & sex matched thirty healthy volunteer.

A thorough physical examination was carried out on all the patients. Routine hematological & radiological investigation was also done. Thirty cases selected from orthopedics OPD diagnosed by orthopedician. The presence of osteoarthritis in patients were diagnosed by carrying out x-ray analysis of joint destruction as well as C –reactive protein & antinuclear antibodies test.

INCLUSION CRITERIA: subjects with normal nutritional habits without supplementing with any vitamins during the last six months included in the study.

EXCLUSION CRITERIA - None of these subjects were alcoholics or chronic smokers, & none of them suffered from any systemic diseases like hypertension , diabetes, (confirmed by clinical & laboratory examination), not having any history of trauma to the joints , also subjects with history of receiving anti-inflammatory drugs in the last six months were excluded from study.
This study was also approved by the institutional ethical committee.

3 ml of fasting venous blood samples were collected in Plain vials for estimation of MDA. & SOD.

Serum for MDA &SOD estimation was separated by centrifuging the blood at 3000 rpm for 10 minute.
Serum MDA was estimated by Thiobarbituric acid method .[5]
SOD (Superoxide dismutase) was estimated by Markland & Markland method .[6]
All estimations were done within 24-48 hrs after specimen collection.
The results were presented as Mean ± SD. Statistical analysis was performed by using the student unpaired t- test&. P value < 0.05 was considered as significant.

RESULT: In present study, serum Malondialdehyde, Superoxide dismutase levels were estimated

P < 0.05 compared to controls – considered as a statistically significant

The serum MDA levels in the osteoarthritis patient was 9.37±5.33 nmol/ml which was increased than that of controls (3.98±1.98 nmol/ml, P<0.05)
Serum SOD levels in the osteoarthritis patient was 4.94±3.0 unit/ml which was increased in OA patients than controls (3.31±0.85, P< 0.05)

DISCUSSION: Osteoarthritis is characterized by increased markers of oxidative stress.
Recent studies have suggested that human articular chondrocyte can actively produce reactive oxygen species. (ROS). [7]

At the site of inflammation increased free radical activity is associated with activation of neutrophils, phagocytosis by macrophages which involve respiratory "burst phenomenon" and uncoupling of variety of cellular redox systems. These processes lead to ultimately increased peroxidation of unsaturated lipids of membrane. Increased lipid peroxidation suggests presence of increased oxidative stress which is the causative factor of joint diseases (K. K. Mane et al 1999). [8]

ROS are released during inflammation of the Synovial membrane of synoviocytes. These radical oxygen species with oxidative activity play an important role in the chondrocyte catabolic program being the mediators and effecters of cartilage damage. The damaging effect of the process is initiated by a chain reaction that provides continue supply of free radicals which initiates further peroxidation. This involves the mechanism of oxidative decomposition of n-3 and n-6 PUFA membrane phospholipids leading to formation of complex mixtures of lipid hydroperoxide aldehydic end products such as MDA. [9]

The present study showed increased level of lipid peroxidation product MDA & increased level of enzymatic antioxidant marker superoxide dismutase (SOD).

Since MDA is an index of lipid peroxidation, its level was estimated in patients with osteoarthritis to estimate the extent of lipid peroxidation. MDA levels were found to be increased in osteoarthritis than in healthy individuals, indicating an increase in the process of lipid peroxidation in osteoarthritis. Results are in agreement with Ruby K B. I et al (1998), [10] and K. K. Mane et al (1999) [08] and Tiku et al (2000 & 2003). [11, 12]

Serum SOD levels were found to be increased in patients with osteoarthritis than in healthy subjects indicating that increased enzymatic antioxidant levels in OA may be an adaptive response to increased oxidative stress. The over expression of SOD might be an adaptive response, and it results in increased dismutation of superoxide anion against hydrogen peroxide. Results are in agreement with M Manesh et al (2005) , [13]
& suprapaneni Krishna Mohan (2007). [14]

From the above discussion it is presumed that oxidative stress involved in pathogenesis of OA which results due to increased free radical production. This leads to alteration in the antioxidant status which varies with the individual antioxidant depending upon their biochemical action.

Further research required in this area to know the status of other antioxidant marker (like Vit E, C, B carotene, reduced glutathione) & about their beneficial therapeutic effect in the management of osteoarthritis & its complications.

REFERENCES:

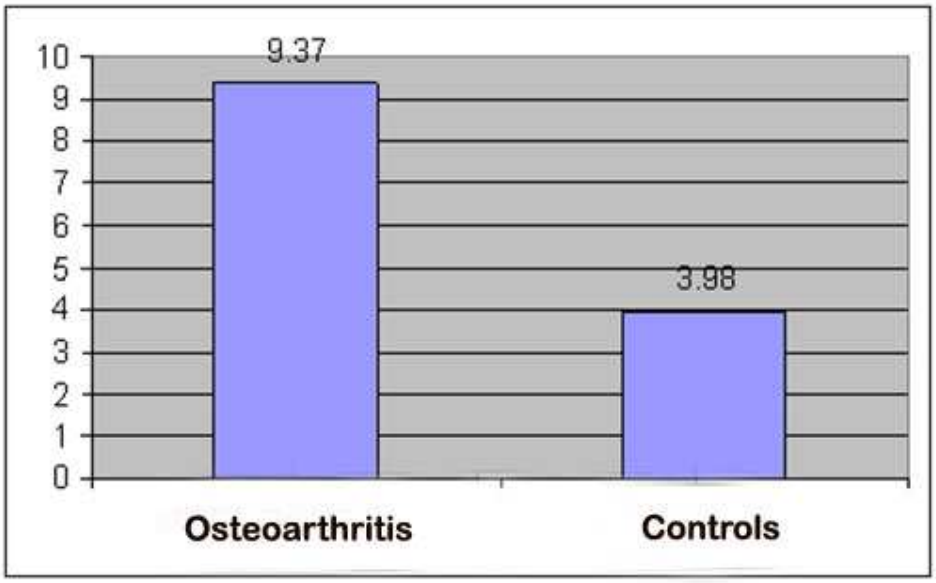
1. Cotran kumar, Collinis. Bones, joints and soft tissue tumors. Robbins pathologic basis of disease 6th edition. , page No.1246-1248.
2. Shin H.C., Hwang H J,Kang K J, Lee B H. An antioxidative and anti-inflammatory agent for potential treatment of osteoarthritis. Ecklonia cava. Arch Pharm Resp. 2006 Feb. [2]:165-71.
3. Sidhaye A, Cheskin L J. Pharmacological treatment of obesity. Adv. Psychosom med. 2006, 27:42-52.
4. Sie H. (1991).Oxidative stress: from basic research to clinical application. Am.J.Med 9, 31-38.
5. Wilbur KM, Bernheim F, ShapiroOW. The thiobarbituric acid method for Malondialdehyde estimation. Arch Biochem Biophys 1943; 250 pg No. 305 – 13.

6. Marklund S, Markuland G. A simple assay for superoxide dismutase using autooxidation of pyrogallol. European Journal Biochem. 1974: page No, 469-72.
7. Loeser R F, Shakoor N. Aging or osteoarthritis which is the problem. Rheum Dis. Clin North Am; 29[4]: 653-673.
8. Mane K. K., Sardeshmukh A. S, Rathi D. B, Suryakar A. N. Lipid peroxide and Antioxidants in arthritis. Medical Journal of western India (1999); vol. 27: 108 – 110.
9. Brunella G, Lina R, Mauro F. Enhanced lipid peroxidation in synoviocytes from patient with OA. J. Rheumatol. 2003 Feb; 30[2]: 345-347.
10. Rubyk BI, Fil chagin NM, Sabadyshin RA. Change in lipid peroxidation in patient with primary osteoarthosis deformans. Ter Arkh 1988; 60 (9): 110 – 113.
11. Moti L, Tiku, Shah Rahul, Allison G. T. Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation. The Journal of Biological chemistry 2000; 275 (26): 20069 – 20076.
12. Tiku M L, Allison G T, Naik K, Karry S K . Malondialdehyde oxidation of cartilage by chondrocytes. Osteoarthritis cartilage 2003 Mar; 11[3]: 159-166.
13. Maneesh M, Jayalekshmi H, Suma T, Chatterjees, Singh T A. Evidence for oxidative stress in osteoarthritis. Indian journal of clinical Biochemistry; 2005; 20[1]: 129-130.
14. Suprapaneni Krishna Mohan, Venkataraman G . Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. Indian journal of medical sciences 2007; 61 [1]: 9-14.

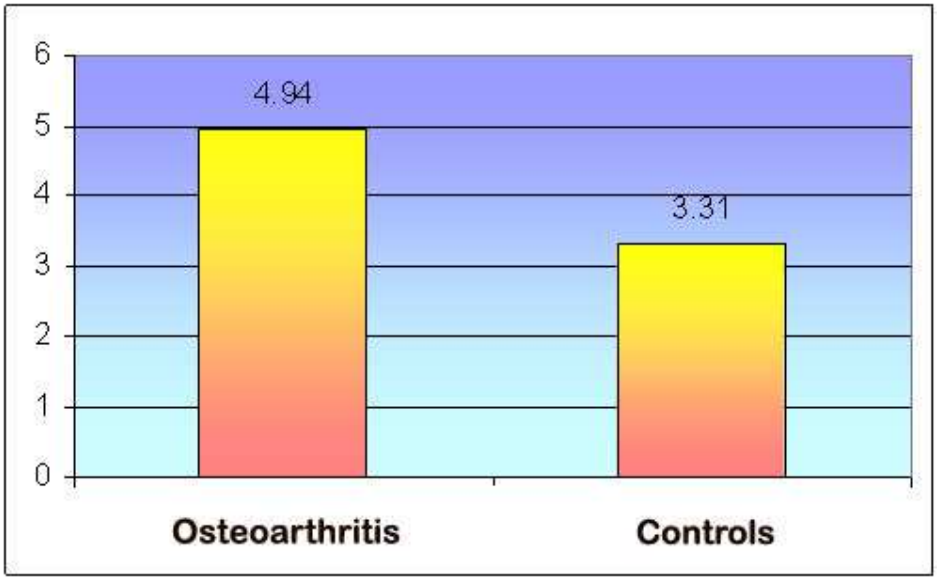
Table 1: Levels of serum MDA, SOD in patients with osteoarthritis and controls.

Study group	Sample size (n)	MDA (nmol/ml) Mean ± SD	SOD Unit/ml. Mean ± SD
Patients	30	9.37±5.33	4.94±3.00
Controls	30	3.98±1.98	3.31±0.85

Graph-1 Serum MDA level in Osteoarthritis Patients & Controls (MDA conc. in nmol/ml)



Graph-2 Serum SOD levels in osteoarthritis patients and controls.



SOD Concentration unit/ml