USES OF HYPERBARIC OXYGEN THERAPY: A REVIEW

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ABSTRACT: In the last three decades great strides in Hyperbaric Oxygen research has raised the value of this unique therapy. Studies have expanded the list of conditions usefully treated with compressed oxygen. Despite the promising experimental and clinical data, the major criticism to most HBO studies has been the lack of controlled prospective analysis for its use. This article discusses the use of HBO, including staffing and equipment considerations, side effects and reviews the published experience in this subject.
KEYWORDS: hyperbaric oxygen therapy, HBO, uses of HBO.

INTRODUCTION: HBO therapy was initially used to treat patients involved in diving accidents or with decompression sickness. However, its indications have increased over the past few decades. Currently, there are twelve indications for HBO therapy approved by the Undersea and Hyperbaric Medical Society (UHMS) in the United States.[1] There are, however, over a hundred indications internationally, although most of them have not been proven by controlled studies. The committee on hyperbaric medicine defines hyperbaric oxygen therapy as "A mode of medical treatment in which the patient is entirely enclosed in a pressure chamber and breathes 100% oxygen at a pressure greater than 1 atmosphere absolute (ATA)". ATA is the units of pressure and 1 ATA is equal to 760 mm of mercury or pressure at sea level.[1]As with most areas of medicine, in hyperbaric medicine there is a constant struggle to balance enthusiasm for progress in the field with the need to apply it on the basis of established evidence.The lack of sound scientific evidence of the efficacy of HBO has bred uncertainty in the wider medical community regarding its legitimacy.

PHYSIOLOGICAL BASIS: The arterial partial pressure of O2 is 100 mm Hg, Hb is 95% saturated and 100 ml of blood carries 19 ml of O2in combination with Hb and 0.32 ml dissolved in plasma. If the inspired O2 concentration is increased to 100%, O2combined with Hb can increase to a maximum of 20 ml when the Hb is 100% saturated and the amount of O2 dissolved in plasma may increase to 2.09 ml. During HBO in addition to the Hb which is 100% saturated the amount of O2 carried in solution will increase to 4.4 ml% at a pressure of 2 ATA to 6.8 ml % at 3 ATA which is almost sufficient to supply the resting total oxygen requirement of many tissues

without a contribution from oxygen bound to hemoglobin. Itis this increased oxygen in plasma which is responsible for most of the beneficial effects of hyperbaric oxygen.[2]

HISTORY OF HYPERBARIC OXYGEN THERAPY: The first pressurized room used to treat health problems was built by an Englishman named Henshaw in 1662.[3]in1788; hyperbaric air was put to large scale use in a diving bell for underwater industrial repairs of an English bridge. The first deep sea diving suit, invented in 1819 by August Siebe, used compressed air supplied to the helmet for generous underwater movement.[4]Dr. John S. developed the first diving tables for the Royal Navy. His legacy gives him the title "Father of Oxygen Therapy" and physicians continue in his line of work to this day.[4] In 1918 Dr. Orval Cunningham built the world's largest functional hyperbaric chamber, a 64' steel sphere "hyperbaric medical hotel" with five floors of living space after he found that denser air helped people fight infection suffering from flu. The Great Depression in the 1930's ended his project and the giant chamber was scrapped for the war effort in the 1940's.[5] The Hyperbaric Oxygen Committee was developed by the UHMS in 1976 to oversee the ethical practice of hyperbaric medicine.[5]

THERAPEUTIC EFFECTS OF HBO THERAPY:

Therapeutic effects of HBO can be attributed to its mechanical or hyperoxygenation effects as shown in the table

Therapeutic effects	Mechanism
Reduces bubble size	Direct mechanical effect.
Immune stimulation	Restores WBC function, enhances phagocytic capabilities and neutrophil mediated killing of bacteria.
Neovascularization	Augmentation of fibroblastic activity which promotes capillary growth.
Reduces edema and tissue swelling	Hyper oxygenation.
Bactericidal	For anaerobic organisms such as Clostridiwelchii, and also inhibits the growth of aerobic bacteria at pressures greater than 1.3 ATA.

ADMINISTRATION: HBO therapy can be given in a monoplace chamber in which a single patient is placed in a chamber pressurized with 100% oxygen or it can be given in a multiplace chamber where many patients can be treated at the same time. To be effective, hyperbaric oxygen must be inhaled in the atmosphere or through an endotracheal tube in a monoplace chamber and in multiplace chamber masks, tight-fitting hoods, or endotracheal tubes can be used. Monoplace chambers are the most common type of chamber used due to their portability, minimal personnel requirements and low cost.[6]

Time: The duration of single treatments varies from 45 minutes for carbon monoxide poisoning to almost 5 hours for some severe decompression disorders. For treatment of wounds - most protocols average 90 minutes for each of 20 to 30 treatments.

GENERAL EQUIPMENT CONSIDERATIONS: All equipment used inside hyperbaric chambers must adhere to the guidelines of the National Fire Protection Association (NFPA) .[7] Chamber fires result in catastrophic consequences.[8]The primary cause of mishaps is the introduction of prohibited items into the chambers, specifically when chamber personnel do not adhere to NFPA fire safety rules. Equipment inside chambers must be intrinsically electrically safe, follow NFPA guidelines, and be tested for the pressures to which they will be exposed.[7]

HYPERBARIC CHAMBER SELECTION, LOCATION AND STAFFING: Hyperbaric oxygen can be offered to critically ill patients inmonoplace and multiplace chamber. Monoplace chambers can be located inside the intensive care unit, where they can be staffed by ICU personnel and are then an extension of the ICU.[9, 10]However; hands-on care cannot be provided to a patient inside a monoplace chamber. Although multiplace chambers do allow hands-on care, experienced staff must be available inside the chamber. Because most hyperbaric chambers are not located within or adjacent to the ICU, the potential benefits of HBO2 to a critically ill patient must be balanced by the risks from transporting the patient as well as the risks from HBO.[11, 12] Personnel working as inside attendants of multiplace chambers must be medically suitable for hyperbaric exposure (e.g., able to equalize ears, no claustrophobia, no pulmonary or cardiac disease, etc). Staff supporting critically ill patients during HBO2 could include Certified Hyperbaric Registered Nurses, physicians; critical care respiratory therapists, and paramedics.

INDICATIONS: The following indications are approved uses of hyperbaric oxygen therapy as defined by the Hyperbaric Oxygen Therapy Committee.

1. Air or Gas Embolism
2. Carbon Monoxide Poisoning
Carbon Monoxide Poisoning Complicated By Cyanide Poisoning
3. Clostridial Myositis and Myonecrosis (Gas Gangrene)
4. Crush Injury, Compartment Syndrome and Other Acute Traumatic Ischemias
5. Decompression Sickness
6. Arterial Insufficiencies:
    Central Retinal Artery Occlusion
    Enhancement of Healing In Selected Problem Wounds
7. Severe Anemia
8. Intracranial Abscess
9. Necrotizing Soft Tissue Infections
10. Osteomyelitis (Refractory)
11. Delayed Radiation Injury (Soft Tissue and Bony Necrosis)
12. Compromised Grafts and Flaps
13. Acute Thermal Burn Injury
14. Idiopathic Sudden Sensorineural Hearing Loss.

USES OF HBO

ARTERIAL GAS EMBOLISM: Arterial gas embolism, occurs when air bubbles in the circulation. There are many causes, including mechanical ventilation; central line placement, haemodialysis, severe diving injury and pulmonary barotrauma.[13]The bubbles cause tissue deformation and vessel occlusion, impairing tissue perfusion and oxygenation. Biochemical effects at the blood-

gas interface cause endothelial damage, changes in haemostasis and activation of leukocytes.[14] Clinical symptoms include muscle and joint pain, arrhythmias, ischemia, confusion, focal neurological deficits and loss of consciousness.

RATIONALE OF TREATMENT WITH HBO:

•HBO reduces bubble size in accordance with Boyle's law—at 3ATA, bubble volume is reduced by about two-thirds.[15]

•Hyperoxia increases the diffusion gradient with the embolized gas, moving gas into solution where it can be metabolized.[16]

HBO is widely accepted as the only life-saving treatment, UHMS suggests maximal benefit with 100% oxygen at 2.8 ATA, and repeated treatments until no further improvement is seen, typically after no more than 5–10 treatments.[17]19 patients in the USA with iatrogenic cerebral arterial gas embolism, showed significant improvement in symptoms with HBO treatment, but the loophole in the study was the control group and end-points were not clearly defined.[18] HBO is most effective when initiated early, but can be successful after hours or even days.[17]

CARBON MONOXIDE POISONING: Loss of consciousness (syncope, seizures, and coma), neurologic deficits, pulmonary edema, myocardial ischemia, and severe metabolic acidosis are the most common symptoms of carbon monoxide poisoning caused primarily from smoke inhalation and suicide attempts. Less severely poisoned patients may have headache, nausea, and other constitutional symptoms. All victims of carbon monoxide poisoning are at risk for delayed neuropsychological sequelae.CO combines preferentially with hemoglobin to produce COHb, displacing oxygen and reducing systemic arterial oxygen (O2) content. CO binds reversibly to hemoglobin with an affinity 200- 230 times that of oxygen.[19]consequently, relatively minute concentrations of the gas in the environment can result in toxic concentrations in human blood. Toxicity includes decrease in the oxygen carrying capacity of blood and alteration of the dissociation characteristics of oxyhemoglobin. It also causes decrease in cellular respiration by binding with cytochrome a3 and binding to myoglobin, which leads to myocardial and skeletal muscle dysfunction.[19]

The rationale for the use of HBO:

- HBO induces cerebral vasoconstriction, which may reduce intracranial pressure and cerebral edema.[20]
- HBO results in more rapid dissociation of CO from respiratory cytochromes.[20]
- HBO may antagonize the oxidative injury that occurs after CO poisoning.[20]

Thom has demonstrated that oxygen at 3 ATA, but not at 1 ATA prevents brain lipid peroxidation when administered to rats beginning 45 minutes subsequent to CO poisoning.[21] undersea and Hyperbaric Medical Society recommends HBO for those patients with signs of serious intoxication regardless of their COHB levels. This includes patients with a history of unconsciousness, presence of neurological signs, cardiovascular dysfunction or severe acidosis. Pregnant women should be evaluated with liberal criteria for HBO due to the increased toxicity risk to the fetus.[1]

GAS GANGRENE: Infection with Clostridium Perfringens in devitalized tissue is the most common cause of gas gangrene. Clostridial microorganisms are anaerobes that produce local and systemic toxins. Wide surgical debridement and appropriate antibiotic therapy remain the standard treatment modality. Adjunctive HBO is known to have antibacterial and anti-toxin effects.[22]case reports support combined therapy with HBO, antibiotics and surgery in these conditions, reducing need for drastic surgery and amputation.[23]

Rationale for treatment:

• HBO therapy induces high oxygen partial pressure in all tissues; achievable tissue oxygen levels are lethal to some obligate anaerobic bacteria such as Clostridium perfringens.[22]

•Anti-edema effect, causes activation of fibroblasts and macrophages, and stimulates angiogenesis.[22]

The UHMS recommends that three 90-min treatments should be given at 3.0ATA in the first 24h, followed by twice-daily treatments for 4–5 days, until clinical improvement is seen.[1]

CRUSH INJURIES, COMPARTMENT SYNDROMES AND OTHER ACUTE TRAUMATIC PERIPHERAL ISCHEMIA'S: Acute traumatic peripheral ischemia's (ATPIs) results in progressive, self-perpetuating ischemia, edema and inadequate healing due to extravasation of intravascular fluid. There is severe damage centrally, with progressive improvement in adjacent tissues. Ischemia and edema may continue even when the primary injury is controlled.[24]

COMPARTMENT SYNDROME: Severe pain on passive stretching of the muscles involved decreased sensation in branches of the involved peripheral nerves and Elevated intra compartmental pressures on direct manometry are the symptoms associated with compartment syndrome.

RATIONALE FOR TREATMENT:

• HBO improves tissue oxygen tensions by increasing plasma-based oxygenation and increasing erythrocyte deformability.[25]

• Intermittent hyperoxia stimulates fibroblast and collagen synthesis, enabling angiogenesis, tissue repair and optimal healing. Hyperoxic vasoconstriction resolves oedema without impairing oxygen delivery, and reverses the ischaemia-oedema cycle.[26]

• HBO also antagonizes free-radical-associated lipid peroxidation, reducing reperfusion injury.[21]

Published research is limited, but a randomized controlled trial in 1996 demonstrated significant improvement in healing with HBO.[27]The UHMS recommends treatment within 4–6h of injury, given at 2.0–2.5ATA at least once daily for several days, although guidelines vary depending on the type of injury.

DECOMPRESSION SICKNESS: Decompression sickness (DCS) occurs mainly when inert gas (mainly nitrogen) comes out of solution during ascent and decompression, forming bubbles in the capillaries and tissues in scuba divers.[18] physical distortion, vessel occlusion, clotting and immune changes lead to symptoms such as fatigue, joint pains, rash, neurological and cardio-respiratory symptoms, coma and death . Predisposing factors include dehydration, injury, exertion at depth and cold exposure.[28] since 1930, HBO is the only established lifesaving treatment for DCS.[29]

Rationale for treatment:

• HBO recompresses bubbles and forces gas back into solution for a more controlled ascent.
• Inert nitrogen is replaced by rapidly-metabolized oxygen, and bubbles move either to the lungs where they are excreted, or to smaller vessels where obstruction is less important, and surface tension forces eventually collapse the bubbles.
• HBO also counteracts platelet and leukocyte activation and endothelial interactions.[30]
UHMS recommend rapid treatment at 2.8 ATA, repeated up to ten times if symptoms persist.[1]

HYPERBARIC OXYGEN THERAPY IN NON-HEALING WOUNDS: Diabetic foot ulcers, non-healing traumatic wounds, and peripheral vascular insufficiency ulcers develop in compromised hosts with local and systemic factors contributing to impairment of tissue repair. Wound healing is slowed down due to decreased collagen production, poor capillary angiogenesis, and impaired oxygen-dependent intracellular leukocyte killing.

Rationale for treatment
• HBO therapy promotes neovascularization and increases endothelial cells, fibroblast proliferation and collagen deposition.[31]
• Modifies the cellular functions of the activated neutrophil, resulting in increased oxidative microbial killing and decreased neutrophil-endothelial adhesion.[32]
• HBO up-regulates platelet-derived growth factor receptor messenger RNA activity.[33]
• Has synergistic effect with growth factor.[34]
Kalani found that the wound-healing rate was 76% in the group with HBO therapy and 48% in the group without HBO therapy in 38 patients treated for diabetic foot ulcers over a period of three years. [35]Patients are generally treated at 2.0 to 2.5 ATA for 90-120 minutes per day and receive 20-30 treatments.[35]

EXCEPTIONAL BLOOD LOSS ANEMIA: Severe hemorrhagic shock and blood loss anemia may lead to tissue hypoxia and ischemia. Where whole blood transfusion is not possible, for religious or practical reasons, HBO may compensate for such a hemoglobin deficiency. HBO is used as a short-term measure, but is inconvenient and expensive, and the risk of oxygen toxicity limits its treatment.
Rationale for treatment:

HBO increases levels of plasma-dissolved oxygen to enable oxygenation while erythrocyte regeneration occurs.[36]
Hart described 70% survival in 26 patients who received HBO after losing >50% of their circulating volume.[37]
UHMS recommend treatments at up to 3ATA for 2–4h periods, three or four times a day, until hypoxic symptoms have resolved and red blood cells have been regenerated.[1]

INTRACRANIAL ABSCESS: HBO can be used as an adjuvant therapy in patients with severe infection or immune compromise, who may be unresponsive to standard aspiration and antibiotic treatment.[1]

Rationale for treatment
- HBO inhibits the predominantly anaerobic micro-organisms.[20]
- reduces cerebral oedema.[20]

- modifies the immune response.[20]

Kurschel reported HBO therapy to be safe and effective in treating children with brain abscesses.[37]

UHMS recommends HBO for multiple, deep or dominantly-located abscesses, or in patients with immune compromise, poor surgical risk, or resistance to conventional treatment.[1] Treatments are once or twice daily, at 2.0–2.5ATA for 60–90min. The average number of treatments is thirteen, and a utilization review is recommended after 20treatments.[1]

NECROTISING SOFT TISSUE INFECTIONS: Necrotising fasciitis is commonly seen in patients with diabetes mellitus, cirrhosis, and intravenous drug abuse. Reports of mortality range from 30% to 75%.[1] It is a rapidly-progressive traumatic bacterial infection of the deep fascia with secondary subcutaneous and cutaneous involvement. Hemolytic streptococci are typical pathogens, but polymicrobial infection, host diabetes and vascular disease are all common. An obliterative endarteritis occurs, causing tissues to become hypoxic, hypovascular and hypocellular. Leukocytes become sequestered in vessels, impairing local immunity, and incomplete substrate oxidation results in hydrogen and methane accumulation in the tissues. Tissue necrosis occurs, with purulent discharge and gas production.[1]

Rationale for treatment

- In animal studies, HBO has a direct bactericidal effect.
- Improves tissue oxygen tension, leukocyte function and bacterial clearance.[38]
- Integrin inhibition decreases leukocyte adherence, reducing systemic toxicity.[21]

HBO has been reported to reduce mortality by up to two-thirds.[39] HBO is particularly indicated in bacterial gangrene and non-clostridial myonecrosis (which have high mortality and morbidity), and in compromised or unresponsive hosts.[40]

The UHMS recommends twice-daily treatments for 90–120min at 2.0–2.5ATA, reduced to once daily when the patient's condition is stabilized. Further treatments may be given to reduce relapse, and a utilization review is recommended after 30 treatments.[1]

HYPERBARIC OXYGEN AS ADJUNCTIVE THERAPY FOR OSTEOMYELITIS: Bone infections that fail to respond to surgical and antibiotic therapy due to systemic host and local immune compromised factors lead to refractory osteomyelitis. Failure of treatment or recurrence of osteomyelitis often leads to amputation. Hyperbaric oxygen can be used as an adjunctive treatment in chronic refractory osteomyelitis along with culture-directed antibiotics, surgical debridement, and nutritional support.

Rationale for treatment:

• HBO enhances oxygen-dependent leukocyte killing through the production of hydrogen peroxide and superoxide by providing increased oxygen tension in the hypoxic tissue.
• Transient reversal of hypoxia might increase clearance of bacteria.[31]
• Optimal tissue oxygen tension enhances osteogenesis and neovascularization.[41]
• HBO has also been shown to enhance osteoaclastic activity on necrotic dead bone to remove bony debris.[42]
• Synergistic effects of HBO on bone healing with bone morphogenic protein were also demonstrated .[43]

• potentiate the antimicrobial effects of aminoglycosides, and possibly sulpha drugs and vancomycin, in the killing of susceptible bacteria.[38]

The results of multiple clinical studies have suggested a beneficial effect with the addition of adjunctive hyperbaric oxygen therapy to conventional treatment regimens for osteomyelitis in terms of reduced hospital stay and amputation rates. [44]whereas other clinical studies have failed to demonstrate such a benefit.[45]Patients with osteomyelitis are usually treated at 2.0 to 2.5 ATA for 90-120 minutes per day and typically receive 20-40 treatments.[1]

SKIN FLAPS AND GRAFTS (COMPROMISED): Skin grafts survive as oxygen and nutrients diffuse into them from the underlying wound bed. Long-term survival depends on a new blood supply forming from the wound to the graft. When the wound bed does not have enough oxygen supplied to it, the skin graft will at least partially fail. Common causes for this are previous radiation to the wound area, diabetes mellitus, and certain infections. Significant improvements with HBO in skin grafts and flaps have been reported since 1967.[46]
Rationale for treatment:

• Increased angiogenesis, healing and increased microvasculature.[47]
• Reduce endothelial leukocyte adherence.[25]
• Prevents progressive vasoconstriction of reperfusion injury.[25]
• Fibroblast stimulation and collagen synthesis.

Nemiroff reported significantly increased microvasculature in animals treated with HBO.[47] The UHMS recommends twice-daily treatment at 2.0–2.5 ATA for 90–120min, reducing to once-daily when the graft or flap has stabilized. A utilization review is recommended after 20 treatments, whether preparing a site for grafting, or maximizing survival of a new graft.[1]

ACUTE THERMAL BURN INJURY: Insufficient oxygen and nutrient supply from the surrounding tissues lead to a central zone of coagulation in cases of severe burn. Burn therapy comprises respiratory care, antibiotics, debridement, and parenteral nutrition, with the aims of reducing oedema, preserving borderline tissue and enhancing host defenses.

Rationale for treatment:
• Reduces haemoconcentration, coagulability and vascular damage in thermal burns.[48]
• Hyperoxic vasoconstriction decreases edema, and increases collagen formation and angiogenesis.[25]
• Phagocytic bacterial killing is also improved, and white cell endothelial adherence is inhibited, preventing capillary damage.[25]
• HBO maintains ATP levels and microvascular integrity, and reduces infection.[25]
HBO has been proved effective in such cases and decreases healing time and reduces need for grafting.[1] However, some studies have found no benefit from HBO in thermal burns and stated that HBO could worsen pulmonary damage in thermal burns.[49] however clinical data is needed to further confirm the benefits. The UHMS recommends three sessions within 24h of injury, and 90-min treatments twice-daily thereafter, at 2.0–2.4 ATA.[1]

IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS: Idiopathic sudden sensorineural hearing loss leads to Tinnitus and a feeling of increased pressure; vertigo is less commonly

associated with the syndrome.[50]Etiology could be a viral infection, such as mumps, trauma, Ménière's disease, acoustic neurinoma, ototoxic medication, multiple sclerosis vascular occlusion, viral infections, labyrinthine membrane breaks, immune associated disease, abnormal cochlear stress response, trauma, abnormal tissue growth, toxins, ototoxic drugs and cochlear membrane damage.

Rationale for treatment:

• Increase of oxygen partial pressure in the inner ear.[51]
•HBO restores the arterial-perilymphatic oxygen concentration difference.

A meta-analysis by Lamm showed a positive effect of HBO in approximately 50% of cases, after failure of classical drug therapy. [52]The UHMS recommends 100% O2 at 2.0 to 2.5 atmospheres absolute for 90 minutes daily for10 to 20 treatments.[1]

OTHER IMPLICATIONS FOR HBO

NEUROLOGICAL ILLNESSES

CEREBROVASCULAR ACCIDENTS (STROKE): Hyperbaric oxygen therapy shows a striking reduction in spasticity possibly due to improved function of neurons in affected areas of the brain and secondly to rise of PO2 in the spastic inactive and hypoxic muscle. Additionally there is an improvement in the cognitive and mental performance.[53]

Potential benefits:

• Increased oxygen delivery.
• decreased cerebral edema.
• decreased lipid peroxidation.
• Inhibition of leukocyte activation.
• Maintenance of blood-brain barrier integrity.[21, 54.]

ACUTE TRAUMATIC BRAIN INJURIES: Acute traumatic brain injury causes a cycle of ischemia, hypoxia, edema and enzymatic derangements. HBO tends to break this vicious cycle. However there has to be a responsive cerebral circulation.

Rationale for treatment

• Improved aerobic metabolism,
• Reduction in lactate levels,
• Increase in creatinine phosphate and ATP levels.
• Elevation of partial pressure of Oxygen increases the diffusion distance, and O2 delivery in abnormal areas is enhanced.[21]

Wang demonstrated that multiple HBOT (3 ATA hourly for 3 or 5 days), delivered 2 days post-injury resulted in significantly reduced overall neurological deficit scores and neuronal apoptosis within brain tissue. [55]

CEREBRAL PALSY: Studies show that HBO therapy can improve some cerebral Palsy symptoms like spasticity, vision, hearing, and speech.[56] however there is a lack of clinical evidence for its use and hence HBO can be used as an adjuvant with other treatment modalities, but it is not a cure.

The rationale for treatment:

- Increased oxygenation of the cerebral ischaemic penumbra.[57]

A study conducted by Collett showed improvement in gross motor function, improved performance in activities of daily living, attention, working memory, and speech in 111 children's with cerebral palsy aged 3-12 years treated with 100% oxygen at 1.75 atmospheres absolute (ATA).[58]

BELL'S PALSY: Steroids and surgical decompression are the only treatment used currently but results are inconclusive as to their benefit. HBO added to other treatment increases the efficacy of the treatment and reduces the period needed for restoration of complete function of the damaged nerve.[59]

Rationale for treatment:

The antioedematous effect and additional oxygenation of hypoxic cranial nerve VII with dissolved oxygen.[60]

In the double blind study, Raeiae in 95.2% cases had full recovery in the average period of 22 days at experimental group of 42 patients treated only with HBO2 under 2.8 ATA and placebo tablets.

ONCOLOGY: A dominant feature of post-radiation change is the obliteration of small blood vessels leading to hypoxia. The oxygen tension inside a tumor drops lower as the tumor enlarges and may drop to zero in the necrotic center of the tumor. Hypoxia increases the resistance of cancer to radiotherapy. With oxygen tension at zero, the amount of radiation required to be effective is three times that required with normal oxygen tension. When irradiation is done immediately after HBO therapy, the well-oxygenated cells will be damaged lethally.

Rationale:

- Stimulates angiogenesis, increases neovascularization, fibroblast and osteoblast proliferation.
- It stimulates collagen formation at wound edges and thus helps in re-epithelialization of ulcers and provides a better nutritive bed to support grafts and pedicle flaps.[61]

AUTISM: Studies have shown that there is cerebral hypo perfusion in approximately 86% of autistic patients.[62] Hypoperfusion could be the result of inflammation around the blood vessels in the brain. HBO is used in successful treatment of vasculitis.

Rationale for treatment:

HBO attenuates the production of proinflammatory cytokines including TNF α, [62] IL-1β and IL-6 and increase the production of anti-inflammatory IL-10.[63]In one case report Heuser treated a 4 year patient with autism using HBO at 1.3 atm and 24% oxygen and reported striking improvement in behavior including memory and cognitive functions.[64]

SIDE EFFECTS OF HBO2: While HBO2 has an admirable safety record, those recommending HBO2 in wound care should be aware of potential side effects and complications.

EAR AND SINUS BAROTRAUMA: Middle ear barotrauma is the most common side effect of HBO2. Patients with a cold, upper respiratory tract infection or allergic rhinitis are not suitable candidates for HBO2. [65]

MYOPIA: Some patients receiving HBO2 will develop reversible myopia. [66] HBO2 may lead to oxidative change of the lens proteins. After cessation of therapy, the refraction usually returns to the pretreatment state within a few weeks.[67]

AGGRAVATION OF CONGESTIVE HEART FAILURE: HBO2 causes increased peripheral vascular resistance from its vasoconstrictive effects. Blood flow to the left ventricle has been noted to decrease during HBO. Patients with a cardiac ejection fraction of less than 35% are generally not treated by HBO.[68]

PULMONARY BAROTRAUMA: injury is related to pressure changes and occurs only on ascent. For the lungs to be injured there must be an obstruction such as a closed glottis or bronchial obstruction. An untreated pneumothorax is an absolute contradiction to HBO2 therapy and patients with a pneumothorax must have a chest tube inserted prior to the treatment dive. If a pneumothorax occurs during the treatment, a chest tube must be inserted prior to ascent to prevent a marked deterioration in the condition of the patient.[69]

COST: analysis has shown that the addition of hyperbaric oxygen to conventional treatment results in significant cost savings due to lesser stay in hospital and shorter course of illness.[53]

CONCLUSION: The Hyperbaric Committee of the Undersea and Hyperbaric Medical Society in the US (UHMS) reviews and publishes once in 2-3 years the indications for HBO, which are supported by adequate medical literature. The Committee usually looks for three kinds of evidence: physiological, animal studies and human studies preferably double blinded, and publishes this list of "approved" indications. In addition to the use of HBO for the "Approved" indications, growth of Hyperbaric Medicine over the past two decades has also led to its popularity and use for some "unapproved" or the so called “Off Label” indications. In diseases for which the use of hyperbaric oxygen is not well supported, the potential benefits must be carefully weighed against the risks of treating. Patient selection for HBOT should be executed carefully and according to accepted guidelines. If safety guidelines are strictly followed, HBO therapy is a modality with an acceptable rate of complications. Doctors in all fields must familiarize themselves with recent evidence on this mode of therapy, so that their patients are not denied the gains of this modern treatment.

REFERENCE:

1. Hampson NB, ed. Hyperbaric Oxygen Therapy: 1999 Committee report. Kensington MD, Undersea and Hyperbaric Medical Society, 1999.
2. Leach RM, Rees PJ, Wilmshurst P. Hyperbaric oxygen therapy.BMJ 1998; 317:1140-3.
3. Simpson A. Compressed air as a therapeutic agent in the treatment of consumption, asthma, chronic bronchitis and other diseases. Edinburgh: Sutherland and Knox, 1857.

4. Acott, C. (1999). "JS Haldane, JBS Haldane, L Hill, and A Siebe: A brief resume of their lives."South Pacific Underwater Medicine Society Journal 29 (3). ISSN 0813-1988. OCLC 16986801.Retrieved 2008-07-12.
5. Kindwall EP, Gottlieb FJ, Larson DL. Hyperbaric oxygen therapy in plastic surgery, plastic and reconstructive therapy.1991; 888:898-908.
6. Hart GB. The monoplace chamber. In: Kindwall EP, ed. hyperbaric medicine practice. Flagstaff, Ariz.: Best,1994:57-82.
7. National Fire Protection Association: NFPA 99: Standard for Health Care Facilities. 2005 Edition.Quincy, MA, NFPA, 2005.
8. Sheffield PJ, Desautels DA: Hyperbaric and hypobaric chamber fires: A 73-year analysis. Undersea Hyperb Med 1997; 24:153–164.
9. Gossett WA, Rockswold GL, Rockswold SB: The safe treatment, monitoring and management of severe traumatic brain injury patients in a monoplace chamber. Undersea Hyperb Med 2010; 37:35–48
10. Weaver LK, Churchill SC, Bell JE: Taking the chamber to the critically ill patient. Abstr. Undersea Hyperb Med 2010; 37:347
11. Waydhas C, Schneck G, Duswald KH: Deterioration of respiratory function after intrahospital transport of critically ill surgical patients. Intensive Care Med 1995; 21:784–789.
12. Shirley PJ, Bion JF: Intra-hospital transport of critically ill patients: Minimising risk. Intensive Care Med 2004; 30:1508–1510.
13. Murphy BP, Harford FJ, Cramer FS. Cerebral air embolism resulting from invasive medical procedures: treatment with hyperbaric oxygen. Ann Surg 1985; 201:242–5.
14. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476–82.
15. Branger AB, Lambertsen CJ, Eckmann DM. Cerebral gas embolism absorption during hyperbaric therapy: theory. J ApplPhysiol 2001; 90:593–600.
16. Dexter F, Hindman BJ. Recommendations for hyperbaric oxygen therapy of cerebral air embolism based on a mathematical model of bubble absorption.AnesthAnalg 1997; 84:1203–7.
17. Moon RE, de Lisle Dear G, Stolp BW. Treatment of decompression illness and iatrogenic gas embolism.Respir Care Clin N Am 1999; 5:93–135.
18. Benson J, Adkinson C, Collier R. Hyperbaric oxygen therapy of iatrogenic cerebral arterial gas embolism. Undersea Hyperb Med 2003; 30:117–26.
19. Gosselin RE, Smith RP, Hodge HC, eds. Clinical Toxicology of Commercial Products, 4th edn, Baltimore, MD: Williams and Wilkins, 1984
20. Tibbles PM, Edelsberg JS. Hyperbaric oxygen therapy (Reviewarticle). NEJM 1996:1642-8.
21. Thom SR. Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning. J ApplPhysiol 1992;73:1584-9.
22. Kaye D. Effect of hyperbaric oxygen on Clostridia in vitro and in vivo. ProcSocExpBiol Med 1967; 124:360–6.
23. Hirn M. Hyperbaric oxygen in the treatment of gas gangrene and perineal necrotizing fasciitis.Eur J Surg 1993; 570:1–36.
24. Wattel F, Mathieu D. Proceedings of the 1st European Consensus Conference on Hyperbaric Medicine. Lille (France), 1994:377–82.
25. Zamboni WA, Roth AC, Russell RC, Graham B, Suchy H, Kucan JO et al. Morphological analysis of the microcirculation during reperfusion of ischaemic

skeletal muscle and the effect of hyperbaric oxygen. Plastic ReconstrSurg 1993; 91:1110–23.
26. Nylander G, Lewis D, Lewis D. Reduction of post-ischaemicoedema with hyperbaric oxygen. PlastReconstrSurg 1985; 76:596–601.
27. Bouachour G, Cronier P, Gouello JP. Hyperbaric oxygen therapy in the management of crush injuries: a randomised double-blind placebo-controlled trial. J Trauma 1996; 41:333–9.
28. Hagberg M, Ornhagen H. Incidence and risk factors for symptoms of decompression sickness among male and female dive masters and instructors: a retrospective cohort study. Undersea Hyperb Med 2003; 30:93–102.
29. Yarbrough OD, Behnke AR. Treatment of compressed air illness utilizing oxygen. J IndustHygToxicol 1939; 21:213–18.
30. Ishii Y, Miyanaga Y, Shimojo H, Ushida T, Tateishi T. Effects of hyperbaric oxygen on procollagen messenger RNA levels and collagen synthesis in the healing of rat tendon laceration. Tissue Eng 1999;5: 279-86.
31. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic. The effect of inspired oxygen on infection. Arch Surg 1984;119:199-204
32. Bonomo SR, Davidson JD, Tyrone JW, Lin X, Mustoe TA.Enhancement of wound healing by hyperbaric oxygen and transforming growth factor beta3 in a new chronic wound model in aged rabbits. Arch Surg 2000; 135:1148-53.
33. Zhao LL, Davidson JD, Wee SC, Roth SI, Mustoe TA.Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. Arch Surg 1994;129:1043-9.
34. Kalani M, Jorneskog G, Naderi N, Lind F, Brismar K. Hyperbaric oxygen therapy in treatment of diabetic foot ulcers. Long-term follow-up. J Diabetes 2002; 16:153-8.
35. Gill, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM. 2004 Jul;97(7):385-95.
36. Hart GB, Lamb RC, Strauss MB. Gas gangrene. J Trauma 1983; 23: 991-100.
37. Kurschel S, Mohia A, Weigl V, Eder HG. Hyperbaric oxygen therapy for the treatment of brain abscess in children. Childs Nerv Syst. 2006 Jan;22(1):38-42.
38. Mader JT, Guckian JC, Glass DL, Reinarz JA.Therapy with hyperbaric oxygen for experimental osteomyelitis due to Staphylococcus aureus in rabbits. J Infect Dis 1978; 138:312–18.
39. Riseman JA, Zamboni WA, Curtis A, Graham DR, Konrad HR, Ross DS et al. Hyperbaric oxygen therapy for necrotizing fasciitis reduces mortality and the need for debridements. Surgery 1990; 108:847–50.
40. Hirn M, Niinikoski J, Lehtonen OP. Effect of hyperbaric oxygen and surgery on experimental multimicrobial gas gangrene. EurSurg Res 1993; 25:265–9.
41. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK et al. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000; 135:1293-7.
42. Barth E, Sullivan T, Berg E. Animal model for evaluating bone repair with and without adjunctive hyperbaric oxygen therapy (HBO): comparing dose schedules. J Invest Surg 1990;3:387-92.
43. Okubo Y, Bessho K, Fujimura K, Kusumoto K, Ogawa Y, Iizuka T et al. Effect of hyperbaric oxygenation on bone induced by recombinant human bone morphogenetic protein-2. Br J Oral MaxillofacSurg 2001;39:91-5

44. Davis JC, Heckman JD, DeLee JC, Buckwold FJ. Chronic non-haematogenous osteomyelitis treated with adjuvant hyperbaric oxygen. J Bone Joint Surg 1986;68A:1210-7.
45. Esterhai JL Jr, Pisarello J, Brighton CT, Heppenstall RB, Gellman H, Goldstein G et al. Adjunctive hyperbaric oxygen therapy in the treatment of chronic refractory osteomyelitis. J Trauma 1987;27:763-8.
46. Perrins DJD. Influence of hyperbaric oxygen on the survival of split skin grafts. Lancet 1967; 7495:868–71.
47. Nemiroff PM, Lungu AL. The influence of hyperbaric oxygen and irradiation on vascularity in skin flaps: a controlled study. Surg Forum 1987; 38:565–7.
48. Grossman AR, Grossman AJ. Update on hyperbaric oxygen and treatment of burns. Burns 1982; 8:176–9.
49. Brannen AL, Still J, Haynes M, Orlet H, Rosenblum F, Law E, et al. A randomised prospective trial of hyperbaric oxygen in a referral burn centre population. Ann Surg 1997; 63:205–8.
50. Lehnhardt, R.E. Die akuteInnenohrschwerhörigkeit. HNO 1991; 39: 378-385
51. Murata, K. and U. Fisch. Effect of CO2 on the perilymphatic oxygen tension in cats. Ann OtolRhinolLaryngol 1977; 86: 164-171
52. Lamm, K., Lamm, H. and W. Arnold. Effect of hyperbaric oxygen therapy in comparison to conventional or placebo therapy or no treatment in idiopathic sudden hearing loss, acoustic trauma, noise-induced hearing loss and tinnitus.A literature survey.AdvOtorhinolaryngol. 1998;54:86-99.
53. Sahni T, Jain M. Status of Status of Hyperbaric Oxygen Therapy in Neurological Illnesses – Review of International and Indian experience. Neurosciences Today 2003; 7:126-134.
54. Mink RB, Dutka AJ. Hyperbaric oxygen after global cerebral ischemia in rabbits reduces brain vascular permeability and blood flow. Stroke. 1995; 26: 2307–2312
55. Wang GH, Zhang XG, Jiang ZL, Li X, Peng LL, Li YC et al.: Neuroprotective Effects of Hyperbaric Oxygen Treatment on Traumatic Brain Injury in the Rat. J Neurotrauma 2010, 27:1733-1743
56. Montgomery D, Goldberg J, Amar M. Effects of hyperbaric oxygen therapy on children with spastic diplegic cerebral palsy: A pilot project. Undersea Hyperb Med 1999;26:235-42.
57. Neubauer RA. Hyperbaric oxygen for treatment of closed head injury. South Med J 1994;87:933-6.
58. Collet JP, Vanasse M. Hyperbaric oxygen for children with cerebral palsy; a randomized multicentre trial. HBO-CP Research Group. Lancet 2001;357:582-86.
59. Heiden CH, Biesinger E, Hoing R. Idiopathic facial palsy (Bell's Palsy): an indication for hyperbaric oxygen therapy? Eur J Underwater Hyper baric Med 2003; 4(1): 20-23.
60. Raèiæ G, Denoble P, Sprem N, Bojiæ L, Bota B. Hyperbaric Oxygen as therapy of Bell,s palsy. Undersea Hyperb Med 1997; 24(1):35-8.
61. TarunSahni, Madhur Jain, S. Hukku, G.K. Jadhav. Role of hyperbaric oxygen therapy in oncology and radiation induced tissue damage: Apollo Medicine, Vol.1, December 2004
62. Weisz G, Lavy A, Adir Y, Melamed Y, Rubin D, Eidelman S, Pollack S et al. Modification of in vivo and in vitro TNF-alpha, IL-1, and IL-6 secretion by

circulating monocytes during hyperbaric oxygen treatment in patients with perianal Crohn's disease. J ClinImmunol. 1997 Mar;17(2):154-9.
63. Benson RM, Minter LM, Osborne BA, Granowitz EV. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. ClinExpImmunol. 2003 Oct;134(1):57-62.
64. G Heuser, SA Heuser, D Rodelander Treatment of neurologically impaired adults and children with mild hyperbaric oxygen (1.38 ATA and 24% oxygen) edited by Joiner JT Flagstaff Arizona:best publications 2002:109-15.
65. Plafki C, Peters P, Almeling M, Welslau W, Busch R. Complications and side effects of hyperbaric oxygen therapy. Aviat Space Environ Med 2000; 71(2): 119-24.
66. Palmquist BM, Philipson B, Barr PO. Nuclear cataract and myopia during hyperbaric oxygen therapy. Br J Ophthalmol 1984; 68(2): 113-7.
67. Lyne AJ. Ocular effects of hyperbaric oxygen. Trans OphthalmolSoc UK 1978; 98(1): 66-8.
68. Bergo GW, Risberg J, Tyssebotn I. Effect of 5 bar oxygen on cardiac output and organ blood flow in conscious rats. Undersea Biomed Res 1988; 15(6): 457-70.
69. Davis JC, Dunn JM, Heimbach RD. Hyperbaric medicine: Patient selection, treatment procedures and side effects. In: Davis JC, Hunt TK, editors. Problem Wounds - the role of oxygen. New York: Elsevier, 1988.

A CORRELATIVE STUDY BETWEEN SERUM URIC ACID AND hs-CRP IN PATIENTS WITH ISCHAEMIC HEART DISEASE

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ABSTRACT: INTRODUCTION: Uric acid (UA) contributes to the development of human vascular disease and atherosclerosis through a pro-inflammatory pathway. Highly-sensitive C-reactive protein (hs-CRP) also has emerged as the most exquisitely sensitive systemic marker of inflammation and a powerful predictive marker of future cardiovascular risk. In this study we estimated the levels of serum uric acid and hs- CRP in patients with ischaemic heart disease and association between them. **METHODS:** Study was carried out among fifty (50) newly diagnosed patients of ischaemic heart disease and fifty (50) age and sex matched healthy controls. Serum uric acid, hs-CRP, random glucose, urea, creatinine and cardiac troponin-I were measured.

RESULTS: The mean serum uric acid level in the control group was 4.66 ± 0.97 mg/dl and in case group was 6.86 ± 0.90 mg/dl. Mean hs-CRP level in control group was 0.19 ± 0.09 mg/dl, in case group was 5.14 ± 2.11 mg/dl. There was a positive association between UA and hs-CRP but the correlation was statistically not significant ($p > 0.05$). **CONCLUSION**: Our study indicated that there was a statistically significant association between ischaemic heart disease patients and elevated serum UA and serum hs-CRP and hence a strong role in the pathophysiology of ischaemic heart disease.

KEY WORDS: Ischaemic heart disease, hs-CRP, uric acid.

INTRODUCTION: With urbanization in the developing world, the prevalence of risk factors for Ischemic heart disease (IHD) is increasing rapidly in these regions such that a majority of the global burden of IHD is now occurring in low-income and middle-income countries. Population subgroups that appear to be particularly affected are men in South Asian countries, especially India (1).

The most common cause of IHD is atherosclerosis disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery (1). It leads to increased uric acid generation (from adenosine breakdown) and reduced excretion (due to lactate competing with urate transporter in the proximal tubule of kidney) (2).

Hyperuricaemia is most commonly defined by plasma uric acid concentration greater than 7.0 mg/dl in men or greater than 6.0 mg/dl in women (3). The association of hyperuricaemia with cardiovascular disease remains controversial. There has been no test of

hypothesis that a reduction in serum uric acid would prevent cardiovascular disease. Serum uric acid probably reflects and integrates different risk factors and their possible interaction. (4)
C-reactive protein is an acute phase reactant initially developed to, evaluate patients with infection. It was originally discovered by Tillet and Francis in 1930 as a substance in the serum of patients with acute inflammation that reacted with the C-polysaccharide of Pneumococcus. A high-sensitivity CRP (hs-CRP) test measures low levels of CRP (5).CRP has been shown to actively participate in both atheromatous lesions formation and plaque disruption (6, 7). CRP increases the expression of endothelial adhesion molecules and monocyte chemoattractant protein-1 (MCP-1), facilitates native LDL uptake into macrophages, promotes monocyte activation and a procoagulant effect by inducing monocytes to synthesize tissue factor (8, 9). CRP can also activate the classic pathway of complement activation and has been demonstrated to co-localize with terminal complement complexes in established coronary plaques (10).

Many recent studies have proved higher serum level of uric acid and high sensitivity C-reactive protein (hs-CRP) associated with ischemic heart disease. But very few comprehensive studies have yet been done to determine the correlation between serum uric acid level and hs-CRP level in ischemic heart disease. This study was therefore undertaken to determine the concentrations of serum uric acid and serum hs-CRP in IHD patients and to evaluate the association and correlation between them if there is any.

MATERIALS AND METHODS: This case-control study was carried out among fifty (50) newly diagnosed patients of ischaemic heart disease ≥ 20 years of age, admitted in Cardiology department of Assam Medical College and Hospital, Dibrugarh. Patients with inflammatory diseases like gout, rheumatoid arthritis, inflammatory bowel disease, renal disease, hypothyroid, diabetes, stroke, malignancy, bacterial infections were excluded from the study. Fifty age and sex matched healthy individuals were taken as control .Blood samples were collected from the subjects and estimation of serum uric acid , hs-CRP, random glucose, urea, creatinine and cardiac troponin-I were measured. Estimation of serum uric acid, random glucose, urea, and creatinine were done in Dimension RxL Max autoanalyzer. Serum UA was estimated by PAP /uricase method. Normal Reference Values with this method were 3.4–7.0 mg/dl for male and 2.5–6.0 mg/dl for female (11).Serum hs-CRP, was analysed in the Dimension RxL Max autoanalyzer using Particle Enhanced Turbidimetric Immunoassay (PETIA) Technique. Normal Reference Value for hs-CRP according to this method was 0.0–0.5 mg/dl (12). Qualitative estimation of cardiac troponin-I was done by using membrane-based immunoassay to confirm the diagnosis of ischaemic heart disease with ready to use Trop I Scan Test Device manufactured by Zydus Pathline.

A database was constructed on Microsoft Excel 2007, and statistical analysis was done using statistical package for social sciences (SPSS) Windows version 14. Student's t-test , and Pearson correlation were done to analyze the data.

RESULTS: The mean levels of serum glucose, urea and creatinine were within reference range in both cases and controls.

The mean serum uric acid level in the control group was 4.66 ± 0.97 mg/dl (in males : 4.98 ± 0.79 mg/dl and in females : 3.53 ± 0.63 mg/dl) and in case group was 6.86 ± 0.90 mg/dl(in males : 7.07 ± 0.86 mg/dl and in females, 6.12 ± 0.58 mg/dl). A highly significant increase in serum uric acid level was found in IHD subjects as compared to that in normal healthy

individuals with $p < 0.001$. Higher values of serum uric acid level was found in the age groups 60–<70 years and 40–<50 years in the control group. The higher values of serum uric acid in the cases were in the age group of 60–<70 yrs and ≥ 80 years. In both the study groups, the mean value of serum uric acid had no relation with age (Table 1).

The mean hs-CRP level in control group was 0.19 ± 0.09 mg/dl(in males:0.18 ± 0.08 mg/dl and in females : 0.25 ± 0.07 mg/dl) and in case group was 5.14 ± 2.11 mg/dl(in males:5.06 ± 2.17 mg/dl and in females : 5.44 ± 1.98 mg/dl). A highly significant increase in serum hs-CRP level was found in IHD subjects as compared to that in normal healthy individuals ($p < 0.001$). Mean value of serum hs-CRP level in controls increased with the increase in age. The highest value was in the age group of ≥ 80 years. In the cases, the mean value of hs-CRP level was high in the age groups of 60–<70 years and ≥ 80 years (Table2).

The correlation coefficient 'r' between serum uric acid and serum hs-CRP was found to be 0.124 with p-value> 0.05 which means that statistically, the correlation is not significant.

DISCUSSION: In the present study, the minimum age of presentation of IHD was found to be thirty-eight (38) years and the maximum was of 85 years. The age-wise distribution revealed the highest percentage of cases in the age group 60–<70 years (26%) followed by 24% in 50–<60 years and 20% in 40–<50 years. Also, there were 2% cases in the age group of < 40 years. Similar findings were also observed by Krisnaswami *et al* (1970) and Jhatakia KU *et al* (1967) (13, 14). Yusuf *et al* (2004) reported that mean age of presentation on with new MI was 52 years in South Indians, 9.7% of these cases were younger than 40 years of age (15).

In the present study, there were 39 (78%) males and 11 (12%) females with male to female ratio of 3.5:1. Almost similar finding was found by Singh *et al* (14). They reported a ratio of 3.8:1 between males and females. The lower incidence of IHD in women, especially in premenopausal age, was probably due to high levels of oestrogens and high density lipoprotein, both of which have anti-atherogenic influence.

In our study, the mean serum uric acid level was found to be significantly higher than in ischaemic heart disease patients (6.86 ± 0.90 mg/dl) that in the control group (4.66 ± 0.97 mg/dl). Similar results were also found by Morris London *et al* (1967) and M. Torun *et al* (1998) (16, 17). The role of uric acid as risk factor for myocardial infarction is controversial. The Rotterdam Study concluded that uric acid is a strong risk factor for myocardial infarction and stroke (18). Hong Evy Lim *et al* (2010) has also found that serum uric acid was higher in patients with coronary artery disease as compared to normal healthy individuals (19).

In the present study, the mean serum hs-CRP level in ischaemic heart disease patients (5.14 ± 2.11 mg/dl) was found to be significantly higher than in control group (0.19 ± 0.09 mg/dl). Similar results were found by Arroyo et al (2004), N. Yilmaz et al (2007) and Suman B Sharma et al (2008) (20,21,22). Significantly higher hs-CRP levels were found in angiographically proven CAD patients with acute coronary syndrome as compared to patients with normal coronary angiography; and the levels of hs-CRP correlated well with the angiographic severity of the CAD (23). Also mean hs-CRP level in females (5.44 ± 1.98 mg/dl) is slightly higher as compared with males (5.06 ± 2.17 mg/dl) with IHD. Ross Arena *et al* (2006) also found a significantly higher hs-CRP level in females as compared with males (24).

These findings may indicate that serum uric acid and serum hs-CRP may have some role in the pathophysiology of ischaemic heart disease. The present study is consistent with earlier studies wherein it was concluded that higher levels of serum uric acid and hs-CRP were associated with poor prognosis of the ischaemic heart disease patients.

In the present study, serum uric acid and serum hs-CRP were found to be positively associated (r = 0.124) but the correlation was not found to be significant. This finding is consistent with the study of Zhang Y H et al in 2009 (25). However a statistically significant correlation was found between serum CRP and serum UA in studies done by N. Yilmaz et al (2007) and Christa Meisinger et al (21, 26).

So, for a definitive conclusion regarding the usefulness of these two parameters as markers of cardiovascular risk factors and to know the mechanism by which uric acid and hs-CRP play role in the pathophysiology of ischaemic heart disease, further studies comprising of large number of cases and longer duration of study are required.

REFERENCES:

1. Anthony S. Fauci , Eugene Braunwald , Dennis L. Kasper ,Stephen L. Hauser, Dan L. Longo , J. Larry Jameson ,et al; Harrison's Principle of Internal Medicine, 17th Edition:1381-3.
2. Pluig JG, Ruilope LM. Uric acid as a cardiovascular risk factor in arterial hypertension. J Hypertens 1999 Jul;17(7):869-72
3. Carl A. Burtis, Edward R. Ashwood, David E. Bruns; Teitz's textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition:803-5.
4. Culleton BF. Uric acid and cardiovascular disease a renal cardiac relationship; Curr. Opin. Nephrol. Hypertens May 2001:10(3):371-5.
5. Leo K. Niskanen, David E. Laaksonen, , Kristiina Nyyssönen, Georg Alfthan, Hanna-Maaria Lakka, et al . Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged man-A prospective cohort study; Arch Intern. Med. 2004 164(14):1546-1551
6. Tenzin Nyandak, Arun Gogna, Sandeep Bansal, Manorama Deb.High Sensitive C-Reactive Protein (hs-CRP) and its Correlation with Angiographic Severity of Coronary Artery Disease (CAD); JIACM 2007; 8(3): 217-21
7. Vincenzo Pasceri, James T. Willerson, Edward T. H. Yeh . Direct proinflammatory effect of CRP on human endothelial cells. Circulation 2000; 102: 2165–8.
8. Thomas P. Zwaka, Vinzenz Hombach, Jan Torzewski, . CRP-mediated LDL uptake by macrophages: implicates for atherosclerosis. Circulation 2001; 103: 1194–7.
9. SP Ballou, Gerard Lozanski. Induction of inflammatory cytokine release from cultured human monocytes to synthesize tissue factor. Blood 1993; 82: 513–20
10. Carolyn Mold, Henry Gewurz, Terry W Du Clos. Regulation of complement activation by CRP. Immunopharmacology 1999; 42: 23–30
11. Town M.H.Gehm, S. Hammer, B. Ziegenhorn; J.Clin. Clin. Biochem(1985):23:591
12. Tie Q. Wei, Steve Kramer, Victor P. Chu, Dave,Hudson, Daniel Kilgore, Sue Salyer, et al. An improved automated immunoassay for C-reactive protein on the DimensionR clinical chemistry system; Journal of Automated Methods & Management in Chemistry, Vol.22, No. 5 (September – October 2000): 125-131.
13. Krishnaswami V, Radhakrishnan T, Michael Johns B and Mathew A. Pattern of ischaemic heart disease: a clinical study; J Indian Med Asso 1970 ;55:153-57.
14. Jhatakia KU, Thakore PB, Vasavada PJ Observations on some facets of coronary artery disease.J Assoc Physicians India. 1967 Mar; 15(3):109-15.
15. Salim Yusuf, Steven Hawken, Stephanie Ôunpuu , Tony Dans , Alvaro Avezum, Fernando Lanas, Matthew McQueen et al. Effect of potentially modifiable risk factors

associated with MI in 52 countries (The Interheart Study). Lancet 2004; 364: 937–52.
16. Morris London and Mary Hums. Distribution patterns of uric acid in CAD; Clinical Chemistry, 1967: Vol. 13:132-41.
17. M. Torun, S. Yardim, B. Simsek & S. Burgaz: Serum uric acid levels in cardiovascular diseases; J Clinn Pharm Ther 1998; 23: 25-9
18. Bos MJ, Koudstall PJ, Hotman A, Wittman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: The Rotterdam Study. Stroke 2006 June; 37(6): 1503–7.
19. Hong Euy Lim, Seong Hwan Kim, Eung Ju Kim, Jin Won Kim. Clinical value of serum uric acid in patients with suspected coronary artery disease;The Korean Journal of Internal Medicine, March 2010: Vol. 25, No. 1:21-6.
20. Ramón Arroyo-Espliguero, Pablo Avanzas, Juan Cosín-Sales, Guillermo Aldama, Carmine Pizzi,Juan Carlos Kaski . CRP elevation and disease activity in patients with coronary artery disease; Eur Heart J 2004 Mar; 25(5): 401–8.
21. Yilmaz N, Çiçek HK, Celik A, Meram I, Kocabas R, Davutoglu V. Diagnostic value of homocysteine, C-reactive protein and bilirubin for coronary artery disease; Eastern Mediterranean Health Journal. May–June, 2007:Vol. 13(3). 522-35
22. Suman B. Sharma, Seema Garg, Abhinav Veerwal, Sridhar Dwivedi. hs-CRP and oxidative stress in young CAD patients: A Pilot Study; Indian Journal of Clinical Biochemistry 2008; 23(4): 334–36.
23. Tenzin Nyandak, Arun Gogna, Sandeep Bansal, Manorama Deb. hs-CRP and its correlation with Angiographic severity of coronary Artery Disease (CAD); Journal, Indian Academy of Clinical Medicine 2007; 8(3): 217-21.
24. Ross Arena, James A. Arrowood, Ding-Yu Fei, Shirley Helm, Kenneth A. Kraft. The Relationship between C-Reactive Protein and Other Cardiovascular Risk Factors in Men and Women; Journal of Cardiopulmonary Rehabilitation & Prevention September/ October 2006, Volume 26 (5): 323-27.
25. Zhang YH, Lü R, Zhao XY, Kang LM, Yang YJ, Zhang J. Association of serum uric acid, plasma NT-proBNP, hs-C reactive protein and invasive hemodynamic parameters in patients with heart failure ; Zhonghua Xin Xue Guan Bing Za Zhi , Chinese journal of cardiovascular diseases :2009 Feb;37(2):126-9.
26. Christa Meisinger, Wolfgang Koenig, Jens Baumert and Angela Doring. The Uric Acid Levels Are Associated With All-Cause and Cardiovascular Disease Mortality Independent of Systemic Inflammation in Men From the General Population. MONICA/KORA Cohort Study; Arterioscler Thromb Vasc Biol 2008; 28:1186-92.

Table 1: Age-wise distribution of serum uric acid

Age group (in years)	Serum uric acid (mg/dl)			
	Range		Mean ± S.D.	
	Controls	Cases	Controls	Cases
< 40	4.3	6.2	4.3	6.2
40–<50	2.6–6.9	5.0–8.2	4.83 ± 1.69	6.63 ± 0.97
50–<60	3.2–5.9	5.2–9.2	4.73 ± 0.77	6.93 ± 1.15
60–<70	3.6–6.2	5.5–8.5	4.85 ± 0.86	7.02 ± 1.00

70–<80	3.9–5.10	5.9–7.8	4.33 ± 0.39	6.78 ± 0.63
≥ 80	3.9–5.0	6.8–7.3	4.32 ± 0.50	7.00 ± 0.19

Table2: Age-wise distribution of serum hs-CRP

Age group (in years)	Serum hs-CRP (mg/dl)			
	Range		Mean ± S.D.	
	Controls	Cases	Controls	Cases
< 40	0.03	3.81	0.03	3.81
40–<50	0.05–0.25	0.8–7.8	0.15 ± 0.07	3.88 ± 1.98
50–<60	0.10–0.29	1.3–9.2	0.17 ± 0.07	4.30 ± 2.68
60–<70	0.10–0.32	3.27–10.0	0.19 ± 0.08	5.05 ± 2.88
70–<80	0.10–0.40	3.72–8.50	0.24± 0.11	4.99 ± 2.90
≥ 80	0.21–0.35	3.9–8.20	0.28 ± 0.06	5.25 ± 2.28

Table–3: Statistical analysis for serum uric acid in the study groups

Study group	Number of Observation	Serum Uric Acid (Mean±S.D.) (mg/dl)	Degree of Freedom	't' Value	'p' Value
Controls	50	4.66 ± 0.97	98	7.6	< 0.001
Cases	50	6.86 ± 0.90			

Table–4: Statistical analysis for serum hs-CRP in the study groups

Study group	Number of Observation	Serum hs-CRP (Mean±S.D.) (mg/dl)	Degree of Freedom	't' Value	'p' Value
Controls	50	0.19 ± 0.09	98	9.2	< 0.001
Cases	50	5.14 ± 2.11			

AN ANALYSIS OF BLOOD USAGE IN AN ELECTIVE SURGERIES AND ITS WASTAGE AT MEDICAL COLLEGE HOSPITAL

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ABSTRACT: BACKGROUND: The demand for large quantities of blood for elective surgeries, of which little is utilized results in exhaustion of valuable supplies and resources both in terms of technician time and reagents. This adds to the financial burden of the patients. The aim of this study is to analyse the usage of blood in elective surgeries and to prevent excessive wastage of blood. **METHODS:** A retrospective study was conducted at Medical College Hospital Blood Bank during the period from January 2010 to December 2010.The number of patients cross-matched & transfused were analysed. The different transfusion indices such as Cross match/Transfusion ratio(C/T), Transfusion Probability (%T), Transfusion Index (TI) were calculated. Maximal Surgical Blood Ordering System (MSBOS) was estimated for each procedure and the degree of over transfusion was calculated. **RESULTS**: A total of 1276 units of blood were cross-matched for 804 patients, but only 399 units were transfused to 213 patients i.e. 26% of blood cross-matched was utilized, leaving 74% unutilized. Significant blood utilization was nil in most of the routine elective cases. The overall C/T was 2.01, MSBOS was 0.465 and in 26% of cases over transfusion was present. **CONCLUSION**: This study showed that there was excessive cross matching of blood. "Type, screen and hold" policy should be implemented. Blood ordering pattern needs to be revised and over-ordering of blood should be minimized.
KEY WORDS: elective surgeries, transfusion indices, over transfusion

INTRODUCTION: The optimal function of the surgical departments depends on an efficient round the clock blood dispensing service of the blood bank.[1] Blood transfusion will be liberal with the ready availability of the blood and blood components. Many units of blood routinely ordered by the surgeons are not utilized, but are held in reserve and are thus unavailable for the needy patient.[2] The consequences of such misuse leads to outdating of blood, overburdening on blood bank personnel, depletion of blood bank resources, and wastage of blood.[3] In South Africa for example, 7-10% of blood is wasted annually because of over-ordering of blood.[4] It has become clear from the studies of United States, Australia and Israel that great savings may be made from rationalizing blood ordering habits.[5] Hence it is quiet necessary to streamline the blood usage by incorporating blood ordering schedule for such procedures which decreases over-ordering of blood, unnecessary compatibility testing, returning of unused blood & wastage due to outdating.

A maximum surgical blood order schedule (MSBOS) provides guidelines for frequently performed elective surgical procedures by recommending the maximum number of units of blood to be cross matched preoperatively. The MSBOS has the following advantages [6]:

1. A reduction in cross matching work load of the blood transfusion laboratory (in some cases in excess of 20%) which allows more time to respond to emergency requests, and also to investigate complex serological problems.
2. A reduction in the level of stress.
3. More efficient use of blood stocks and a reduction in wastage due to out-dating.

The ratio of the number of units cross matched to the number of units actually transfused, that is C: T ratio, T ratio should not exceed 2:1. Although MSBOS has improved the efficiency of blood utilization, there are certain drawbacks, the most significant are being the absence of accountability for individual, differences in transfusion requirements between different persons undergoing the same surgical procedure.[3]

The aim of this study is to analyse the usage of blood in elective surgeries and to study the measures to prevent excessive wastage of blood bank resources .

MATERIALS AND METHODS: A retrospective study was conducted at Medical College Hospital Blood Bank during the period from January 2010 to December 2010. There are seven surgical departments which include Obstetrics & Gynaecology, Surgery, Orthopaedic Surgery, Urology, Neurosurgery, ENT and Ophthalmolgy and perform between 7500- 8000 elective surgeries a year. The patients included in this study are adult patients who underwent elective surgical procedures for which requisition of blood was made. Postponed elective surgeries for various reasons and patients who underwent massive transfusion were excluded from the study to eliminate bias.(Massive transfusion is defined based on the absolute number of transfused packed red blood cells (RBCs) either the transfusion of 10 units or more of packed RBCs in 24 hours or the transfusion of 20 units of packed RBC or more in the course of the hospital stay).[3] The details of the patient who underwent elective surgical procedures was obtained from the operation theatre records. The number of units cross matched, the number of units issued and transfused during the procedure were obtained from blood bank records. The pre-operative haemoglobin (Hb) levels, the post–operative Hb levels of patient between first and fourth post-operative days were recorded The following transfusion indices were used to determine the blood utilization for each surgical procedure.[5]

A) **CROSS-MATCH TO TRANSFUSION RATIO (C/T RATIO)=** No. of units cross-matched /No. of units transfused

A ratio of <2.5 is considered indicative of significant blood usage.

B) **TRANSFUSION PROBABILITY (%T) =** No. of patients transfused x100 /No. of patients cross-matched.

A value of >30 was considered indicative of significant blood usage.

C) **TRANSFUSION INDEX (TI)=** No. of units transfused /No. of patients cross-matched

A value of >0.5 was considered indicative of significant blood utilization.

Next Maximal Surgical Blood Order Schedule (MSBOS) and degree of over transfusion was calculated by using the following formula:

1. **MSBOS= 1.5xti**

MSBOS estimates the amount of blood that will be needed for the individual procedure.

2. **DEGREE OF OVERTRANSFUSION**= No. of patients with post-transfusion Hb more than 11g/dl /Total no. of patients transfused

RESULTS: The patients who had elective surgeries for which request for blood (packed red cell concentrate) was made from six surgical departments, except ophthalmology. Total number of patients included were 804. Male patients were 228, while female were 576.The age range was 15-65 years. The mean pre-operative Hb in the study group was 10.2+/- 0.8g/dl. On calculation of all the transfusion indices, a total of 1276 units of blood were cross-matched for 804 patients, but only 399 units were transfused to 213 patients i.e;26% of blood cross-matched was utilized, leaving 74% unutilized. The overall C/T was 2.01 (as shown in Table- 1). Maximum number of patients (61%) who had requested for blood was from the department of obstetrics & gynaecology with 47% listed for LSCS and 53% being gynaecology cases. The department of Surgery, Orthopaedic, Urology, Neurosurgery, ENT recorded 14%, 12%, 10%, 2%, 1% respectively.

1.OBSTETRICS AND GYNAECOLOGY: The blood usage indices for obstetrics & gynaecology cases (as shown in Table-2). LSCS (general) was the most frequently performed elective surgery(190 cases) and had the highest total number of units cross-matched (272cases).All the three transfusion indices (C/T,%T,TI) showed less utilization of blood. In LSCS-placenta praevia all the 3 indices showed significant utilization and MSBOS is more in this procedure. In dilatation and curettage (D&C) and total abdominal hysterectomy & bilateral salphingo-oopherectomy (TAHBS), two indices showed blood utilization. The C/T ratio was significant in LSCS-placenta praevia and vaginal hysterectomy.

2. GENERAL SURGERY: Blood usage for surgical cases (as shown in Table-3). Modified radical mastectomy was the most frequently performed elective surgery (26cases) and had the highest total number of units cross-matched (32 units).The C/T and TI was significant. All the transfusion indices were nil in patients who have undergone laparoscopic cholecystectomy, thyroidectomy and oesophagectomy. Blood utilization was more in right hemicolectomy. In large bowel resection 2 indices were significant. But %T was high in open cholecystectomy and spleenectomy. In breast mass lumpectomy C/T was high and other indices were insignificant indicating less utilization of blood. MSOBS was more in large bowel resection.

3. ORTHOPAEDICS: Blood usage for orthopaedic surgical cases (as shown in Table-4). All the three transfusion indices were significant in hemiarthroplasty, disc surgery, tibial fractures and total hip replacement indicating more blood utilization. In trochantric fracture and shoulder repair, all the three transfusion indices were nil, because number of units transfused was nil. In femur shaft fracture C/T was high and %T &TI was insignificant. MSBOS in tibial fractures was more.

4. UROLOGY: Blood usage for urology cases (as shown in Table-5). Transurethral resection of prostate (TURP) was the most frequently performed elective surgery and the three transfusion indices was nil. In nephrectomy and radical cystectomy the three indices showed significant blood utilization and MSBOS in nephrectomy was more.

5. NEUROSURGERY: Blood usage for neurosurgery cases (as shown in Table-6). Craniotomy was the only one elective surgery performed and requested for blood. Fifty units of blood were cross matched for eighteen patients. All the three indices were significant.

6. ENT: Blood utilization was nil (as shown in Table-7), because no patient received blood transfusion.

The overall MSBOS was 0.465.Among 804 cases, we were able to trace 121 cases who had been transfused with red cells with in 24 hours of surgery and had documented post-transfusion Hb levels. Using the formula stated above, 32 (26%) cases of the patient has been over transfused and may be the cause of low C/T ratio.

DISCUSSION: Blood is a precious commodity and its proper utilization is the key for efficient management of blood bank resources. Blood transfusion no doubt plays a major role in the resuscitation and management of surgical patients, but surgeons most of the time overestimate the anticipated blood loss thereby over-ordering blood.[7] Many a times blood requisition is made by 'force of habit'. Analysis of the data indicated that the majority (77%) of operations will need no pre-operative preparations of blood.[8] The demand for large quantity of blood for elective surgeries of which little is utilized causes wastage of valuable supplies and resources both in terms of technician time and reagents. Hence it is essential that the usage of blood and blood product be rationalized and in crisis situations only.

A number of studies in many countries of the world have shown over-ordering of blood by surgeons with utilization ranging from 5-40%. [9] Basnet et al [2] showed 13.6% of utilization. Present study showed 26% of cross matched blood being utilized. This suggests that a significant amount of time spent by overworked blood bank technicians as well as reagents used for cross matching were wasted. In our study significant blood utilization using all three indices was obtained in placenta praevia, right hemicolectomy, hemiarthroplasty, disc surgery, tibial fractures, total hip replacement, nephrectomy, radical cystectomy and craniotomy. Various published studies elsewhere have shown similar findings.[4,10] The study also shown that in ENT surgeries, laparoscopic cholecystectomy, thyroidectomy, oesophagectomy, trochantric fracture, shoulder repair, TURP, none of the blood that was cross matched was utilized which was similar to the findings of Olawuni Ho et al.[4] This indicates that the routine cross matching of blood in elective surgery is a culture rather than a necessity.[11] The lack of confidence on the part of surgeons in the ability of the blood bank staff to supply blood immediately if required.[12] The ordering of blood appeared to be even more indiscriminate. The ordering of blood for surgery was left to the inexperienced house surgeons who have been known to over order blood due to lack of communication with the senior doctors.[13]

In the present study, pre-operative cross matching for elective surgery was 1276 units for 804 patients. The overall C/T ratio was 2.015. A low C/T ratio may represent a low cross match incidence or alternatively a high transfusion incidence conversely, a high C/T may represent a high cross match incidence or low transfusion incidence.[14] In the authors study the overall C/T ratio showed 4.4, which represents only about one fifth of the blood cross matched for elective surgery is transfused.[1] In order to reduce excessive cross-matching "type, screen and hold" procedure must be implemented. Here, blood is screened for antibodies by using internationally accepted techniques and reagents a few days prior to the procedure. If no antibodies are detected, no blood will be cross-matched. If need does arise for transfusion, cross matching may be accomplished in 10 minutes using the immediate spin method. If antibodies are detected in the antibody screening tests, suitable blood units lacking the corresponding antigen and compatible with the patient will have to be provided prior to surgery. Several studies have shown the "type, screen & hold" to be safe if done according

recommended technique.[15] This technique proved to be 99.99% efficient in preventing incompatible blood.[10]

The degree of over transfusion obtained here is similar as compared to that of various other centers, where over transfusion ranged from 27% to 39%.[13] Dodsworth[16] found 33% and Maha SA[1] found 45.5%. This study suggests that blood has been further wasted and patient have been unnecessarily exposed to the very significant risk of blood transfusion.

In the present study MSBOS in placenta praevia, nephrectomy and craniotomy was high. But the overall MSBOS is 0.465. In a similar study by Vibhute [9] the blood evaluation and transfusion practices for 500 elective general surgical procedures were evaluated and MSBOS was put into immediate effect after formulation. As a result, the blood ordering pattern changed for the next 150 patients.[9] This shows that MSBOS definitely improves the blood utilization and reduces the wastage rate. However, it does not take into consideration the individual differences in transfusion needs between different patients undergoing the same surgery. Surgical blood ordering equation (SBOE) is an extended MSBOS incorporating patient and surgical variables, such as pre- and postoperative hemoglobin levels of the patient and the amount of surgical blood loss during each surgical procedure.[3] By establishing such an SBOE, each surgical team can develop its own transfusion system. They can also audit the operative blood loss for each procedure.[10] A strong institutional hospital transfusion committee is required to uplift the profile of blood transfusion.

CONCLUSION: The present study showed 26% of cross matched blood being utilized. The overall C/T ratio is 2.015 and the over transfusion is 26%. Blood ordering pattern needs to be revised and over-ordering of blood should be minimized. It is an ideal method in saving hospital resources and manpower. In order to reduce unnecessary cross matching, “type, screen and hold” procedure must be implemented. However, one must confirm the availability of blood for emergency situation before starting the surgery.

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REFERENCES:

1. Maha SA, Abdelhadi, Layla AM, Bashawari. Blood conservation in elective surgery. Kuwait Medical Journal. 2001; 33(3):232-234.
2. Basnet RB, Lamichhane D, Sharma VK. A study of blood requisition and transfusion practice in surgery at Bir Hospital. PMJN. July-Dec 2009; 9(2):14-19.
3. Arulselvi Subramanian, Kanchana Rangarajan, Sudeep Kumar, Kamran Farooque, Vijay Sharma, Mahesh Chandra Misra. Reviewing the blood ordering schedule for elective orthopaedic surgeries at a level one trauma care center. Journal of emergencies, trauma and shock. 2010; 3(3):225-230.
4. Olawumi Ho, Bolaji Bo. Blood utilization in elective surgical procedures in Ilorin. The Tropical Journal Of Health Sciences. 2006 ; 13:15-17.
5. Smallwood JA. Use of blood in elective general surgery: an area of wasted resources. BMJl. 1983 ; 286:868-870.
6. Guidelines for implementation of a maximum surgical blood order system schedule. The British committee for standards in haematology Blood Transfusion Task Force. Clin Lab Haematol. 1990 ; 12:321-7.

7. Efraim K.Blood conservation in South Africa. A vital need .Bloodless Medicine and Surgery. 2001 ; 43:7.
8. Chawla T, Kakepoto GN, Khan MA. An audit of blood cross- match ordering practices at the Aga Khan University Hospital: First step towards a maximum surgical blood ordering schedule. J Pak Med Assoc. 2001 ; 51:251-4.
9. Vibhute M, Kamath SK, Shetty A. Blood utilization in elective general surgery cases: Requirements, ordering and transfusion practices. J Postgrad Med. 2000 ; 46:13-4.
10. Friedman BA, Oberdman HA, Chandnick AR, Kingdon KI. The maximum surgical Blood order schedule and surgical blood use in United States.Transfusion.1976; 16:380-7.
11. Friedman BA. An analysis of surgical Blood use in United States hospitals with application to the maximum surgical blood order schedule. Transfusion. 1979; 19:268-78.
12. 12. Davis SP, Barrasso C, Ness PM. Maximizing the benefits of type and screen by continued surveillance of transfusion practice. Am J Med Technology. 1983 ; 49:579-82.
13. Sarma DP. Use of blood in elective surgery. JAMA. 1980 ; 243:1536-7.
14. Boral L, Henry JB. The type and screen: a safe alternative and supplement in selected surgical procedures. Transfusion.1977 ; 17:163.
15. Garratty G. The role of compatibility tests. In: Report of a meeting sponsored by the Bureau of Biologics for the Blood Products Advisory Committee. Transfusion. 1982; 22:169-72.
16. Dodsworth H, Dudley H. Increased efficiency of transfusion practice in routine surgery using pre-operative antibody screening and selective ordering with an abbreviated crossmatch.Br J Surg. 1985;72:102-104.

ANALYSIS OF TRANSFUSION DATA IN ELECTIVE SURGERIES

Table-1 :Shows blood usage from various surgical departments

Department	No.of patients cross matched	No. of units cross matched	No.of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.OBG& Gynaec	488 (61%)	838	128	248	3.37	26	0.29	0.43
2.Surgery	112 (14%)	181	26	54	3.35	23	0.29	0.43
3.Orthpaedics	98 (12%)	105	24	28	3.75	24	0.26	0.39
4.Urology	80 (10%)	86	20	28	3.07	24	0.32	0.48

5.Neurosurgery	18 (2%)	50	15	41	1.2	83	2.27	3.4
6.ENT	8 (1%)	16	Nil	Nil	Nil	Nil	Nil	Nil
Total	804 (100%)	1276	213	399	2.015	26	0.31	0.465

Table -2: Department of Obstretics and Gynaecology.

Procedure	No.of patients cross matched (Total=)	No. of units cross matched (Total=)	No. of patients transfused (Total=)	No. of units Transfused (Total=)	C/T	% T	TI	MSBOS
1.D&C	101	226	41	72	3.13	41	0.71	1
2.TAHBS	98	190	28	49	3.87	39	0.50	0.75
3.Vaginal Hystrectomy	28	36	07	12	2.33	25	0.42	0.6
4.Partial Hystrectomy	33	42	02	06	5.50	06	0.18	0.27
5.LSCS								
-General	190	272	28	46	4.13	15	0.24	0.36
-Placenta praevia	38	72	22	63	0.60	58	1.65	2.47

D&C=Dilatation & Curettage, TAHBS=Total abdominal hysterectomy and bilateral salphingo-oopherectomy, LSCS=Lower segment caesarean section

Table-3: Department of General Surger

Procedure	No. of patients cross matched	No. of units cross matched	No. of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.Open Cholecystectomy	06	14	01	02	07	33	0.3	0.45
2.Laproscopic Cholecystectomy	09	12	Nil	Nil	0	0	0	0
3.Thyroidectomy	08	09	Nil	Nil	0	0	0	0
4.Modified radical mastectomy	26	32	05	18	1.77	19	0.69	1.03
5.Right hemicolectomy	18	22	08	11	02	44	0.61	0.91
6.Oesophagectomy	04	10	Nil	Nil	0	0	0	0
7.Large bowel resection	12	30	03	14	2.14	25	1.16	1.74
8.Spleenectomy	12	22	05	05	4.4	41	0.41	0.61
9.Breast mass lumpectomy	07	12	02	02	06	17	0.28	0.42
10.Hemorrhoidectomy	10	18	02	02	09	05	0.2	0.3

Table-4: Department of Orthopaedics

Procedure	No. of patients cross matched	No. of units cross matched	No. of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.Hemiarthroplasty	24	29	12	12	2.4	50	0.5	0.75
2.Trochantric fracture	23	25	Nil	Nil	0	0	0	0
3.Femur shaft fracture	18	11	02	02	5.5	11	0.1	0.15
4.Disc surgery	09	15	05	07	2.14	56	0.7	1.05
5.Tibial fractures	02	06	02	04	1.5	100	02	3
6.Total hip replacement	04	07	03	03	2.3	75	0.75	1.12
7.Shoulder repair	18	12	Nil	Nil	0	0	0	0

Table-5: Department of Urology

Procedure	No. of patients cross matched	No. of units cross matched	No. of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.TURP	41	41	Nil	Nil	0	0	0	0
2.Nephrectomy	04	06	04	06	1.0	100	1.5	2.25
3.Urethroplasty	12	06	03	04	1.5	25	0.3	0.45
4.Radical cystectomy	23	27	13	18	1.5	57	0.78	1.17

TURP= Trans – urethral resection of prostate

Table-6: Department of Neurosurgery

Procedure	No. of patients cross matched	No. of units cross matched	No. of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.Craniotomy	18	50	15	41	1.2	83	2.27	3.4

Table-7: Department of ENT

Procedure	No. of patients cross matched	No. of units cross matched	No. of patients transfused	No. of units transfused	C/T	%T	TI	MSBOS
1.Parotidectomy	06	08	Nil	Nil	0	0	0	0
2.Laryngectomy	02	08	Nil	Nil	0	0	0	0

A HOSPITAL BASED STUDY ON LIPID PROFILE IN SMOKERS AND NON SMOKERS- A COMPARATIVE STUDY

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ABSTRACT: BACKGROUND: Smoking is one of the environmental factors which can alter normal lipid profile. It is one of the major risk factors in the genesis of coronary atherosclerosis and development of coronary heart disease. **AIMS:** To evaluate and compare the lipid profile in both groups and to evaluate the existence of dose dependent relationship and durational significance between smoking and lipid profile among smokers. **SETTINGS AND DESIGN:** Out of 100 apparently healthy male subjects of age group 21-40yrs, 50 were smokers and 50 were non smokers. All the subjects were non alcoholic, non –obese, normotensives and from same socioeconomic status. Subjects who smoke more than equal 10 cigarettes for more than 2 years were considered as smokers group. **METHODS AND MATERIAL:** The subjects were asked to fast overnight and early morning blood samples collected and analyzed for lipid profile by appropriate methods. **STATISTICAL ANALYSIS USED:** The student's unpaired "t" test was used for statistical analysis. P-value of < 0.05 or P value <0.01 was considered statistically significant. **RESULTS:** In our study we found serum TC, TG, LDL and VLDL were higher in smokers as compared to non smokers and the serum HDL level was significantly decreased in smokers compared to non smokers showing greater risk of these persons to atherosclerosis and coronary heart disease.
Conclusions: - We conclude our study with the observation that smoking causes alteration in lipid profile. Increased amount and duration of smoking causes more dyslipidaemia .This alteration in serum lipid levels increases risk for coronary artery disease.
KEY WORDS: Cigarette Smoking, Coronary Heart Disease, Dyslipidaemia, Lipid profile and Tobacco.

INTRODUCTION: Smoking is generally considered to be associated with increased risk of a variety of medical disorders. Diet and environmental factors influence the lipid profile. Smoking is one of the environmental factors which can alter normal lipid profile and several studies provide the evidence that tobacco is strongly associated with altering the normal status of the lipid profile. Tobacco smoking is one of the largest single preventable causes of ill health in the world.[1] People smoke for many reasons like for relieving stress, for enjoyment, social reinforcement etc. Usually smoking starts with a curiosity to know how it feels at a tender age of

12-14 years. Kessler[2] defined smoking as a "Pediatric Disease". The prevalence of smoking has reached its peak among teenagers.

Teenagers are not aware of harmful effects of smoking. Smoking parents, attractive smoking and tobacco chewing advertisements, unhealthy bets on maximum smoking challenges, inadequate legislation to control smoking have made the teenage smokers to become adult chronic smokers. All these factors have contributed tobacco smoking as a major factor of preventable cardiovascular and respiratory morbidity and mortality. Relationship of Coronary Heart Disease (CHD) and smoking was first developed by White et al.[3] and Doll et al.[4] Incidence of developing CHD is directly related to the number of cigarettes smoked. Sudden death is 2-4 times more in heavy smokers than in non smokers.[5] It has been suggested that cigarettes when consumed more than 10 per day on regular constitute a major risk factor for CHD.[6]

This study was carried out to assess the impact of active tobacco smoking on lipid profile and to compare the effect of smoking in apparently healthy adult male smokers and non-smokers.

AIMS:

1. To evaluate and compare the lipid profile in both smokers and non-smokers.
2. To evaluate the existence of dose dependent relationship and durational significance between smoking and lipid profile among smokers.

MATERIALS AND METHODS: This study was conducted in the Department of Physiology and Biochemistry, M.R.M.C. and Basaveshwar teaching hospital Gulbarga, after obtaining the permission of the Ethical committee of our institution. The present study includes apparently healthy male subjects in the age group of 21–40 years of Gulbarga city. All the subjects gave the informed consent. The inclusion criteria of study subjects includes Non Smokers [control group, n=50] non alcoholic, non smokers, non-obese and Smokers group [n=50], subjects who smoke more than 10 cigarettes for more than 2 years and non-alcoholic subjects. The exclusion criteria of our study includes subjects below 21yrs and above 40yrs of age, obese, persons with angina, diabetics, alcoholics, subjects with renal failure, hepatic failure and females excluded from the study as female smokers are almost non-existent in our area. All subjects were of same socioeconomic status. Detailed history, name, age, sex, occupation, personal history and personal habits of the subjects were taken. Smoking history was taken in detail. Family history of hypertension, diabetes and obesity were enquired. These were noted in a personal Performa and parameters concerned with the study were recorded. The lipid profile parameters include serum Total Cholesterol [TC], Triglycerides [TG], Low Density Lipoproteins [LDL], High Density Lipoproteins [HDL] and Very Low Density Lipoproteins [VLDL].

The subjects were asked to fast overnight and early morning blood sample from the antecubital vein of each subject [5 ml blood] was collected under all aseptic precautions. Samples were processed within 1 hour for quantitative lipoprotein cholesterol measurements using the vertical spin ultracentrifugation technique. Serum was obtained by centrifugation for 4 min at 3000 rpm and was then transferred into properly labeled sterile vials and stored at -20°C till the performance of lipid profile. Serum TC was measured by CHOD-PAP method[7], serum TG by GPO -TRINDER method[8] and HDL-C tests by Phosphotungstic Acid method[9] whereas LDL-C and VLDL-C was determined by calculation method.[10] All the tests were done on ERBA Chem-7 semi autoanalyser with in 1 hour after collection of sample in biochemistry laboratory at Basaveshwar Teaching and General Hospital.

The student's unpaired "t" test was used for statistical analysis. P-value less than 0.05 or P value less than 0.01 was considered statistically significant.

RESULTS: In our study out of 50 smokers and 50 non smokers 44% were in the age group of 21to 30 years and 56% were in the age group of 31 to 40 years. Out of 50 smokers 23 (46%) smokers were smoking 10 cigarettes per day and 27(54%) smoking more than 10 cigarettes per day. Majority i.e. 32(64%) of smokers were smoking for more than 5 years duration and 18(36%) smoking for less than 5yrs.

Table 1 depicts the age wise distribution of subjects in both smokers group and non smokers group with mean age distribution of 29.15$\pm$ 5.91 and 33.8 $\pm$ 5.15 years. This table reveals that majority 68% of smokers were in the age group of 31-40 years.

Table 2 shows the comparison of lipid profile in both smokers group and non smokers group. A significant ($P \leq 0.005$) rise of lipid profile i.e. Total Cholesterol, Serum Triglycerides, Low density lipoproteins and Very low density lipoproteins was observed among smokers group than their control group. On the other hand no significant rise of lipid profile was observed among non smokers. HDL was decreased in smokers group compared to non smokers; this decrease was statistically significant with p value ≤ 0.005.

Table 3 indicates that the number of total cigarettes smoked per day has a significant impact on lipid profile as mean Total cholesterol, Triglycerides, LDL, VLDL were higher in smokers who smoke more than 10 cigarettes per day in comparison to those who smoke 10 cigarettes per day . This observed difference is statistically highly significant with $p<0.001$ and <0.005 respectively for LDL and Total cholesterol. HDL level was lower in subjects who were smoking more than 10 cigarettes per day.

The Table 4 reveals that duration of smoking in years has an impact on lipid profile as Total cholesterol (243mg/dl), Triglycerides (185mg/dl), and LDL (161mg/dl) were high among smokers who were smoking for more than 5 years in comparison to smokers of less than 5 years of duration With $P < 0.001$, 0.01and 0.05 respectively.HDL level was significantly lower 33.5$\pm$3 among smokers smoking for more than 5 years in contrast to those smoking for less than 5years of duration i.e. HDL 39.7$\pm$ 2 with $P<0.05$.

DISCUSSION AND CONCLUSION: Tobacco smoke contains many constituents; nicotine is one of the main constituents. Nicotine and other toxic substances from tobacco smoke are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances narrow or damage the blood vessel walls; hence plaques form at a faster rate in smokers.[11] There is definite relationship between tobacco use and atherosclerosis. Therefore even modest cigarette smoking during adolescence and early adulthood adversely alters the serum lipid and lipoprotein levels. [12, 13, 14]

We conducted this study to assess the impact of smoking on lipid profile. In our study all subjects in both smokers and non smokers group were apparently healthy adult males who were non-diabetic, non-alcoholic and normotensives. In our study Serum TC, TG, LDL and VLDL were significantly higher in smokers as compared to non-smokers and the serum HDL level was significantly lower in smokers as compared to non-smokers.

Our findings are in accordance with the findings of many research workers. The change in the serum lipoprotein levels became more marked with the number of cigarettes smoked per day and duration of smoking in years. This finding has been substantiated by N S Neki.[15] Contrary to the above findings Diricana M et al did not find significant differences in serum TC,

TG, LDL HDL levels between smokers and nonsmokers.[16] Nesje LA et al also found no significant difference between smokers and non-smokers concerning triglycerides and total cholesterol.[17] Dyslipidemia is a well-established risk factor for the development of coronary artery disease. Our study demonstrated presence of dyslipidemia in chronic smokers.

Increased levels of Triglycerides, Total cholesterol, LDL, VLDL and lower HDL in smokers points to coronary artery disease. HDL is an anti-atherogenic substance; its fall raises cardiovascular disease. The rapid reduction of its risk after cessation of smoking implies that tobacco enhances thrombosis and contributes in formation of atherosclerotic plaque. The health benefits of smoking cessation occur faster for cardiovascular diseases compared to other conditions.

We conclude our study with the observation that smoking causes dyslipidemia. Increased amount and duration of smoking also enhances this condition .This dyslipidemic state is an increased risk for coronary artery disease.

As statistical analysis were dependent on the accuracy of self reported smoking habits and age this could limit significance of the result. The result of our study cannot be generalized at this stage to the entire population because of small sample size and local geographical representation.

The policies that prevent and reduce smoking will have immediate and large benefits for reducing cardiovascular mortality.[18] Creating awareness regarding health consequences of smoking in schools and colleges by establishment of mandatory public health education and other antismoking advice should be made an important part of public health system.

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REFERENCES:

1. (WHO) World Health Organization (1983). Chronicle 37: 86-90.
2. Kessler DA, Natanblut SL, Wilkenfeld JP, Lorraine CC, Mayl SL, Bernstein IBG (1997). Nicotine addiction: a pediatric disease. J. Pediatr. 130: 518-524.
3. White, P.D; Coronary disease & coronary thrombosis in youth. J.Med. Soc. J. 1935:32: 596-605
4. Doll, R, & Hill, A.B. Mortality in relation to smoking. Br.Med. J. 1964: ii: 399-440.
5. Stanler, J.Wentworth D. Is the relationship between serum cholesterol & risk of premature death from coronary heart disease continuous & graded? JAMA 1986: 256:2823-28.
6. Garrison, R.J. Kannel, W.B. Cigarette smoking & HDL cholesterol. Atherosclerosis: 1979: 30,17.
7. Roeschalu P Bernt E and Gruber W A Clin Biochem 1974; 12(226).
8. Mc Gowan, M .W etal.Clin.Chem, 1988 ;29:538.
9. Burstein M. Scholnic HR,Morfin R.J Lipid Res,1970.
10. Carl.A.Burtis, Edward Ashwood, Cardiac function teitz Textbook of clinical Biochemistry and molecular Diagnostic; 4th edition; Saunders publications: 1619-1660.

11. Mitchell B (1999). Tobacco use and cessation: The adverse health effects of tobacco and Tobacco-Related Products. Primary Care: Clin. Office Pract., 26: 463-498.
12. Glueck CJ, Heiss G, Morrison JA, Khoury P, Moore M (1981). Alcohol intake, cigarette smoking and plasma lipids and lipoproteins in 12-19 year children. Circulation 64: 48-56.
13. Freedman DS, Srinivasan SR, Shear CL, Hunter SM, Croft JB, Webber LS (1986). Cigarette smoking initiation and longitudinal changes in serum lipids and lipoproteins in early adulthood: The Bogalusa Heart Study. Am. J. Epidemiol. 124: 207-219.
14. Craig WY, Palomaki GE, Johnson AM, Haddow JE(1990). Cigarette smoking-associated changes in blood lipid and lipoprotein levels in the 8- to 19-year-old age group: a meta-analysis. Pediatr. 85: 155-158.
15. Neki NS. Lipid profile in chronic smokers-A clinical Study. JIACM; 3:51-4,(2002)
16. Dirican M , Sarandol E , Ulukaya E, Tokullugil HA. Effects of smoking on Serum lipid and lipoproteinconcentrations and lecithin: cholesterolacyltransferase activity. J. Med. Invest. 46:169-172, (1999)
17. Nesje LA, Mjøs OD. Plasma HDL cholesterol and the subclasses HDL2 ISSN 0975-6299 Vol.1/Issue-4/Oct-Dec.2010 www.ijpbs.net Biochemistry B 113 and HDL3 in smokers and non-smokers .Artery. ; 13 (1):7-18, (1985)
18. Ezzati M, Henley SJ, Thun MJ, Lopez AD, . Role of Smoking in Global and Regional Cardiovascular Mortality. Circulation.; 112: 489-497, (2005).

Table-1: Age distribution of subjects studied

Age in years	Non-Smoking group		Smoking group	
	Number	%	Number	%
21-30	28	56.0	16	32.0
31-40	22	44.0	34	68.0
Total	50	100.0	50	100.0
Mean ± SD	29.14±5.91		33.80±5.15	

Table 2:- Comparison of lipid profile between smokers and non smokers

Lipid profile	Smokers n=50 Mean ±SD(mg/dl)	Non – smokers n=50 Mean ±SD(mg/dl)	P value
Total cholesterol[TC]	220±40.3	162±29.1	0.005
Triglycerides[TG]	182.4±41.1	109.1±25.4	0.005
LDL	142.3±32.4	101.4±20.2	0.005
VLDL	22.7±5.6	21.1±5.4	0.98
HDL	38.8±3.9	45.7±5.8	0.005

Results are presented in Mean ± SD, P value obtained by student t test.

Table 3:- lipid profile among smokers based on number of cigarettes smoked per day

Lipid profile	10 cigarettes per day n=23 Mean ±SD(mg/dl)	>10 cigarettes per day n=27 Mean ±SD(mg/dl)	P value
Total cholesterol	191±30	259±32	0.005
Triglycerides	164±30	183±50	0.1
LDL	102±21	166±22	0.001
VLDL	24±18	26±16	0.17
HDL	38±7	35±4	0.1

Results are presented in Mean ± SD, P value obtained by student t test.

Table 4:- lipid profile among smokers based on duration of smoking.

Lipid profile	Duration of smoking <5yrs [n= 18] Mean ±SD(mg/dl)	Duration of smoking >5yrs [n=32] Mean ±SD(mg/dl)	P value
Total cholesterol	197±24	243±51	<0.001
Triglycerides	169±40	185±48	<0.01
LDL	112±26	161±24	<0.05
VLDL	25±13	28±13	0.132
HDL	39.7±2	33.5±3	<0.05

Results are presented in Mean ± SD, P value obtained by student t test.

PREVELANCE OF ANTI-TPO ANTIBODY IN TYPE-1 DIABETES AND THYROID DYSFUNCTION IN TPO ANTIBODY POSITIVE DIABETICS.

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ABSTRACT: BACKGROUND: The appearance of TPO-Abs precedes thyroid dysfunction and increases in autoimmune diseases like type1diabetes.Thyroid peroxidase (TPO) antibodies are one of the major secondary antibodies associated with autoimmune thyroid disease and can be used as diagnostic marker. The prevalence of thyroid auto antibodies is increased when patients have non-thyroid autoimmune diseases such as type 1 diabetes and pernicious anemia. Thyroid dysfunction is common among diabetic patients and can produce metabolic disturbances. Therefore, regularly screening diabetic patients allows early treatment. **OBJECTIVE:** The objective of our study is to measure TPO-Abs in young Type-1Diabeticindividuals and to find Thyroid abnormalities in TPO-Abs positive individuals. **MATERIALS AND METHODS:** This was a cross-sectional study conducted at a rural clinic in Tiruchirappalli, Tamil Nadu. 60 persons in the age group of 10 to 35 years were selected for this study. Fasting blood samples were collected from the study population and glucose, lipid profile, thyroid profile and TPO- Ab were estimated using standard kits by standard methods. **RESULTS**:16 persons showed high levels of anti TPO-Abs(> 40 IU).In the anti TPO-Ab Positive group, all values were statistically significant according to the Pearson R formula $P < 0.001$. There was significant correlation between age and anti TPO-Ab level, between weight, BMI and TPO Positive and Negative levels, as per the T-Test $P < 0.001$.56.30% of anti TPO-Ab Positive subjects had high TSH. **CONCLUSION:** Our results indicate that thyroid dysfunctionis common in Type-1 diabetes but more in anti TPO-Ab positive subjects. Hence all Type-1 diabetic individuals should undergo annual screening of serum anti TPO-Ab and TSH measurement in anti TPO-Ab positive individuals.
KEY WORDS: Type-1 diabetes, lipid profile, thyroid profile, TSH and TPO-Ab

INTRODUCTION: There are a number of auto antibodies associated with the autoimmune thyroid diseases, which are characterized as either primary or secondary antibodies. Primary antibodies are directly pathogenic and often directed against cell membrane receptors, whilst secondary antibodies do not appear to be involved in pathogenesis but can serve as useful diagnostic markers for the presence of autoimmune thyroid disease. Thyroid peroxidase (TPO) antibodies are one of the major secondary antibodies associated with autoimmune thyroid disease.

TPO was previously known as thyroid microsomal antigen (1). It is a 107 KD enzyme which is involved in thyroid hormone synthesis. TPO is located both on the cell surface and within the

cytoplasm of thyroid acinar cells, bound to the vesicle which transports newly synthesized thyroglobulin, where it is involved in the iodination of thyroglobulin. High affinity antibodies (predominantly IgG) directed against TPO is found at elevated levels in the serum of patients with autoimmune thyroid disease such as Graves's disease, Hashimoto's thyroiditis, Diabetes mellitus and myxedema. Autoimmune thyroid disease (AITD) causes cellular damage and alters thyroid gland function by humoral and cell-mediated mechanisms (1).

Cellular damage occurs when sensitized T-lymphocytes and/or auto antibodies bindto thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking auto antibodies on cell membrane receptors. Three principal thyroid auto antigens are involved in AITD. These are thyroperoxidase (TPO), thyroglobulin (Tg) and the TSH receptor (1). Other auto antigens, such as the Sodium iodide Symporter have also been described, but as yet have no diagnostic role in thyroid autoimmunity.

CLINICAL SIGNIFICANCE OF THYROID AUTOANTIBODIES: TPO antibodies (TPO-Abs) appear to be involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's and atrophic thyroiditis(2). The appearance of TPO-Abs usually precedes the development of thyroid dysfunction. Some studies suggest that TPO-Abs may be cytotoxic to the thyroid. TPO-Ab and/or Tg-Ab are frequently present in the sera of patients with AITD.

The prevalence of thyroid auto antibodies is increased when patients have non-thyroid autoimmune diseases such as type 1diabetes and pernicious anemia. Ageing is also associated with the appearance of thyroid auto antibodies and increased prevalence of AITD.

THYROID DISEASE AND DIABETES: Thyroid disease is widespread and prevalence increases with advancing age. However, as assessing thyroid function is reliable and inexpensive, certain high risk groups such as neonates, the elderly and diabetics – should undergo regular screening.

Diabetes mellitus (DM) is a multisystem disease and is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin. In type 1 diabetes, insulin is functionally absent because of the destruction of the beta cells of the pancreas. Type 1 DM occurs most commonly in juveniles but can occur in adults, especially in those in their late 30s and early 40s.Diabetes mellitus is thought to be, in some cases, an auto-immune disease caused when antibodies attack certain cells of the pancreas, affecting the production of insulin. In patients or families where auto-immune thyroid disease exists, this type of diabetes mellitus may develop, sometimes in younger members of the family. This should be suspected particularly if symptoms of tiredness and weight loss develop together with increased thirst and passing of large volumes of urine. Clinically, thyroid dysfunction may undermine diabetes control. For example, hyperthyroidism may worsen glycemic control and increase insulin requirements. Indeed, thyrotoxicosis may unmask subclinical diabetes. While hypothyroidism markedly alters carbohydrate metabolism, such changes are rarely clinically significant. However, as less insulin is degraded, the exogenous insulin requirement may be lower. Moreover, hypothyroidism often produces dyslipidemias, including elevated triglyceride and low- density lipoprotein (LDL) cholesterol concentrations. Therefore, hypothyroidism can exacerbate coexisting dyslipidemias in type 2 diabetes. Thyroxin reverses these lipid abnormalities. But diagnosing thyroid dysfunction can be difficult. For example, poor glycemic control produces symptoms similar to hyperthyroidism, such as weight loss despite increased appetite as well as fatigue. Clinicians need to be careful not to confuse severe diabetic

nephropathy and hypothyroidism, both producing edema, fatigue, pallor and weight gains. Finally, poorly controlled diabetes may alter thyroid function(3).

Against this background, the serum TSH immunoassay offers the most reliable and sensitive screening test for thyroid dysfunction. However, screening for anti-thyroid peroxidase (TPO) antibodies in people with type 1 diabetes may predict autoimmune thyroid disorders.

Management is generally similar to that in the non-diabetic population. However, L-thyroxin therapy may exacerbate angina by increasing myocardial contractility and heart rate. Clinicians should consider treating subclinical hypothyroidism if patients either have elevated serum LDL cholesterol exacerbated by hypothyroidism or detectable serum anti-TPO antibodies.

Thyroid dysfunction is common among diabetic patients and can produce metabolic disturbances. Therefore, regularly screening diabetic patients allows early treatment(4). Type 1 patients expressing anti-TPO antibodies should be screened annually(5). In anti-TPO negative patients, a TSH assay every two to three years suffices. Among patients suffering from type 2 diabetes, clinicians should consider a TSH at diagnosis and then at least every five years.

OBJECTIVE OF THE STUDY:

1. To detect and measure TPO-Ab in young Type-1 DM individuals.
2. To find out the Thyroid abnormalities in TPO-Ab positive individuals in IDDM – using Thyroid function tests.

MATERIALS AND METHODS: This was a cross-sectional study conducted at a rural clinic in Tiruchirappalli, Tamil Nadu. 60 persons in the age group of 10 to 35 years were selected for this study. Subjects with any anti-thyroid treatment (or) hormone (or) lipid lowering drugs were excluded from the study. All the participants were enquired by a questionnaire about their complaints and physically examined to rule out features suggesting any hypo (or) hyper thyroid status.

Fasting (10-12 hrs of fasting) blood specimen was collected from each participant and analyzed for the following parameters. Plasma fasting glucose level was estimated by GOD/POD method in auto analyzer. Serum tri-iodothyronine, serum thyroxin and thyroid stimulating hormone were estimated by enzyme immunoassay method. Serum total cholesterol, serum triglycerides were estimated by enzymatic methods and serum high density lipoprotein cholesterol biphosphotungstate method using standard kits in auto-analyzer in the fasting serum sample. Serum low density lipoprotein cholesterol was estimated using Friedwald's formula. Estimation of TPO antibodies by accubind ELISA micro wells, a sequential ELISA method. Normal TPO-Ab level is below Forty (40) IU / ml

RESULTS: In our study 60 individuals withType-1 DM in the age group of 10 to 35 years were included. Based on the level of anti TPO antibody, the biochemical parameters T3, T4, TSH and lipid profile were tabulated, statistically analyzed and evaluated. In this study 16 persons showed high levels of anti TPO- Ab greater than 40 IU. (Table-1/ chart-1).

In anti TPO-Ab Positive Individuals, T3 levels were low in 5 persons (31.3%) (Chart 2).T4 levels were low in 6 persons (37.5%) (Chart 3) and TSH levels high in 9 persons (56.3%) (Chart 4) (Table- 2).

The value of high TSH was statistically significant according to the Pearson R formula P < 0.001.There was no correlation between duration of IDDM and anti TPO Ab level (Table - 3).There was no statistical correlations between high anti TPO Ab titer and Lipid profile (Table-4).TPO Ab titer was positively correlated with TSH and negatively correlated with T3 and T4 significantly (Table-5).

Table-3 shows overall correlation between TPO, Age, Duration of the Disease, Blood – Sugar level and BMI. There was no significant correlation between Blood-Sugar level and TPO level. Prevalence of TPO-Ab with 95% CI is 23 to 30. In our study that was 26.7 %.

DISCUSSION: Auto immune mechanisms are involved in many cases of Type-1 DM (6). The role of screening for thyroid levels in Type-1 DM is controversial. Substantial prevalence of thyroid abnormalities were noted in patients with Type-1 diabetes mellitus (7).Hypothyroidism is more common in females than males (2). TPO positive patients are more prone to develop thyroid dysfunction (8). This statement correlates with our study. Thyroperoxidase being responsible for iodination of tyrosine moieties is essential for active thyroid hormone T4 and T3 synthesis. When inhibited by anti TPO Abs, the active T4, T3 synthesis decreases, resulting in low T4, T3 level and there is a compensatory increase in TSH level, which with increased anti TPO titer and duration leads to deterioration of thyroid function from sub clinical dysfunction to fully manifest clinical hypothyroidism.

Our results indicate that all Type-1 diabetic individuals with positive TPO-Ab should undergo annual screening of serum TSH measurement to detect asymptomatic thyroid dysfunction. In Type-1 diabetes 21.6 % has high level of antibodies to TPO and TG. Thyroid autoimmunity was more common in girls and this predominance was observed in all age groups. In a study to detect sub-clinically associated AITD, 22 % of patients showed thyropathy with the assessment of thyroid auto antibodies and TSH.

The screening for auto antibodies in Type-1 diabetic patients will reveal sub-clinical cases of AITD. The sub-clinical thyroid dysfunction has no influence on diabetic control. There is a need for regular follow up of patients with positive auto antibodies to detect further deterioration of other organs(9).

Type-1 Diabetic patients with thyroid abnormalities have shown an increase in thyroid volume ultrasonographically (10). The expression of involvement of the thyroid in an auto immune disorder is not limited to the islet cells.

The use of high sensitive immunometric methods in clinical laboratories to assay anti thyroid anti bodies had expanded in recent years. The agreement of qualitative results is close to 97% for anti-TPO (11). In individuals with Type-1 DM, the measurements of anti TPO-Abs and TSH are the most efficient and cost effective combination of screening tests in the early detection of AITD (5 and 10).

The positive predictive value of anti TPO-Abs and TSH is 90%. In our study there was increased level of anti TPO-Abs and increased level of TSH and low T4 and T3 level indicating sub clinical hypo thyroidism (12, 13 and 17).

In a three years follow up study of Type-1 DM the prevalence of thyroid dysfunction increased from 5 to 8 %. The prevalence of TPO-Abs is unchanged. All Type-1 DM patients with increased TSH level has ultra sound abnormalities(14) while ultra sound abnormalities were not always associated with increase in TSH level. Thyroid ultrasound abnormalities were a sensitive but nonspecific marker of auto immune thyroid diseases. This shows that it is unsuitable for screening purposes.

So we recommend regular annual screening of serum TSH in the follow up of young Type-1 DM (15 and 16). Sub clinical phase of thyroid dysfunction in Type-1 diabetes due to auto antibody positivity did not affect control of diabetes while fully manifested hyper, hypothyroidism impair diabetes control. So there is no influence on diabetic control(9).In our study fasting blood glucose level in cases with positive TPO–Abs did not reveal statistical significance which is similar to previous studies.

CONCLUSION: Type-I DM being a chronic auto immune disease is associated with different auto-antibodies to thyroid, viz anti TPO-Abs, anti TG- Abs and anti-thyroid antibodies. The Anti TPO- Abs is the autoimmune parameter measured in Type-1 DM patients. In reference 16, the authors proposed the following screening protocol Thyroid auto – antibodies should be measured at diagnosis of Type-1 DM and should be repeated if TSH level exceeded the reference range and free T4 and TSH level should be measured at diagnosis and annually thereafter.

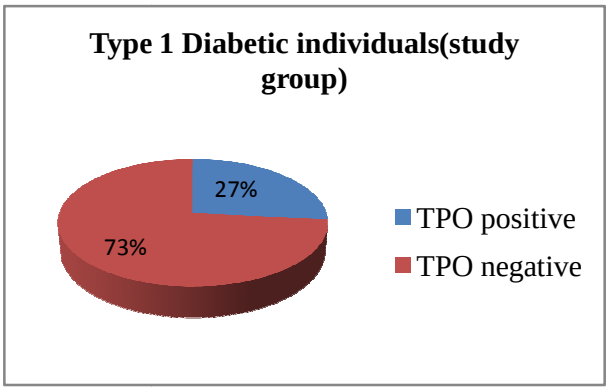
While the other studies suggest , Type-1 DM young patients with anti TPO Abs develop clinical thyroid disease with a mean latent interval of 10 years from the onset of Type-1 DM, during which sub clinical thyroid dysfunction occurs and it should be suffice to measure TSH level annually in anti TPO positive Type-1 DM patients with which we concur.

REFERENCE:

1. Manorama Swain, Truptirekha Swain, Binoy Kumar Mohanty. Autoimmune Thyroid Disorders - An Update. Indian Journal of Clinical Biochemistry. 2005; 20 (1):9-17
2. SeyedMahmoodGhoraishian, SeyedHosseinHekmatiMoghaddam and Mohammad Afkhami. Relationship Between Anti-Thyroid Peroxidase Antibody and Thyroid Function Tests.W J Med Sci2006 Jan-Jun; 1(1) : 44-47
3. Moayeri H and Rabbani A.Prevalence of autoantibodies to thyroid Peroxidase and autoimmune thyroid disease in Type I diabetes mellitus.ActaMedicaIranica. 2004;42(4): 267-271.
4. Riley WJ, Maclaren NK, Lezotte DC, Spillar RP, Rosenbloom AL. Thyroid autoimmunity in insulin-dependent diabetes mellitus: the case for routine screening. J Pediatr . 1981;99:350–354.
5. Kordonouri O, Klinghammer A, Lang EB, Grüters-Kieslich A, Grabert M, Holl RW. Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. Diabetes Care 2002Aug; 25(8):1346–1350.
6. Shimura.H, Kobayashi.T, Autoimmune Endocrine Diseases. JMAJ. 2004 Sep;47(9): 419–424.
7. Guillermo E. Umpierrez, Md,Kashif A. Latif, Md,Mary Beth Murphy, Rn, Ms, Cde, Mba, Helen C. Lambeth, Rn, Frankie Stentz, Phd, Andrew Bush, Phd, Abbas E. Kitabchi, Phd, Md Thyroid Dysfunction in Patients With Type 1 Diabetes. *Diabetes Care* 26:1181–1185, 2003
8. Bjoro T, Holmen J, Kruger O*et al*. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur. J. Endocrinol. 2000; 143: 639-647.
9. Prázný M, Škrha J, Límanová Z, Vaníčková Z, HilgertováJ, Prázná J et al. Screening for Associated Autoimmunity in Type 1 Diabetes Mellitus With Respect To Diabetes Control.Physiol. Res. 2005; 54: 41-48.

10. BianchiG, MontanariP, FabbriA, Gamberini A, Zoli M,MarchesiniG. Thyroid volume in type 1 diabetes patients without overt thyroid disease.ActaDiabetologica. 1995March; 32(1): 49-52.
11. Tozzoli R,Bizzaro N, Tonutti E, PradellaM,Manoni F,Vilalta D et al. Immunoassay of Anti-Thyroid Autoantibodies: Clinical Chemistry and Laboratory Medicine 2002 Jun;40(6):568-573.
12. Bilimoria KY, Pescovitz OH, DiMeglio LA. Autoimmune thyroid dysfunction in children with type 1 diabetes mellitus: screening guidelines based on a retrospective analysis. PediatrEndocrinolMetab. 2003 Oct-Nov; 16(8):1111-1117.
13. Sarah J. Glastras, Maria E. Craig, Charles F. Verge,Albert K. Chan, Janine M.Cusumano,
14. Kim C. Donaghue. The Role of Autoimmunity at Diagnosis of Type 1 Diabetes in the
15. Development of Thyroid and Celiac Disease and Microvascular Complications.*Diabetes Care.* 2005September; 28(9):2170-2175
16. HansenD, BennedbaekFN, Hoier-MadsenM, HegedusL, JacobsenBB. A prospective study of thyroid function, morphology and autoimmunity in young patients with type 1 diabetes. European Journal of Endocrinology. 2003; 148(2): 245-251
17. Lorini R, d'Annunzio G, Vitali L, Scaramuzza A. IDDM and autoimmune thyroid disease in the pediatric age group. J PediatrEndocrinolMetab. 1996 Mar; 9(1):89-94.
18. Kordonouri O, Hartmann R, DeissD, WilmsM,Grüters-KieslichA.Natural course of autoimmune thyroiditis in type 1 diabetes: association with gender, age, diabetes duration, and puberty. Archives of Disease in Childhood. 2005;90:411-414
19. De Graaff L.C.G. , Martín-Martorell P, Baan J, Ballieux B, Smit J.W.A., Radder J.K. Long-term follow-up of organ-specific antibodies and related organ dysfunction in type 1 diabetes mellitus. Netherlands The Journal of Medicine. 2011 Feb; 69(2) : 66-71.

Chart-1



	Frequency	Percent
Negative	44	73.3
Positive	16	26.7
Total	60	100.0

Table 1 Percentage of TPO-AbposItive& negative type I diabetics

Table-2 TPO-Ab and thyroid function tests(T3, T4 and TSH)

	TPO-Ab negative			TPO-Ab positive		
	Low	Normal	High	Low	Normal	High
T3 count percent	 3 6.8%	 41 93.2%		 5 31.3%	 11 68.8%	
T4 count percent	 3 6.8%	 41 93.2%		 6 37.5%	 10 62.5%	
TSH count percent		 42 95.5%	 2 4.5%	 1 6.3%	 6 37.5%	 9 56.3%

Chart-2

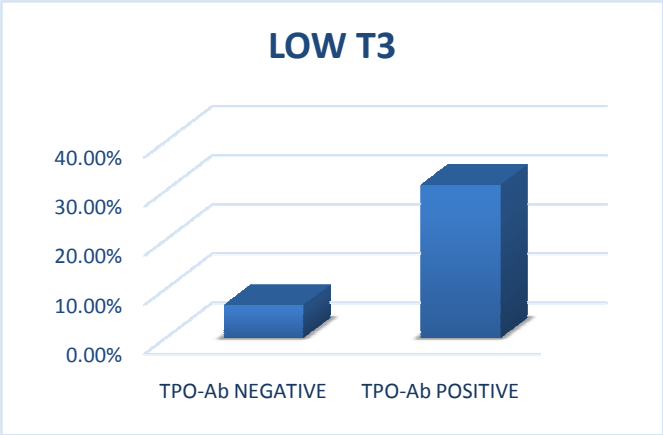


Chart-3

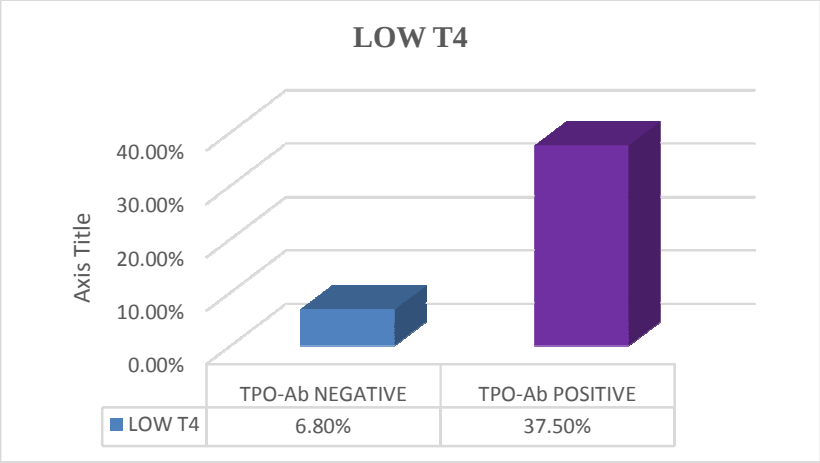
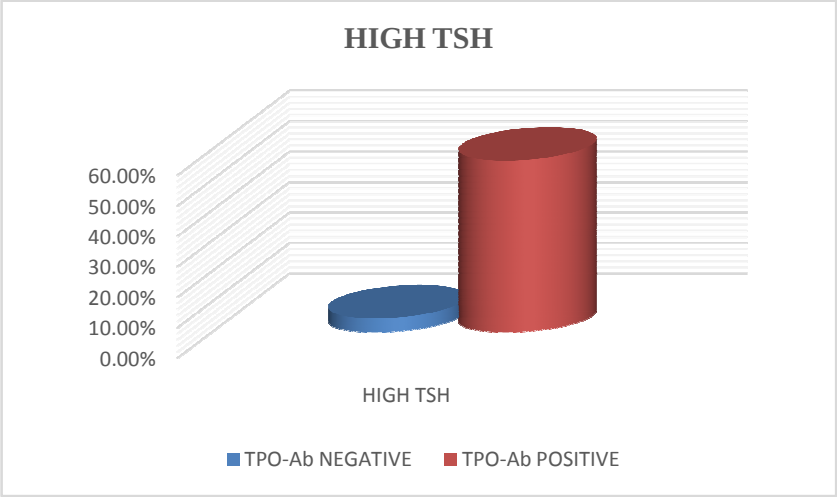


Chart-4



Correlations

		TPO	AGE	DURATION	BLD_SUG	BMI	ATHRO_IN
TPO	Pearson Correlation	1	.172	.029	-.076	.598**	.179
	Sig. (2-tailed)	.	.189	.823	.566	.000	.172
	N	60	60	60	60	60	60
AGE	Pearson Correlation	.172	1	.354**	.167	.407**	.046
	Sig. (2-tailed)	.189	.	.005	.201	.001	.729
	N	60	60	60	60	60	60
DURATION	Pearson Correlation	.029	.354**	1	.038	.170	-.210
	Sig. (2-tailed)	.823	.005	.	.772	.193	.108
	N	60	60	60	60	60	60
BLD_SUG	Pearson Correlation	-.076	.167	.038	1	-.018	-.105
	Sig. (2-tailed)	.566	.201	.772	.	.889	.425
	N	60	60	60	60	60	60
BMI	Pearson Correlation	.598**	.407**	.170	-.018	1	.274*
	Sig. (2-tailed)	.000	.001	.193	.889	.	.034
	N	60	60	60	60	60	60
ATHRO_IN	Pearson Correlation	.179	.046	-.210	-.105	.274*	1
	Sig. (2-tailed)	.172	.729	.108	.425	.034	.
	N	60	60	60	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table-4

Correlations

		TPO	TGL	HDL	LDL	VLDL
TPO	Pearson Correlation	1	.032	.043	.057	-.041
	Sig. (2-tailed)	.	.810	.743	.668	.756
	N	60	60	60	60	60
TGL	Pearson Correlation	.032	1	.115	.030	.573**
	Sig. (2-tailed)	.810	.	.380	.821	.000
	N	60	60	60	60	60
HDL	Pearson Correlation	.043	.115	1	.717**	.053
	Sig. (2-tailed)	.743	.380	.	.000	.688
	N	60	60	60	60	60
LDL	Pearson Correlation	.057	.030	.717**	1	-.159
	Sig. (2-tailed)	.668	.821	.000	.	.224
	N	60	60	60	60	60
VLDL	Pearson Correlation	-.041	.573**	.053	-.159	1
	Sig. (2-tailed)	.756	.000	.688	.224	.
	N	60	60	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

Table-5

Correlations

		TPO	T3	T4	TSH	CHOLESTR
TPO	Pearson Correlation	1	-.296*	-.403**	.580**	.043
	Sig. (2-tailed)	.	.022	.001	.000	.746
	N	60	60	60	60	60
T3	Pearson Correlation	-.296*	1	.457**	-.585**	-.257*
	Sig. (2-tailed)	.022	.	.000	.000	.048
	N	60	60	60	60	60
T4	Pearson Correlation	-.403**	.457**	1	-.768**	-.480**
	Sig. (2-tailed)	.001	.000	.	.000	.000
	N	60	60	60	60	60
TSH	Pearson Correlation	.580**	-.585**	-.768**	1	.368**
	Sig. (2-tailed)	.000	.000	.000	.	.004
	N	60	60	60	60	60
CHOLESTR	Pearson Correlation	.043	-.257*	-.480**	.368**	1
	Sig. (2-tailed)	.746	.048	.000	.004	.
	N	60	60	60	60	60

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

THE PREVALENCE OF CHIKUNGUNYA ARBOVIRAL INFECTION IN AND AROUND BELLARY DISTRICT, KARNATAKA.

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ABSTRACT: BACKGROUND: An arbovirus is one that multiplies in a blood sucking arthropod and is transmitted by the bite to a vertebrate host. Chikungunya fever is a crippling disease caused by an arbovirus transmitted to human through mosquitoes. The sudden onset of very high fever along with rash and severe arthralgia are main symptoms. High morbidity with severe arthralgia persisted for several months made the people both physically and mentally weak. **OBJECTIVES:** To know the prevalence of chikungunya arboviral infection in and around Bellary district. **MATERIAL AND METHODS:** The laboratory records of clinically suspected chikungunya patients from January 2009 to December 2011 analyzed retrospectively and results of Ig M anti chikungunya antibodies tested by Ig M capture enzyme linked immunosorbant assay (Mac ELISA). **RESULTS AND CONCLUSION:** A total of 1386 chikungunya suspected serum samples were analyzed, out of which 343 (24.75%) samples were fond positive for chikungunya virus infection. Maximum number of positive cases was seen in 2010 (28.40%). The present study emphasizes the continuous sero- epidemiological surveillance for the effective chikungunya arboviral infection control programme.

KEY WORDS: Chikungunya and Ig M antibody capture ELISA

INTRODUCTION: The arboviruses are transmitted by blood sucking arthropods from one vertebrate host to another. The vector acquires a lifelong infection through the ingestion of blood from a viremic vertebrate host. The viruses multiply in the tissues of the arthropod without evidence of disease or damage. Some arboviruses are maintained in nature by transovarian transmission in arthropods [Figure No: 1]. The major arboviral diseases distributed worldwide are yellow fever, dengue , Japanese B encephalitis, chikungunya , St. Louis encephalitis, western equine encephalitis, eastern equine encephalitis, Russian spring summer encephalitis, westnile fever and sand fly fever[1]. Chikungunya virus (CHIK V) is a RNA virus belonging to family Togaviridae, genus Alphavirus. The disease caused by bite of Aedes aegypti mosquito (Man-Mosquito-Man). The incubation period one to seven days, characterized by abrupt onset of fever, severe arthralgia and disease is almost self limiting. The literal

meaning of chikungunya is "that which bends up" that stooped posture developed due to arthritic symptoms of the disease [2]. The disease is generally non-fatal and the acute phase resolves within three to four days leaving the arthralgic syndrome persisting for some more time. The serological studies have repeatedly demonstrated the presence of antibodies in human [2,3]. Incubation period one to seven days, CHIK V produces disease about 48 hours after mosquito bite. Viraemia declines within three to four days [2,3]. Neutralizing antibodies can usually be detected after five days fading viraemia[2]. All age groups were affected including newborns. Recovery from the disease varies by age and longer for elders. Younger patients recover within 5-15days and middle agers recover in one to three months [2]. Chikungunya fever is an acute illness characterized by a sudden onset of high fever, rash and joint pain. The most significant symptom of CHIK V related disease consists of a painful arthralgia that occurs in almost 100% of patients. Most infections completely resolve within weeks but there are reported cases of CHIK V induced arthralgia lasting for months or even for years in the form of recurrent or persistent episodes [2,4].The clinically suspected cases screened using immunochromatography test kits (Rapid tests) and followed by ELISA detection of Ig M antibodies and confirmed by virus isolation and virus nucleic acid in sera by RT-PCR [5] The vector-borne disease and mosquitoes breeding sites are playing an important role in the transmission and propagation of chikungunya. The present study briefly describes the disease in general, laboratory diagnosis and the prevalence of chikungunya in and around Bellary.

MATERIAL AND METHODS: The study was conducted at a tertiary care Hospital from January 2009 to December 2011. A total of 1386 serum samples from suspected chikungunya cases included in our study. Aseptic precautions, two to five ml of blood samples were collected by venipuncture for chikungunya suspected cases. The samples transported to the Microbiology laboratory in vaccine carriers with duly filled requisition forms. The serum was separated by centrifugation of the whole blood sample and labeled with the particulars of the patient and stored in the refrigerator at -20^{o}C [6]. The test kits used chikungunya Ig M antibody capture ELISA supplied by Group leader, Arbovirus Diagnostics, National Institute of Virology, Pune, India. The tests performed strictly as per the manufacturers' instructions.

RESULTS: During the three years of study period, 1386 Chikungunya suspected serum samples were analyzed, out of these 343 (24.75%) samples were positive for Chikungunya virus infection [Table No: 1]. The prevalence of Chikungunya is high in 2010 (28.40%) [Table No: 2]. Male to Female ratio of fever diagnosis in suspected cases is 0.98 [Table No: 3] and majority of cases belong to age group more than 15 years [Table No: 4].

DISCUSSION: The 2005-2006 epidemics in Indian Ocean island was the most devastating and had very complicated clinical manifestations associated with encephalopathy and hemorrhagic fever [7]. Arthralgia persisted for months and years with excruciating pain in joints and ankles [7,8]. The most affected were the aged adults and suffering from diabetes, alcoholic hepatopathy and impaired renal functions [7,9]. In India, Ahmedabad city, Gujarat and Kerala states experienced large scale out breaks with morbidity and extensive incapacitation [7]. The Kerala state had worst epidemic as the infection run through 2006 to 2008 affecting the whole state. Unique complications such as swollen limbs with painful arthralgia which persisted for long periods were witnessed among the patients [7]. Karnataka state also more affected during 2006 outbreak, 27 Districts of the state reported over (54.74%) suspected cases. Several districts of

the state such as Bellary, Gulbarga, Tumkur, Bidar, Raichur, Dharwad, Chitradurga, Davangere, Kolar and Bijapur have reported more number of chikungunya cases during epidemic outbreak [10]. More than 64% of the entire suspected cases in reported in India during 2008 [10]. We have noticed that chikungunya outbreak in and around Bellary. The number of confirmed cases are more in 2010 (28.40%) than in 2009 (23.07%) and subsequently decreased in 2011(19.05%) [Table No: 2]. All three years statistics revealed that majority of the cases belong to age group of 15 years and above [Table No: 4]. The serological study indicated that arthropod-borne virus Chikungunya was prevalent in and around Bellary district, although the prevalence differed according to age, sex, geographic location and the individual virus. The geographical distribution had a significant influence on the prevalence of antibodies to the virus. This might be explained by the possible impact of ecological characteristics of the areas on the natural cycles of the arthropod-borne viruses under consideration [11]. Chikungunya disease recently emerged as an important public health problem in India. The epidemic occurs in the Indian Ocean islands during 2006[12]. More than 64% of the entire suspected cases reported in India during 2008 [13]. We have noticed that Chikungunya outbreak in and around Bellary district. The number of confirmed cases were more in 2010 (28.40%) than in 2009 (23.07%) and subsequently decreased in 2011 (19.05%).

CONCLUSION: The arboviral infections mainly dengue, chikungunya and Japanese B Encephalitis are most common in tropical and subtropical regions. Until the recent epidemic, CHIK V did not receive much attention due to low mortality, infrequent occurrence and absence in the developed countries. The serological results (Ig M antibody capture ELISA) clearly establish the etiology.

KEY MESSAGE: Prevention is better than cure.

REFERENCES:

1. Jawetz, Melnick, Adelberg, Arthropod borne and Rodent borne viral diseases In: Medical Microbiology, Chapter 38, 23rd edition, Singapore, The Mc Graw Hill Companies; 2004:514.
2. Chhabra M, Mittal V, Bhattacharya D, Rana UVS, Lal S, Chikungunya fever: A re-emerging viral infection, Indian Journal of Medical Microbiology, 2008; 26(1): 5-12.
3. Srikanth P, Sarangan G, Mallilankaraman, Nayar SA, Barani R, Mattew T, et al, Molecular Characterization of Chikungunya virus during an outbreak in South India, Indian Journal of Medical Microbiology, 2010; 28(4): 299-302.
4. Cavrini F, Gaibani P, Pierro AM, Rossini G, Landini MP, Sambri V, Chikungunya: an emerging and spreading arthropod-borne viral disease, Journal of Infection in Developing Countries, 2009;3(10): 744-752.
5. Alyson Kelvin A, Outbreak of Chikungunya in the Republic of Congo and the Global picture, Journal of Infection in Developing Countries, 2011; 5(6): 441-444.
6. Anuradha SK, SurekhaYA, Sathyanarayan MS, Suresh S, Krishna S, Satish SP, Mariraj J, Ravikumar R , Japanese Encephalitis virus; common cause of viral encephalitis in paediatric age group in Bellary, Karnataka, India, Journal of Clinical and Diagnostic Research, 2011; 5(3): 480-482.
7. Sudeep AB, Parashar D, Chikungunya: an overview, Journal of Bioscience, 2008; 33(4): 443-449.

8. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M, Chikungunya : an epidemic arboviruses, Lancet Infectious Diseases, 2007; 7:319-327.
9. Couderc T, Chretien F, Schilte C, Disson O, Brigitte M, Guivel BF, Touret Y, Barau G, et al, A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease, PLoS Pathogens, 2008; 4:29.
10. Talawar AS, Pujar HS, An outbreak of Chikungunya epidemic in South India-Karnataka, International Journal of Research and Reviews in Applied Sciences, 2010; 5(3): 229-234.
11. Padbidri VS, Wairagkar NS, Joshi GD, Umarani UB, Risbud AR, Gaikwad DL, Bedekar SS, Divekar AD, Rodrigues FM, A serological survey of Arboviral diseases among the Human population of the Andaman and Nicobar islands, India, Southeast Asian Journal of Tropical Medicine and Public Health, 2002; 33 (4): 749-800.
12. Ukey PM, Bondade SA, Paunipagar PV, Powar RM, Akulwar SL, Study of seroprevalence of dengue fever in Central India, Indian Journal of Community Medicine, 2010; 35: 517-519.
13. Kalantri SP, Joshi R, Riley LW, Chikungunya epidemic: An Indian Perspective, National Medical Journal of India, 2006; 19: 315-322.

Table No: 1 Distribution of suspected and confirmed Chikungunya cases

Suspected Cases	Confirmed Cases	Percentage
1386	343	24.75

Table No: 2 Distribution of Chikungunya suspected and confirmed cases according to year

Year	Suspected Cases	Confirmed Cases	Percentage
2009	802	185	23.07
2010	500	142	28.40
2011	84	16	19.05
Total	1386	343	70.52

Table No: 3 Distribution of clinically suspected cases according to gender

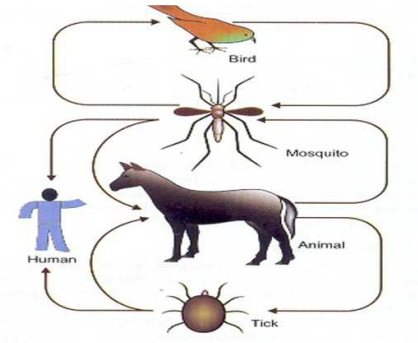
Gender	Suspected Cases
Male	689
Female	697
Total	1386

Table No: 4 Distribution of clinically suspected cases according to age

Age in Years	Suspected Cases
Less than 5	12
5 to 10	38
10 to 15	261
More than 15	1075
Total	1386

Figure No: 1

Arbovirus life cycle



CO-INCIDENCE OF MICROFILARIA & TOXOPLASMA IN LYMPH NODE -A RARE DIAGNOSIS BY FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)

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ABSTRACT: Toxoplasma lymphadenitis is a rare disease entity, microfilaria also rarely found in lymph node aspirate but coincidence of toxoplasma gondii and microfilaria in lymph node is very rare. We report here one such case of a four year old child presented with cervical lymphadenopathy, fine needle aspiration cytology showed microfilaria and bradyzoides of toxoplasma gondii, granuloma in a background of reactive lymphoid cells. On serology, serum IgM anti toxoplasma antibody was positive and IgG was negative.
KEY WORDS: Fine needle aspiration cytology (FNAC), Lymph node, Toxoplasma, Microfilaria.

INTRODUCTION: Filariasis is a major public health problem and it is endemic all over India. [1, 2] About 120 million people infected in this region and in need of treatment including 40 million people with overt disease. [3] Despite its high incidence, it is infrequent to find microfilaria in FNAC smears and only incidentally detected in various sites such as lymph node, breast lump, bone marrow, bronchial aspirate, pleural and pericardial fluid, ovarian cyst fluid in clinically unsuspected cases of filariasis with absence of microfilaria in the peripheral blood. [4] Microfilaria has been observed as a coincidental finding with other infective, inflammatory conditions and neoplastic lesions. We present here a very rare case in which microfilaria and Toxoplasma gondi (bradyzoides) together were observed in the fine needle aspirates of cervical lymph node. No such case report has been documented till date.

CASE REPORT:A 4-year-old male child belongs to a Nepali tribal community of low socioeconomic condition in Dooars region, presented with multiple enlarged right cervical lymph nodes since two months with flu like symptoms. On examination, the lymph nodes were at the right posterior triangle of neck, mildly tender, firm; together measuring 3.5cm × 3.2cm. Axillary and inguinal lymph nodes were not palpable. He had no visual or neurological symptom. FNAC of the lymph nodes were done. Aspirate material were whitish granular and microscopic examination of the air dried Leishman stained and alcohol fixed Hematoxylin & Eosin (H&E) stained smears showed small cluster of epithelioid histiocytes without necrosis associated with sheathed microfilaria of Wuchereria bancrofti and cyst of Toxoplasma gondii (bradyzoides) in a background of heterogeneous population of reactive lymphoid cells [Figure 1 & 2]. Aggregates of activated large ovoid lymphoid cells also seen in the background [Figure 3].

Peripheral blood smear were prepared from blood sample taken for three consecutive nights showed no microfilaria but eosinophilia was noted (12%). In urine examination, there was no evidence of chyluria and microfilaria in the centrifuged deposit. Serological study by Enzyme Linked Immunosorbent Assay (ELISA) for serum IgM anti toxoplasma antibody was positive but IgG was negative. Patient was non-reactive for HIV-1 & 2. All the test results were negative in patient's mother.

DISCUSSION: Microfilaria is transmitted by the Culex mosquito and is caused by two closely related nematodes, Wuchereria bancrofti and Brugia malayi. About 95% of cases lymphatic filariasis is caused by infection with Wuchereria bancrofti. [1] Infective larvae penetrate through the skin wound, enter the lymphatics and travel to the lymph nodes. Adult worms are thread like creamy white. Females are viviparous, once fertilized they discharge as many as 50,000 microfilaria per day. [5] The sheathed microfilaria of Wuchereria bancrofti having multiple, coarse, discrete nuclei extending from the head to the tail, except in the small terminal portion of the caudal end. Thus species are identified by seeing the larval form. [2] Microfilaria displays a nocturnal periodicity; this is basically a biological adaptation to the nocturnal biting habit of vector mosquitoes. That is why at least three consecutive night blood sample were examined. The clinical manifestations varies, may be asymptomatic, or may have acute or chronic manifestations e.g. lymphangitis, lymphadenitis, lymphedema, and elephantiasis. [1]

Toxoplasmosis is cosmopolitan zoonotic infection caused by a single-celled obligate intracellular parasite called Toxoplasma gondii. It exists in three form e.g. oocysts, tachyzoites and bradyzoides (tissue cysts) and transmitted by eating vegetables or drinking water contaminated with the oocysts commonly passed by cats or from ingestion of mutton, pork, chicken etc. Early maternal infection (first and second trimester) results in spontaneous abortion and intrauterine foetal death (IUFD).

Toxoplasma is an infection which is highly variable in its clinical manifestation, these include myocarditis, encephalomyelitis, chorioretinitis especially in the immunocompromised host and the healthy host who harbour the infection usually remains asymptomatic.

Superficial enlarged lymph nodes are the most common presenting sign of acquired toxoplasmosis. [6] Usually involves the posterior cervical lymph nodes. [7] In cytology smear, some lymphoid cells with relatively large, ovoid, pale nuclei may be seen. These cells probably correspond to the pale monocytoid B-cells in histological sections. Microcysts and organisms of Toxoplasma gondii are rarely seen. Diagnosis is usually done by identifying tissue cysts and serological tests. [6], [8],[9], [10]

Here in our case also, right posterior cervical lymph nodes were involved and the smears showed loose aggregates of activated large ovoid lymphoid cells [Figure 3].

The objective of the study is to document the value FNAC in the diagnosis of filariasis and toxoplasmosis in lymph node and thus demonstration and identification of these parasites in cytological smears played a significant role in the prompt recognition of the disease and initiation of specific treatment, thus avoiding the more severe manifestations of lymphatic filariasis and toxoplasmosis. Secondly to highlight such a rare coincidence of two infection in lymph node.

CONCLUSION: Although microfilaria and toxoplasma cysts in cytological smears are considered incidental findings, this case illustrates the value of FNAC in the detection of asymptomatic and clinically unsuspected cases of filariasis & toxoplasmosis. Absence of microfilaria in the

peripheral blood does not exclude filarial infection. Filariasis may be detected in a clinically unsuspected case, especially in an endemic zone. The spectrum of host response may vary from no reaction to a marked inflammatory response. [1] The entire spectrum of changes should be kept in mind while practicing cytopathology in an endemic area. In such situations, a strong clinical suspicion and meticulous screening of cytology smears may lead to a correct diagnosis. At the same time, the cytopathologist should search for a coexisting pathology thoroughly. In the present case, we feel that the presence of microfilaria in association with Toxoplasma gondii is a very rare coincidental finding. The patient was harboring subclinical filariasis when he was suffering from toxoplasmosis. Microfilaria was not detected in the peripheral blood smears. This also highlights the importance of screening smears for parasites even in the absence of clinical symptoms, particularly in highly endemic areas.

REFERENCES:

1. Park K. Park's Textbook of Preventive and Social Medicine. 21st ed. Jabalpur, India: Banarasidas Bhanot publisher; 2011.Chapter 5; page: 244.
2. Chatterjee KD. Parasitology (Protozoology and Helminthology) 12th ed. Calcutta, India: Chatterjee Medical Publishers; 1980; 190–8.
3. WHO (2009), Weekly Epidemiological Record, No. 11/12, 13th March, 2009.
4. Varghese R, Raghuveer CV, Pai MR, Bansal R. Microfilariae in cytologic smears: A report of six cases. Acta Cytol 1996; 40 (2): 299-30.
5. Nelson GS. Filarial infections. *Med Int* 1981; **1**: 77–82.
6. Stansfelt. AG. Histological diagnosis of Toxoplasmic lymphadenitis. J. clin. Path 1961; 14: 565.
7. Leopold GK. Koss' Diagnostic Cytology and its Histopathologic Bases: 5th ed, Lippincott Williams & Wilkins Publisher; 2006. Vol-II, Chapter 31, Page: 1195.
8. Argyle JC, Schumann GB, Kjeldsberg CR, Anthens JW. Identification of a Toxoplasma cyst by fine needle aspiration. Am J Clin Pathol 1983; 80: 256-258.
9. Christ ML, Feltes-Kennedy M. Fine needle aspiration cytology of Toxoplasmic lymphadenitis. Acta Cytol 1982; 26: 425-428.
10. Jayaram N, Ramprasad AV, Chatan M, Sujay AR. Toxoplasma lymphadenitis. Analysis of cytologic and histologic criteria and correlation with serologic tests. Acta Cytol 1997; 41: 653-658.

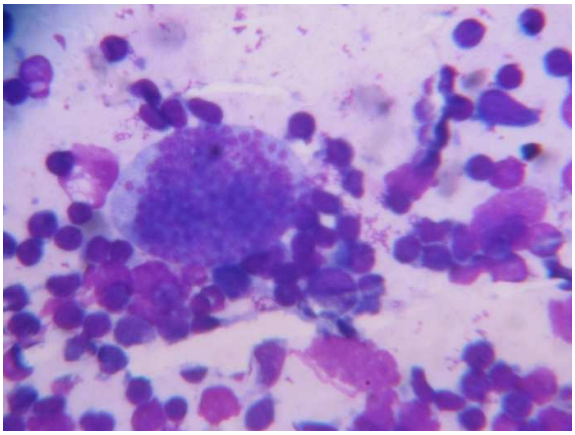

Figure 1: Tissue cyst of Toxoplasma gondii (Leishman's stain x 40)

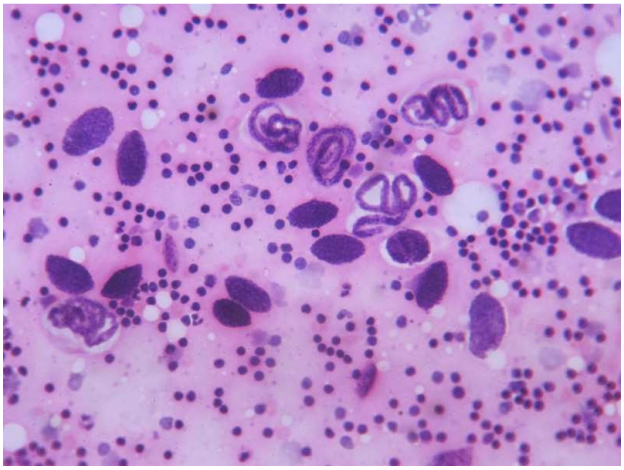

Figure 2: Tissue cysts Toxoplasma gondii & coiled microfilaria of Wuchereria bancrofti (H/E stain x10)

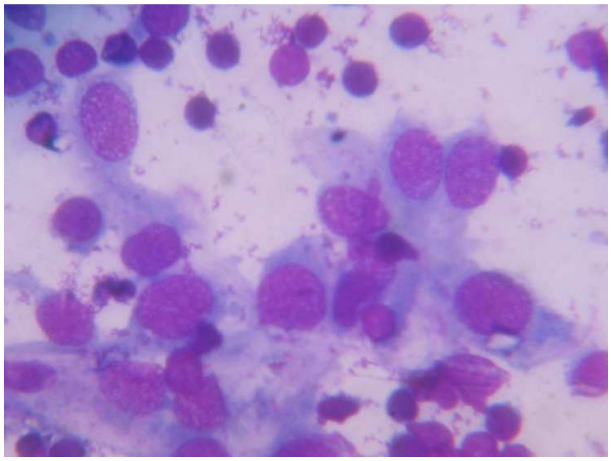

Figure 3: Activated large ovoid lymphoid cells (Leishman's stain x 100)

MULTIPLE LIVER ABSCESS BY MIXED BACTERIAL ETIOLOGY: AN UNUSUAL CASE REPORT

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ABSTRACT: Pyogenic liver abscess is a serious disease, which is potentially fatal if left untreated. In developed countries, pyogenic abscesses are the most common but worldwide, amoebae are the most common cause. We report a 60-year-old diabetic woman with a 2 months history of pain abdomen, 1 month history of high fever and anorexia who had multiple liver abscess caused by anaerobic Actinomyces species and Enterococcus faecalis and was complicated by peritonitis. Treatment included prompt percutaneous drainage coupled with long-term intravenous administration of Amikacin, Metronidazole and Piperacillin/ Tazobactum. The patient later died due to peritonitis.

KEY WORDS: Liver abscess, Enterococci, anaerobic Actinomyces

INTRODUCTION: Pyogenic liver abscess (PLA) is a serious infrequent disease caused by bacterial, parasitic or fungal organisms and is potentially fatal if left untreated. In previous years it was the disease of the young adults but recently the disease has evolved as disease of the elderly [1]. Liver abscess may be solitary or multiple; they may arise from hematogenous spread of bacteria or from local spread from contiguous sites of infection within the peritoneal cavity. In the past, appendicitis with rupture and subsequent spread of infection was the most common source for a liver abscess. Currently, associated disease of the biliary tract is most common. Pylephlebitis, usually arising from infection elsewhere in the peritoneal cavity is another common source for bacterial seeding of the liver [2].

CASE HISTORY: A 60 year old female diabetic patient came with the history of pain abdomen in right upper quadrant since 2 months, associated with on and off fever with chills, malaise and anorexia since 1 month. Patient gave past history of epigastric pain which was treated with antacids. On examination she had marked tenderness in the right upper quadrant with hepatomegaly of 4 cm below the right costal margin. Her temperature was 39^0C, pulse 96 beats/min and blood pressure 104/80 mm Hg. Laboratory data were as follows: hemoglobin 11.6 gm/dl; white blood cell count 16300/mm^3; platelet count 4.80 lakh/mm^3; PT 14 seconds; PCV 33%; MCV 79.7 fl; MCH 28 pg; MCHC 35.2 gm/dl; Red blood cell count 4.14 million/mm^3; Differential count: Polymorphs 90%, Lymphocytes 7%, Monocytes 3%, Basophils & Eosinophils 0%; Total Protein 5.8 gm/dl; Albumin 2.5 gm/dl; Globulin 3.3 gm/dl; A/G ratio 0.8; total bilirubin 0.7 mg/dl; bilirubin direct 0.0 mg/dl; bilirubin indirect 0.7 mg/dl; Alkaline phosphatase 114 IU/L; SGOT 19 IU/L; SGPT 18 IU/L; GGT 23 U/L; Glucose (Random) 161

mg/dl; PPBS 200 mg/dl; urine analysis was normal except for presence of 8 – 10 pus cell/ hpf and traces of albumin. The test for HIV and HBsAg was found to be negative.

Ultrasonography of the abdomen revealed that there was a well defined hypo-echoic lesion with internal debris in right lobe of liver with sub-capsular collection in the right lobe, suggesting the presence of abscess with sub-capsular extension. CT scan of the abdomen confirmed the presence of multiple irregular abscesses varying in size, predominantly in the right lobe of the liver. Gram stain of the aspirated pus showed the presence of gram positive filamentous bacilli which was broken into coccoid and bacillary forms, gram positive cocci in pairs and short chains and scanty of gram negative fusiform bacilli along with plenty of pus cells. Wet mount of pus did not reveal the presence of trophozoites of Entamoeba histolytica. The Ziehl- Neelsen stain did not show presence of acid fast bacilli. A definitive diagnosis of mixed infection was made when an anaerobic Actinomyces species and Enterococcus faecalis was cultured from aspirated pus by conventional method. The antibiotic sensitivity testing of Enterococcus faecalis was done by Kirby-Bauer disc diffusion method. The isolate was found to be sensitive to ampicillin, ampicillin/sulbactum, cotrimoxazole, piperacillin/tazobactum, vancomycin, ceftriaxone, amikacin, high level gentamicin (HLG) and teicoplanin and was resistant to ciprofloxacin and linezolid. The patient was treated by percutaneous drainage and with long-term intravenous administration of amikacin, metronidazole and piperacillin/tazobactam. Later patient developed peritonitis and succumbed to death.

DISCUSSION: Pyogenic liver abscess is a serious infrequent disease caused by bacterial, parasitic or fungal organisms and is potentially fatal if left untreated [1]. The microorganisms most often recovered from pyogenic liver abscess are Escherichia coli, anaerobic Streptococci, gram negative bacilli, Bacteroides fragilis, Staphylococcus aureus, Clostridium species etc., but the incidence varied considerably among previous reports. Pyogenic liver abscesses were more commonly seen in elderly patients and in patients with diabetes mellitus. The abscesses were mostly single and located more frequently in right lobe of the liver [1, 3]. Multiple liver abscesses are not frequently reported in the literature. The overall mortality rate of pyogenic liver abscess is 11%- 31% and the mortality rate is high in patients with multiple liver abscess. Patients with diabetes mellitus, immune deficiency, sickle cell anemia, malignancy and liver transplants are at greater risk for developing liver abscess and in the majority of the cases more than one organisms have been isolated. Without appropriate diagnosis and treatment, the pyogenic liver abscesses are almost uniformly fatal. Early diagnosis as well as treatment with appropriate antibiotics and selective drainage can subsequently reduce the mortality [4].

A high incidence of anaerobic microorganisms (15-46%) as the cause of liver abscess has been reported recently and because of this high incidence, antibiotics effective against anaerobes and aerobic microorganisms should be used in all patients [3, 5]. CT scan is probably the most useful aid in diagnosing and localizing hepatic abscesses [6]. Biliary tract disease is the etiology of the abscess in most cases, but sometimes the origin remains unidentified [7]. In our case the cause of multiple liver abscess is probably due to extension from a small perforation and extension of pus from healed peptic ulcer, as no other aetiology was found in the patient for the presence of liver abscess. Hepatic actinomycosis should also be considered in the differential diagnosis of liver abscesses and space-occupying lesions of the liver in immunocompetent patients [8]. The strain of Enterococcus faecalis isolated was resistant to linezolid, which is relatively rare and appears either due to previous treatment with linezolid or transmission from other patients [9]. The source of linezolid resistant enterococci in our case

could not be determined. Linezolid resistant Enterococcus faecalis is an emerging pathogen and hence it is important to minimize the emergence of resistance by using it only if the therapeutic indications exist and keeping the duration of treatment as short as possible. In summary, multiple liver abscess due to mixed infection with Enterococcus faecalis and an anaerobic Actinomyces species is a rare entity, but a life threatening disease. Hence such cases requires timely recognition using CT scan and guided aspiration to start an early and specific therapy to prevent mortality among high risk and elderly patients.

REFERENCES:

1. Galdfelter T, Brooks M. Liver abscess. JMSMA 1981;7:173-4.
2. Baron MJ, Kasper DL. Intraabdominal infections and abscesses. In: Longo DL, Fauci AS, Kasper DL, editors. Harrison's principles of internal medicine. 18th ed. USA: Mc Graw Hill; 2010. P. 1077-83.
3. Ergas D, Hai AA, Sthoeger Z, Menahem BH, Miller R. Multiple pyogenic liver abscesses following hemorrhoid banding. IMAJ 2007;9:753-4.
4. Bahloul M, Chaari A, Khlaf NB, Kallel H, Herguefi L, Chelly H, et al. Multiple pyogenic liver abscess. World J Gastroenterol 2006;12(18):2962-3.
5. Alvarez JA, Baldonedo RF, Bear IG, Jorge JL. Anaerobic liver abscesses as initial presentation of silent colonic cancer. HPB (Oxford) 2004;6(1):41-42.
6. Tzur T, Liberman S, Felzenstein I, Cohen R, Rivkind AI, Almogy G . Liver abscesses caused by Streptococcus milleri: An uncommon presenting sign of silent colonic cancer. IMAJ 2003;5:206-7.
7. Wang YJ, WenSC, Chien ST, King J, Hsuea CW, Feng NH. Liver abscess secondary to sigmoid diverticulitis. A case report. J Intern Med Taiwan 2005;16:289-94.
8. Tiftikci A, Vardareli EN, Kaban K, Peker O, Akansel S, Tozun N. Actinomycotic hepatic abscess. Hepatol Int 2008;2(1):133–5.
9. Burleson BS, Ritchie DJ, Micek ST, Dunne WM. Enterococcus faecalis resistant to linezolid: case series and review of the literature. Pharmacotherapy 2004;24(9):1225-31.

RECURRENT MARJOLIN'S ULCER WITH REGIONAL LYMPH NODE METASTASIS

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ABSTRACT: Marjolin's ulcer is a malignant tumour developing in a chronic skin lesion (burn scar, vaccination scar, non-healing wound etc.). The majority of cases reported are squamous cell carcinoma. Surgery remains the first treatment of choice (resection with 2cms. safety margin of healthy skin for primary squamous cell carcinoma Marjolin ulcers and 2.5cms. safety margin for recurrent cases). Recurrence after surgery and regional lymph node metastasis are not uncommon (17% & 30% respectively).We presents a case report and literature review of Recurrent Marjolin's Ulcer with regional Lymph Node Metastasis. Marjolin's ulcer should be considered as a significant post-burn complication; it should be treated with full emphasis on adequate local clearance and regular follow up for many years; if not treated adequately, it may lead to complicated recurrence.
KEY WORDS: Marjolin's Ulcer, Recurrent, Lymph Node Metastasis.

INTRODUCTION: Marjolin's ulcer is a malignant tumour developing in a chronic skin lesion (burn scar, vaccination scar, non-healing wound etc.).[1] The majority of cases reported are squamous cell carcinoma but other type of malignancy such as basal cell carcinoma, malignant melanoma, liposarcoma, osteosarcoma, and fibrosarcoma can also be seen although rare.[2] The incidence of burn scar undergoing malignant transformation has been reported to be 0.77-2 %.[3]

CASE REPORT: A 22 year old male was presented to our surgical department with exophytic ulcerated growth of size 7x7 cm. near angle of right scapula. He had a past history of burn over anterior chest wall, back and left arm 12 years ago that healed completely by conservative management. Approx. 11 years after initial insult, the patient developed an ulcer on the back over burn scar. It gradually increased in size to become exophytic growth of size 12x12cms. No regional lymph node enlargement was noted at that time. Whole of the ulcer with a 2 cm. margin of healthy skin was excised. The defect in skin was closed by partial thickness skin graft. Histopathological examination of the excised tissue was suggestive of poorly differentiated squamous cell carcinoma.

8 months after surgery & skin grafting, the patient came with exophytic ulcerated growth of size 7x7 cm. near angle of right scapula. It was fixed to underlying muscles of back.

Patient was also having another fungating growth in left axilla which was 6x6 cms. in size, fixed to underlying structures causing restriction of movements at shoulder. Serous discharge was present from axillary growth. There was no vascular or neurological impairment present in left upper limb. Biopsy from axillary growth was positive for squamous cell carcinoma.

LITERATURE REVIEW: The eponym "Marjolin's ulcer" was derived from French surgeon Jean Nicholas Marjolin in 1828 who observed and classified cellular change in burn skin and coined the term "ulcere cancroide".[4] The skin lesion underlying development is predominantly burn scar (75%), traumatic non healing wound (8%), venous stasis ulcer (6%), pressure ulcer (3%) and other e.g. Frost bite, vaccination scar .[1]

The latency period is inversely proportional to patient's age at the time of skin injury. In chronic Marjolin's ulcer it ranges from 20-40 year as transformation from non-healing wound to malignant disease is slow. The average time lag between the burns and subsequent malignant ulceration is 19 years. [5] In acute Marjolin's ulcer it occurs within few weeks to one year.[1] Average age at diagnosis of Marjolin's ulcer is in 5th decade of life with a range of 18-84 years.[2] It is seen more frequently in males as compare to females with ratio of 3:1.[2] Most commonly it is located on lower extremities (53%), upper extremities (18%), trunk (12%), face and nape (5%), scalp (9%). [4]

Aetiology of Marjolin's ulcer is not yet clear. Many hypotheses have been suggested. Slow healing and scar instability characterized by chronic irritation and the induction of constantly proliferating epidermal unit have been blamed. Repeated cycle of healing and breakdown, wound that never healed and application of irritant medication are all result in reduced ability to withstand carcinogens.[6] As a result of constant breakdown of ulcer, a nutritional deficiency develops, owing to release of toxins by autolysis and heterolysis of scar. This yields an epithelium that is unable to withstand the carcinogens produced by skin because of excessive heat and radiation. [7] Ultraviolet rays are also found to be associated with Marjolin's ulcer. On histological examination of sun damaged skin, dyskeratosis and vacuolated keratinocytes known as sun burn cell are seen. Marjolin's ulcer least frequently founds on trunk which is not frequently exposed to sunlight. [8] It has also been suggested that relatively avascular scar tissue act as immune privileged site that allows the tumour to resist body defences against foreign cell.[6]

Scar tissue acts as a barrier for the tumours, if we release this barrier, the virulent spread of the tumour will be permitted. [8] Regional lymph node metastasis and recurrence after surgery is not uncommon. Metastasis to regional lymph nodes is seen in 30% of cases and local recurrence occurs in 17% of patients. The median interval to recurrence after surgery is 15 months. Locoregional recurrence is more common in female patients and those with high-grade tumours. [9] Poorly differentiated squamous cell carcinomas have a tendency to spread to lymph nodes earlier. Squamous cell carcinomas resulting from the Marjolin's ulcer have a much greater tendency to metastasize than squamous cell carcinomas resulting from the other causes [10].

Various studies suggested that all chronic wound should be closed surgically either by skin graft or skin muscle flap. Large wound should not be left for secondary intention healing. Burn scar from childhood should be carefully monitored; biopsy of any suspected lesion should not be delayed. At present no standard treatment for Marjolin's ulcer is suggested. Surgery remains the first treatment of choice. Marjolin's ulcer should be excised with a 2 cm. margin of normal healthy tissue, which may necessitate amputation with lesion involving joint space or deep local extensive invasion. Although classically 2cm safety margin is still widely used for resection of primary squamous cell carcinoma Marjolin ulcers, 2.5cm safety margin is better for

resection in recurrent cases.[11] Axillary & inguinal lymph node dissection should be done if nodes are found to be positive. Radiotherapy and chemotherapy can be instituted on individual basis.[1, 2] Chemotherapeutic agents most commonly used are 5-fluorouracil, methotrexate, bleomycin, cisplatin.[8] Indication of radiotherapy include – tumour >10 cms. in size with positive regional lymph node (to be given after regional lymph node dissection), head & neck lesion with positive regional lymph node, patient with inoperable regional lymph node metastasis.[8]

Lifeso and Bull describe histological classification with include grade I (well differentiated), grade II (moderately differentiated), grade III (Poorly differentiated) tumours.[12] Tumours which have latent period of >5 years, situated over trunk and lower limb, infiltrative form, poorly differentiated and presence of regional lymph node metastasis have worse prognosis.[4] Stage and grade of the tumor, presence of metastases and presence of local recurrence are the main predictors of death.[13]

CONCLUSION: Marjolin's ulcer should be considered as a significant post-burn complication; [5] it should be treated with full emphasis on adequate local clearance and regular follow up for many years; if not treated adequately, it may lead to complicated recurrence.

REFERENCES:

1. Wojciech MW, Andrzej LK, et al. Two Different Cases of Marjolin's Ulcer and Recommendations for Practice. The Open Surgical Oncology Journal, 2010; 2: 83-85.
2. Interesting Case Series, Chronic Ulcer. Correspondence: williamaboumd@hotmail.com William Abouhassan, MD, Johns Hopkins Burn Center, Johns Hopkins University, Baltimore, Md. http://www.eplasty.com/images/PDF/eplasty-d-10-00007.pdf Date of access 01/10/2012.
3. Copcu, Eray. Marjolin's Ulcer: A Preventable Complication of Burns? Plastic & Reconstructive Surgery, July2009; 124(1): 156e-164e.
4. Tomasz Wojewoda, Wojciech M. Wysocki, Jerzy Mituś. Marjolin's ulcer – case report and literature review. Polish Journal of Surgery 2009; 81(9): 414–418.
5. E. Copcu, A. Aktas, N. Şişman, Y. Oztan. Thirty-one cases of Marjolin's ulcer Clinical and Experimental Dermatology March 2003; 28 (2): 138–141.
6. Ifeanyi IO, Bismark Okwor, Wilson IBO. Penetrating scalp Marjolin's ulcer involving bone and dura mater in a Nigerian hospital: Case report and literature review. Burns (2009), doi:10.1016/j.burns.2009.04.010.
7. Malheiro E., Pinto A., et al. Marjolin's Ulcer of the scalp: case report and literature review. Annals of Burns and Fire Disasters - vol. XIV - n. 1 - March 2001.
8. Eser Aydoğdu Serkan Yildirim, Tayfun Aköz. Is surgery an effective and adequate treatment in advanced Marjolin's ulcer? Burns June 2005; 31(4): 421-43.
9. Dr A. Eroğlu, S. Çamlibel. Risk factors for locoregional recurrence of scar carcinoma British Journal of Surgery 1997; 84(12): 1744–6.
10. Ozek Cuneyt, Celik Naci, et al. Marjolin's Ulcer of the Scalp: Report of 5 Cases and Review of the Literature. Journal of Burn Care & Rehabilitation Jan./Feb. 2001; 22(1): 65-69.

11. Abdolazim Ghalambor. Marjolin ulcer: How much of safety margin needs resection along marjolin ulcer squamous cell carcinoma in recurrence cases Pak J Med Sci May-June 2007; 23(3): 394-397.
12. Lifeso RM, Bull CA. Squamous cell carcinoma of the extremities. Cancer. 1985; 55:2862-2867.
13. Phillipo L Chalya, Joseph B Mabula1 et al. Marjolin's ulcers at a university teaching hospital in Northwestern Tanzania: a retrospective review of 56 cases. World Journal of Surgical Oncology 2012; 10:38.

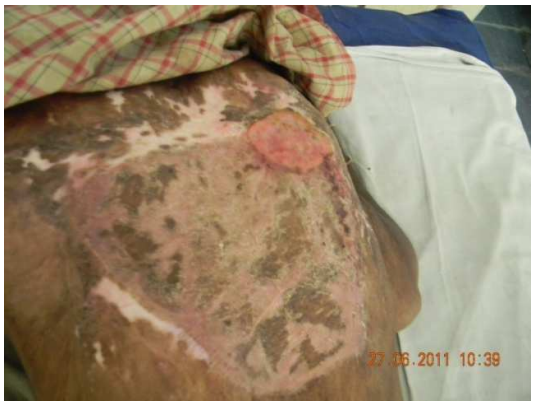

Figure 1: Marjolin's Ulcer arising in a Burn Scar

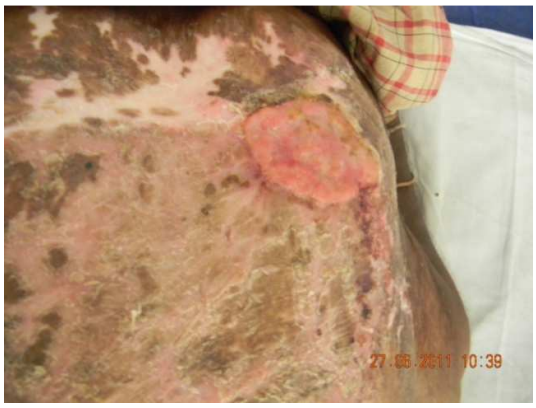

Figure 2: Exophytic Marjolin's Ulcer

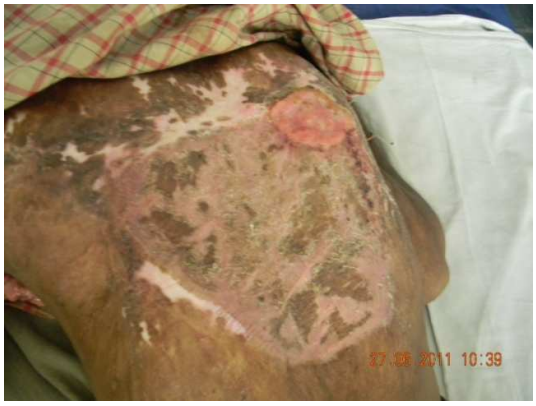

Figure 3 : Marjolin's Ulcer on back

Figure 4: Regional Lymph Node Metastasis in case of Recurrent Marjolin's Ulcer on back

KLIPPEL TRENAUNAY SYNDROME: REPORT OF A RARE, MILD FORM OF SYNDROME

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ABSTRACT: Klippel Trenaunay Syndrome (KTS) is a congenital vascular disease characterized by malformations of capillary, venous and lymphatic vessels with bony and soft tissue hypertrophy. KTS is a sporadic disease with unknown etiology, and there is no predilection for gender or any particular ethnicity. The disease appears more frequently at birth, childhood or adolescence. It generally affects only one extremity and lesions are present at birth or appear by the age of 12 years. Clinical presentation of this syndrome is protean ranging from minimal asymptomatic disease to life threatening bleeding and embolism. Management of this syndrome includes careful diagnosis, prevention and treatment of complications. We report a case of KTS in a 25 year old female who presented with mild form of disease.
KEYWORDS: Soft tissue hypertrophy, vascular malformation, congenital disease.

INTRODUCTION: Klippel Trenaunay is a rare, congenital disorder characterized by a triad of capillary vascular malformation, venous malformation and soft tissue or bone hypertrophy.[1-7] It usually affects lower extremity[6] and lesions are present since birth in approximately 90% of patients presenting before 12 years of age.[6,8] KTS has a wide spectrum of presentation, from truncular to extratruncular, from infiltrating to limited forms, containing primarily three anomalous vascular elements: veins, capillaries and lymphatics.[7] However patients present with protean manifestations.[2] We report a case of KTS in a 25 year old female who presented with mild form of disease.

CASE REPORT: A 25 year old married female presented to dermatology department of our hospital with multiple red colored lesions on the left lower extremity since birth which were increasing in size with age. She developed difficulty in walking at the age of 6 years. There was no history of similar symptoms in other family members. On examination, she had multiple, erythematous, hyper pigmented plaques on lateral side of left lower extremity with largest plaque of 10x8 cm (Figure 1a).The patient also difficulty in extension of the limb with presence of hypertrophy and varicose veins (Figure 1b). Hematological investigations showed: Hemoglobin of 10.2 gm%, WBC of 10,000/mm^3, Platelets of 2,10,000/mm^3 and microcytic hypochromic anemia on peripheral smear examination. The vascular anomalies of internal organs were ruled out using abdominal ultrasonography, Doppler studies and CT scan of

abdomen and pelvic region. The histopathological examination of the skin biopsy showed ectatic blood vessels in upper dermis with stasis of erythrocytes (Figure 2). The histopathological diagnosis of nevus flammeus was offered and considering the clinical features, the case was diagnosed as Klippel Trenaunay Syndrome (mild form).

DISCUSSION: Klippel-Trenaunay Syndrome was first described by two French doctors, Klippel and Trenaunay in 1900.[6,7] The incidence and genetic predisposition has not been proved despite various case reports in world literature.[7] KTS occurs sporadically, and shows no particular racial, sexual or geographical predilection. It affects skin, veins, lymphatic system, bone and soft tissues of an extremity. Clinical presentation of patients with KTS has a wide spectrum from incomplete, mild form of port wine stains and few varicose veins causing only cosmetic deformity to severe disability associated with massive limb overgrowths, chronic pain syndrome, skin infections, arthritis, thrombo- embolism and life-threatening pelvic or recurrent rectal bleeding from venous malformations.[7] Capillary malformation is usually a port wine stain or nevus flammeus. The port-wine stain is usually red to purple in color and is flat. It usually has an irregular margin and clear, sharp border but rarely crosses the midline. It may or may not blanch on pressure. It usually occurs on the ipsilateral side as the affected limb.[9] Varicosities are extensive, atypically very large, take an erratic course and begin to manifest in early childhood.[9] Varicose veins is the characteristic feature affecting the lower limbs which was present in our patient.[2] Deep vein anomalies like venous hypoplasia to frank aneurysm, valve hypoplasia to avalvulia have been described with lymphatic malformations.[7] The bony abnormalities may affect all bones in an extremity or limited to one or two bones. Single limb involvement is found in 80-85% patients. Apart from hypertrophy or sometimes hypotrophy of bones other deformities includes macrodactyly, syndactyly, split hand deformity, phalangeal agenesis and dislocation of hip joint.[7] At least two of three main symptoms (port-wine stains, varicosity, and hypertrophy of soft tissues and bones) must be present for the diagnosis Klippel-Trenaunay syndrome to be accepted.[2] Association with arteriovenous malformation is called as Klippel Trenaunay Weber syndrome.[6] Various authors have described other systemic involvements in patients of KTS. Central nervous system abnormalities may include microcephaly, macrocephaly, cerebral arteriovenous malformations and orbito- frontal varices.[10] Patients may present with neurological symptoms due to compression of spinal cord by hemangiomas. Gastrointestinal bleeding and genitourinary lesions like hematuria and vascular malformations of scrotum, penis, vulva, vagina and bladder may also occur.[10]

Several theories have been proposed which include (1) Servelle's theory of a primary obstruction of the venous system resulting in venous hypertension and therefore development of abnormal venous pathways and tissue overgrowth; (2) failure of regression of the lateral limb bud vein; and (3) alteration of the tight balance between angiogenesis and vasculogenesis, which is controlled by numerous genes, among other theories.[7] Most recent being mutation in angiogenic factor VG5Q leading to vascular abnormalities.[3]

The management of KTS has been largely conservative with compression therapy as mainstay of treatment.[7] Conservative measures for varicose veins like compression stockings, alleviating pain by painkillers, antibiotics for infections, anticoagulant therapy for thrombophlebitis, laser for ulcers and port wine stains is indicated. Surgery is indicated for cosmetic reasons or for complications of venous insufficiency and for bone or soft tissue overgrowth. The conservative management is sufficient in mild form of KTS but in female patients of reproductive age group, KTS increases obstetric risk and can exacerbate

complications mainly thromboembolism and hemorrhagic. Probably risk is 10 times higher than in the normal population.[1,2] The other factors which exacerbate this risk are oral contraceptives, surgery and pregnancy.[1,2,4,11]

So these patients have to be managed with multidisciplinary approach and must be counseled for: a) Use of contraceptive measures other than oral contraceptive pills b) Pregnancy related complications such as deep vein thrombosis, thromboembolism, and coagulopathy c) Surgery for cosmetic appearance of the leg and d) Risk of abnormalities in the fetus.
The multidisciplinary management with long term, regular follow up is mandatory in female patients with Klippel-Trenaunay Syndrome.

CONCLUSIONS: Klippel Trenaunay syndrome is a rare condition with protean manifestations which should be extensively investigated for the reason of being associated with various systemic manifestations and these patients have to be managed accordingly. The clinicians need to be aware of mild form of KTS which can be managed conservatively and has better prognosis. KTS occurring in female patients of reproductive age group has different implications and has to be managed by a multidisciplinary team of obstetrician, physician and surgeon.

REFERENCES:

1. González-Mesa E, Blasco M, Andérica J, Herrera J. Klippel-Trenaunay syndrome complicating pregnancy. *BMJ Case Rep* doi: 10.1136/bcr-2012-006534.
2. Gundogan TG, Jacquemyn Y. Klippel - Trenaunay syndrome and pregnancy. Obstetr & Gynaecol Int doi: 10, 1155/ogt-2010706850.
3. Tian XL, Kadaba R, You SA, Liu M, Timur AA, and Yang L, et al.: Identification of an angiogenic factor that when mutated causes susceptibility to Klippel-Trenaunay syndrome. Nature. 2004; 427:640-5.
4. Martin JR, Pels SG, Paidas M, Seli E. Assisted reproduction in a patient with Klippel-Trenaunay syndrome: management of thrombophilia and consumptive coagulopathy J Assist Reprod Genet 2011; 28:217–9.
5. Tanaka R, Fujita Y, Hiasa KI, Yumoto Y, Hidaka N, Fukushima K. Wake N. Successful management of pregnancy complicated by Klippel Trenaunay syndrome using MR angiography based evaluation. Case Rep in Obstetr and Gynaecol doi: 0.1155/2011/723467.
6. Leon Cdo A, Braun Filho LR, Ferrari MD, Guidolin BL, Maffessoni BJ. Klippel-Trenaunay syndrome: case report. An Bras Dermatol. 2010; 85:93-6.
7. Zea MI, Hanif M, Habib M, Ansari A. Klippel-Trenaunay Syndrome: a case report with brief review of literature. J Dermatol Case Rep 2009; 3:56-9. doi: 10.3315/jdcr.2009.1038
8. Verhelst H, Van Coster R. Neuroradiologic findings in a young patient characteristic of Sturge Weber syndrome and Klippel Trenaunay Syndrome. J Child Neurol 2005; 20:911-13.
9. Abdul-Rahman NR, Mohammad KF, Ibrahim S. Gigantism of the lower limb in Klippel-Trenaunay syndrome: anatomy of the lateral marginal vein. Singapore Med J 2009; 50: e223-e225.
10. Chhajed M, Pandit S, Dhawan N, Jain A. Klippel- Trenaunay and Sturge- Weber overlap syndrome with phakomatosis pigmentovascularis. J Pediatr Neurosci 2010; 5:138-40.

11. Billingham R. Klippel Trenaunay Weber syndrome- a case report. J Lymphoedema 2010; 5:99-103.

Figure 1a: Multiple, erythematous, hyperpigmented plaques on left lower extremity with soft tissue hypertrophy.

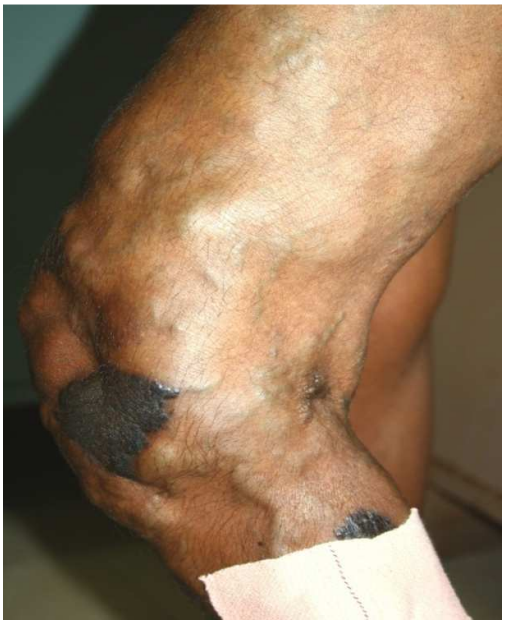

Figure 1b: Multiple tortuous varicosities on the limb.

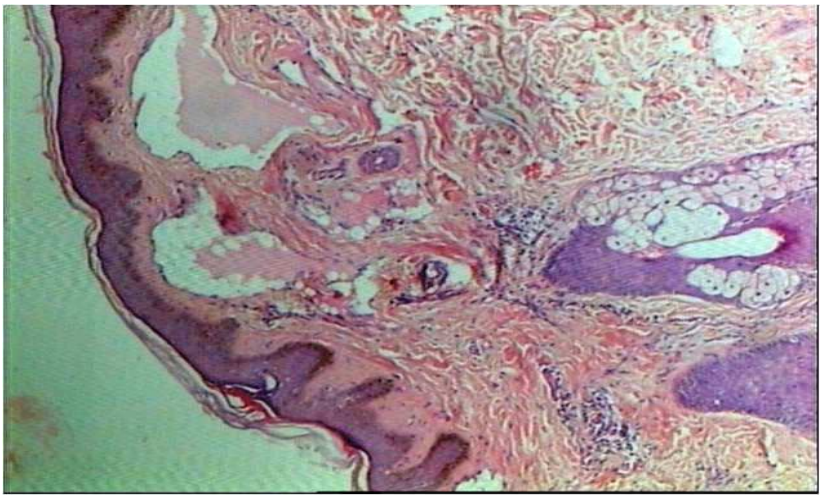

Figure 2: Nevus flammeus showing ectatic blood vessels in upper dermis with erythrocyte stasis.

CASE REPORT: UNUSAL FOREIGN BODY IN SUBMANDIBULAR SPACE

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ABSTRACT: A rare case report of submandibular space infection secondary to a vegetative foreign body (stalk of wheat crop) which penetrated the floor of mouth to reach the submandibular space and resulted in the formation of abscess. The abscess was drained and the stalk of wheat measuring 2.5 cms in length, removed in toto.
KEYWORDS : foreign body, submandibular space, Wharton's duct

INTRODUCTION : Lodgement of a vegetative foreign body in Submandibular space is a rare case. In chronic and advanced inflammatory process due to foreign body in the gland itself or in the Wharton's duct, submandibular gland excision is mandatory. This case is reported as submandibular abscess due to lodgement of a stalk of wheat crop, in the submandibular space penetrating into the submandibular gland.

The pathology was attributed to the entry of the straw of wheat crop into the mouth with subsequent migration of the stalk into the submandibular space through the floor of mouth as a result of masticatory movements.

CASE REPORT: A 40 years old female, farmer by occupation , presented with the complaints of a swelling in the left submandibular region of neck of 15 days duration with pain in the swelling for last 10 days. She gave history of entry of a stalk of wheat crop into the mouth 20 days back while she was cutting the crops. Patient tried to manually remove the stalk with her finger and possibly pushed it deeper, as a result of which the stalk penetrated the floor of mouth and reached the submandibular space. There was no history of increase in the size of swelling during meals.

On local examination, there was a firm, tender, immobile swelling of size 5 X 4 cms in the left submandibular region. The swelling had smooth surface, diffuse margins, raised temperature.

On examination of oral cavity, there was slight edema and congestion in floor of mouth. No ulcer or entry point seen.

Other relevant ENT examination was normal.

FNAC of the swelling was suggestive of an abscess.

The Ultrasonography report showed a hyperechoic foreign body in the left submandibular space penetrating the submandibular gland which was surrounded by oedema and abscess.

Patient was started on intravenous antibiotics and anti inflammatory drugs. The swelling subsided in 4 days.

Then the patient was planned for surgical removal of the foreign body under general anaesthesia. A fragment of vegetable crop of length about 2.5 cms was found embedded within the submandibular space penetrating the submandibular gland surrounded by abscess.
The abscess was drained and the foreign body was removed.

Post-operative antibiotics and anti inflammatory drugs were given for 7 days. Post-operative period was uneventful and patient was discharged on 7th day.

DISCUSSION:

Submandibular space

- The submandibular space is bounded above by mucous membrane of the floor of mouth and tongue and below by the deep cervical fascia that extends from the hyoid to the mandible. It is divided into two by the mylohyoid muscles. The space superior to mylohyoid is called the sublingual space and contains the sublingual gland, the space inferior to muscle is the submaxillary space and contains the submandibular gland.
- Sublingual space communicates with the submaxillary space through the posterior margin of the mylohyoid muscle, around which pus can easily travel.
- Common causes of infection in the submandibular space are oral trauma, dental abscess of mandibular teeth, submandibular or sublingual sialadenitis. Odontogenic infection in adults is the most common cause among these, and foreign bodies being one of the rarest.[1-3] Prior to this, only a single case of deep neck space infection secondary to foreign body in submandibular space has been reported in literature.[3] The passage of the foreign body, in this location, can cause local pain. So, surgical removal of the foreign body of the submandibular space is the treatment left because the spontaneous expulsion is not possible due to its anatomy. For proper removal of foreign body, preoperative localization is very important. CT scan is unreliable in detecting unpainted wooden foreign bodies.[4] In our case, USG proved very useful.
- In the case of foreign body which cannot be accurately localised or is situated posterioly, a traditional submandibular approach through horizontal cervical skin incision is a preferable choice as it is simple and safe method for removal of foreign body and complications can be prevented.[5] In our case there was no complication, except painful swelling of the neck and trismus.

  Initial care of patients with a submandibular abscess includes broad spectrum intravenous antibiotics to cover for both aerobic and anaerobic organisms. Other treatment modalities for submandibular abscess include needle aspiration and drainage. Open surgical drainage is mandatory in the cases of abscess formation with suspected foreign body.

REFERENCES:

1) Maran AG, Mackenzie IJ, Murray JA. The parapharyngeal space. J Laryngol Otol 1984; 98:371-380. 6325567
2) Abemayor E, Lufkin R. Enhancing accesss to the parapharyngeal space. Laryngoscope 2002; 112:757-759. 12150536

3) Coales UF, Tandon P, Hinton AF. Limitations of imaging for foreign bodies in parapharyngeal abscess and the importance of surgical exploration. J Laryngol Otol 1999; 113:683-685. 10605573
4) Limitations of imaging for foreign bodies in parapharyngeal abscess and the importance of surgical exploration. J Laryngol Otol 1999; 113:683-685. 10605573
5) Kalsotra PK, Thakar A, Kumar R. Foreign body in the Wharton's duct. Ind J Otolaryngol Head Neck Surg, 1995; 47(4): 297-98.

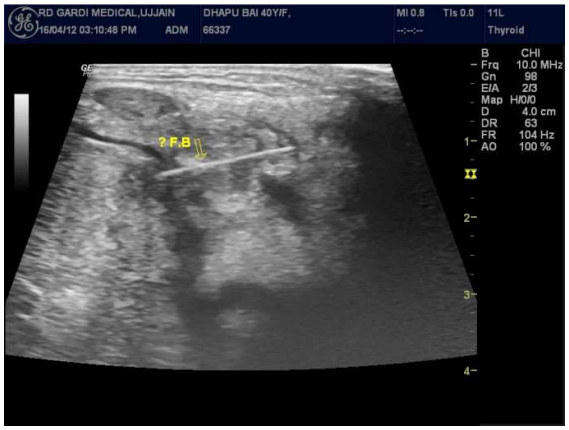

Figure 1 : USG showing FB in submandibular space penetrating the submandibular gland

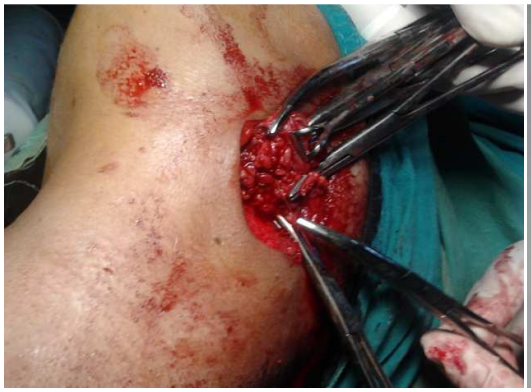

Figure 2 : showing F.B. wheat stalk in left submandibular space

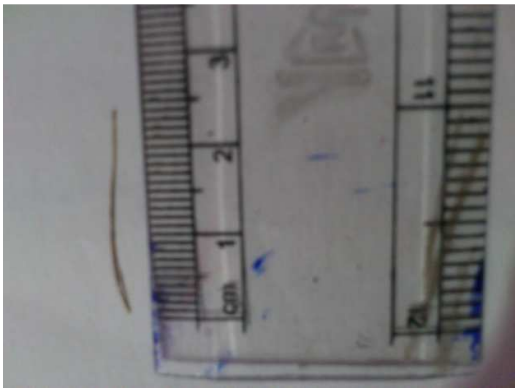

Figure 3 : showing wheat stalk removed from the left

ANAESTHETIC MANAGEMENT OF BILATERAL PHEOCHROMOCYTOMA

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ABSTRACT: A 27 year old female patient with bilateral pheochromocytoma presented with hypertension. Confirmation of diagnosis was done by CT scan and raised 24 hour urinary vanillylmandelic acid levels. Preoperative BP was controlled with prazosin, labetolol, indapamide and clonidine. The anaesthetic technique used was general anaesthesia with epidural analgesia.

KEY WORDS : Pheochromocytoma, hypertension, anaesthetic management

INTRODUCTION: Pheochromocytoma is a catecholamine secreting tumour of chromaffin tissue which can produce excessive amounts of catecholamines. These tumours are most often found in adrenal glands but can also occur in chromaffin cells in or about sympathetic ganglia. These tumours are not common, occurring in 0.1% of hypertensive population only.[1] Pheochromocytoma typically occurs in a patient who is 30-50 years of age.[2] A "rule of 10" has been applied to these tumours- 10% are bilateral, 10% are malignant and 10% are extra-adrenal.[1,5]

CASE REPORT: A 27 year old, 50 kg, female patient presented with confirmed diagnosis of bilateral adrenal pheochromocytoma on CT scan for removal of tumour. On pre-operative check up, her BP was 240/126 mmHg. 24 hour urinary vanillylmandelic acid level was 47.70mg. Plain X-ray KUB showed inferior displacement of right kidney. On echocardiography, LVEF was 60% at rest with normal global and segmental wall motion. There were occasional premature atrial contractions on E.C.G. Serum cortisol level was 26.09 µg/dL. FBS was 98.0 mg/dL. Other investigations like haemoglobin, renal profile, serum electrolytes, serum calcium, thyroid profile, liver function tests and P.T.I. were normal. Her Blood pressure (BP) was controlled with prazosin 10mg OD, labetolol 100mg BD, indapamide 1.25mg OD, clonazepam 1mg OD and clonidine 250µg OD. She was taken up for surgery when her BP was 140/90mmHg with no signs of postural hypotension. Her haematocrit was 31.7%.

Anaesthetic management

Morning dose of oral antihypertensives and clonazepam were given. Injection butorphanol 1mg I.V. was given as premedication. Preanaesthetic BP was 140/86 mmHg and heart rate was 74/min. IV line was maintained with 18G cannula. Monitors were attached for continuous monitoring of NIBP, IBP, CVP, ECG, SpO_2 and temperature. Radial artery cannulation

and right subclavian central line insertion was done. Initial CVP was 8 cm H_2O. 18G epidural catheter was inserted in L2/3 inter-vertebral space and after a test dose of 3ml injection lignocaine 2% with 1:200000 adrenaline, 10ml of 0.5% plain bupivacaine with 50µg clonidine was given. Urinary catheterisation was done. After preoxygenation for three minutes, patient was induced with propofol 100mg, vecuronium 5 mg, N_2O :O_2 (50:50) and isoflurane 1%. 75mg xylocard was given one minute before laryngoscopy to avoid stress response and intubation was done. $EtCO_2$ monitoring was done throughout surgery. Maintenance of anaesthesia was done with O_2:N_2O, isoflurane, vecuronium and positive pressure ventilation. Intra-operative BP surge of >200/110mmHg was controlled by 0.01% infusion of sodium nitroprusside (SNP) and heart rate more than 100/min by intermittent bolus of metoprolol 2 mg. After three hours of surgery, 10ml of 0.5% plain bupivacaine was given through epidural catheter. After the removal of tumour, there was precipitous fall of BP and the same was restored by rapid infusion of Ringer lactate 2.5L, Haemaccel 500 ml, 2 units of blood and noradrenaline infusion. Injection hydrocortisone 200mg was given after removal of the tumour. Urine output was maintained throughout the procedure. At the conclusion of the surgery, patient was reversed with neostigmine and glycopyrrolate and extubated. Patient was fully awake and maintained her vitals with support of noradrenaline. Post-operative analgesia continued through epidural catheter. Patient was shifted to ICU for continuous monitoring of vitals. Post-operative steroid cover was continued. Patient remained stable in ICU and in ward. She was discharged on 15th day with stable BP.

DISCUSSION: Primary pre-operative goal should be good pharmacological control of adverse effects of circulatory catecholamines and restoration of blood volume by α- adrenoreceptor blockade. Several authors suggest the use of calcium channel blockers (verapamil 120-240 mg every day, nifedipine 30-90 mg, diltiazem 180 mg daily) to prepare the patient in preoperative period. These agents do not cause postoperative hypotension and can control the rhythm and heart rate. [7,8] Our patient was given prazosin, a selective α-blocker that causes less tachycardia and postural hypotension than other α-adrenoreceptor blockers.[4] Labetalol was added later on to control tachycardia. Another approach involves the administration of metyrosine (alpha-methyl-para-tyrosine), which inhibits catecholamine synthesis. In one report, the patients given metyrosine had a smoother perioperative course than those given phenoxybenzamine alone.[9]

The main aim of anaesthetic management is to provide optimal surgical conditions and suppress the response of endotracheal intubation, surgical stimulation, tumour handling and devascularisation. Combined regional and general anaesthetic technique is preferred, so we used epidural anaesthesia with general anaesthesia to expand the vascular bed and provide pre-emptive and post-operative analgesia[4]. It is most appropriate to administer an anxiolytic sedative preferably a benzodiazepine to decrease catecholamine release.[3] Our patient was already taking clonazepam so we gave 1mg IV butorphanol as premedication. Monitoring in our case was performed as per recommendation.[1] Drugs causing histamine release were avoided. The anaesthetic agents preferred were propofol, isoflurane and nitrous oxide. Isoflurane was used in our patient because it does not sensitise the heart to catecholamines and decreases the peripheral resistance too.[1] Vecuronium was preferred as a relaxant due to it's lack of cardiovascular effects and histamine release.[1] Lignocaine 1.5mg/kg IV was given 1 minute before laryngoscopy to attenuate the stress response.[2] To control BP and tachycardia, we used continuous IV infusion of SNP and metoprolol IV boluses. There are recent reports of usage of I/V infusions of dexmedetomidine and magnesium sulphate in perioperative management of

pheochromocytomas.[6] Steroid cover is mandatory for patients undergoing bilateral adrenalectomy. We also used hydrocortisone in intra-operative and post-operative period.

In conclusion, the proper anaesthetic management of pheochromocytoma is a truly rewarding challenge. Mortality has been reduced in recent years due to better knowledge of these bizarre tumours, particularly the chronic hypovolemia produced, and more adequate pre-treatment regimens, better intra and post- operative management.

REFERENCES:

1. Brown R. Brunell Jr. Anaesthesia for Pheochromocytoma. In: Robert C Prys and Brown R. Brunell Jr. Editors International practice of anaesthesia.
2. Stoelting RK, Dierdosrf SF. Endocrine diseases. Anaesthesia and Co-existing diseases 2002; 430-434.
3. Published – butterworth-Heinemann 1996; 1/83/1-7 G.Singh, P.Kam. An overview of anaesthetic issues in Pheochromocytoma.Ann acad Singapore 1998;27:843-8.
4. Roberts C. Prys. Pheochromocytoma- Recent progress in it's management. Br J Anaesth 2000; 85(1): 44-57.
5. Joet T. Alder, Goswin Y et al. Phaeocchromocytoma: Current approaches and future directions. The Oncologist 2008; 13:779-793.
6. Bryskin R, Weldon BC. Dexmedetomidine and magnesium sulfate in the perioperative management of a child undergoing laparoscopic resection of bilateral pheochromocytomas. J Clin Anesth. 2010 Mar;22(2):126-9.
7. Domi R, Laho H. Management of pheochromocytoma: Old ideas and new drugs. Niger J Clin Pract 2012;15:253-7.
8. Lebuffe G, Dosseh ED, Tek G, Tytgat H, Moreno S, Tavernier B, et al. The effect of calcium channel blockers on outcome following the surgical treatment of pheochromocytomas and paragangliomas. Anaesthesia 2005;60:439-44.
9. Steinsapir J, Carr AA, Prisant LM, Bransome ED Jr. Metyrosine and pheochromocytoma. Arch Intern Med 1997; 157:901.

CONGENITAL DIAPHRAGMATIC HERNIA ASSOCIATED WITH FRYNS SYNDROME –AN AUTOPSY STUDY

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ABSTRACT: BACKGROUND: Congenital Diaphragmatic Hernia (CDH) is an anatomical defect that permits abdominal contents inside the thoracic cavity and affects 1 in 2000 to 5000 children each year and is associated with high morbidity and mortality. CDH may be associated with other anomalies like dysmorphic features, genitourinary, musculoskeletal, cardiovascular, neurological and gastrointestinal malformations.CDH is a devastating birth defect that can occur in isolation or part of complex malformation of various syndromes. Here we report an autopsy study of CDH with Fryns syndrome**. CASE PRESENTATION:** An autopsy study of still born foetus born to a 35-year old, fifth gravida presented with features of CDH with left lung hypoplasia, hypoplasia of distal parts of digits of upper and lower limbs and dysmorphic features. **CONCLUSION:** CDH is a life threatening pathology in infants and a major cause of death due to pulmonary hypoplasia and pulmonary hypertension. An early diagnosis with increased understanding of this disease is a crucial factor for a timely approach to manage the critically ill infant and to offer potential treatment for improved outcome and substantial reduction in morbidity.

KEY WORDS: Congenital diaphragmatic hernia (CDH), Fryn syndrome, pulmonary hypoplasia, Hypoplasia of digits.

INTRODUCTION: Congenital Diaphragmatic Hernia (CDH) is a life threatening congenital anomaly which needs intensive neonatal care and is associated with high morbidity and mortality with an incidence ranging between 1 in 2000 to 5000 per year. CDH may be associated with other congenital anomalies like dysmorphic features, genitourinary, musculoskeletal, cardiovascular, neurological and gastrointestinal malformations in 30% to 40% of the cases. These associated anomalies acts as major factor influencing the outcome of the affected patients. According to some authors, the most commonly associated major congenital anomaly was neural tube defect followed by cardiac and chromosomal anomalies. Severe pulmonary hypoplasia was the major cause of mortality in stillborn babies and in all infants of CDH dying within one week of birth.[1,2] CDH can occur in isolation or as part of a various syndromes such as Pallister-Killian syndrome(PKS), Ghersoni-Baruch syndrome, WAGR syndrome, Fryn syndrome, Denys-Dash syndrome, Brachman-De Lange syndrome and Wolf-Hirschhorn syndrome. Fryns syndrome is a rare autosomal recessive disorder characterized by diaphragmatic hernia and multiple anomalies[2].

CASE SUMMARY: A 35-year old, fifth gravida presented with 24 week of gestation. She gave past obstetric history of one abortion and history of neonatal death of one child. Antenatal ultrasound scan was performed which revealed congenital anomaly of diaphragm. Based on ultrasound findings, radiological diagnosis of CDH was done for which therapeutic abortion was performed in the present pregnancy at 24 week of gestation, and a still born foetus was delivered which was sent for post mortem study.

AUTOPSY FINDINGS: Received a male foetus with placenta and attached umbilical cord. Placenta was 275 gm in weight and measured 16x10x3 cm. Weight of the foetus was 600 gm. Crown rump and crown heel length was 17 and 29cm respectively. Head circumference, chest circumference and abdominal girth were 23, 20 and 18 cm respectively. External examination showed widely spaced protuberant eyes, broad and depressed nasal bridge, extra space between nose and upper lip, large mouth, small chin and hypoplasia of distal parts of digits of upper and lower limb (Figure 1). On cut open, it was found that left part of the diaphragm was deficient & left lobe of liver, spleen and coils of intestine were protruded through this deficiency in the thoracic cavity (Figure 1). Left lung weighed 4gms and measured 1.5x1.5x0.5cm; Right lung weighed 6gms and measured 3x2x0.5cm (Figure 2, Inset). Lung weight to body weight (LW: BW) ratio was 0.01. Heart, liver, spleen, right and left kidneys were normal. Microscopic examination of right lung showed thick walled lung alveoli lined by cuboidal epithelium. Dense connective tissue and bronchial cartilage was noted in inter and intralobular connective tissue. In the left lung features as described above with decrease in number of alveoli were noted. Based on above findings, a diagnosis of CDH associated with dysmorphic facial features, hypoplasia of digits and pulmonary hypoplasia suggestive of Fryns syndrome was given.

DISCUSSION: Development of the diaphragm is divided into two phases such as development of the diaphragmatic pericardium and development of the pleural cavity and closure of the pleuroperitoneal canal (PPC). General hypothesis is that the defect in CDH may results from failure of complete closure of the PPC at the embryonic period of 8th to10th gestational week. Association of CDH with various congenital anomalies, varying pathological patterns and clinical presentation suggests that it is a result of multiple, complex developmental abnormalities.[1] CDH is a cardinal feature of Fryns syndrome.[3] Studies indicate that Fryns syndrome is diagnosed in 1.3% to 10% of all the cases having CDH. This syndrome is generally difficult to diagnose.[3,4] Diagnostic criteria for Fryns syndrome reformulated by Lin AE et al[5] were 4 out of following 6 symptom groups such as diaphragmatic defect, facial characteristics, distal digital hypoplasia, pulmonary hypoplasia, parental consanguity and other associated anomalies such as cardiovascular malformation, renal dysplasia/renal cortical cysts, gastrointestinal malformation, genital malformation should be present.[4,5] In the present case, 4 symptom groups such as CDH, dysmorphic facial features, pulmonary hypoplasia and distal digital hypoplasia were seen. Hence this case was concluded as Fryns syndrome. LW: BW ratio is the most widely used parameter for diagnosis of pulmonary hypoplasia. Pathologic postmortem diagnosis of pulmonary hypoplasia was suggested when the LW: BW was found to be less than 0.12.[6] Based on this finding, diagnosis of pulmonary hypoplasia was made in the present case. Considerable phenotypic overlap was noted between Fryns and PKS. In both syndromes CDH, coarse facial features and minor limb anomalies were seen. Features more common for, Fryns syndrome are CDH, cleft palate, and distal phalange and/or nail hypoplasia, cardiovascular malformations, and renal malformations. Features more common for PKS are high forehead, streaky skin hyper

pigmentation, and sparseness of hair bi-temporally.[7] In the present case, CDH was associated with pulmonary hypoplasia, dysmorphic facial features and distal digital hypoplasia hence the case was concluded as Fryns syndrome. Association of diaphragmatic defects and upper limb anomalies were not reported commonly.[8-10] McCredie and Reid [8] reported 4 single cases of diaphragmatic defect associated with limb anomalies. The limb defects ranged from a radial hypoplasia to a transverse deficiency, with a block of bone distal to the hummers. Lerone et al[9] reported a case of left congenital diaphragmatic hernia associated with ipsilateral thumb hypoplasia. A similar case of congenital left diaphragmatic hernia with ipsilateral thumb hypoplasia and absent radius was reported by Wallerstein et al.[10] In the present case, CDH is associated with hypoplasia of distal parts of digits of upper & lower limbs which is one of the uncommon finding.

CONCLUSION: Incidence of mortality associated with CDH will become apparent only when the autopsy study of infant and fetuses that have died with a diagnosis of CDH will be included in the analysis.[1] In this case, abortion of previous pregnancy and neonatal death of sibling may be due to presence of CDH and associated syndrome. As Fryns syndrome is autosomal recessive disorder, autopsy study of present case will assist in counseling to the parents about recurrence of the disease in next pregnancies and advising regular antenatal ultrasound scan and screening for maternal serum Alpha fetoprotein assay at 13 weeks in next pregnancy.

REFERENCES:

1. Arafah M, Boqari DT, Alsaad KO. Left-sided congenital diaphragmatic hernia with multiple congenital cardiac anomalies, hernia sac, and microscopic hepatic heterotopia: A Case Report. Pathology Res Int 2011; 96:710 -7.
2. Tovar JA. Orphanet Journal of Rare Diseases 2012; 7:1 doi: 10.1186/1750-1172-7-1. Available at: http://www.ojrd.com/content/7/1/1
3. Slavotinek A, Lee SS, Davis R, Shrit A, Leppig KA, Rhim J et al. Fryns syndrome phenotype caused by chromosome microdeletions at 15q26.2 and 8p23.1. J Med Genet 2005; 42:730–736.
4. Slavotinek A. Fryns syndrome. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 2007 [cited 2010 Jun 1]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1459/
5. Lin AE, Pober BR, Mullen MP, Slavotinek AM. Cardiovascular malformations in Fryns syndrome: is there a pathogenic role for neural crest cells. Am J Med Genet A 2005; 139:186–93.
6. Askenazi SS, Perlman M. Pulmonary hypoplasia: lung weight and radial alveolar count as criteria of diagnosis. Arch Dis Child 1979; 54:614-618.
7. Pober BR, Russell MK, Ackerman KG. Congenital Diaphragmatic Hernia Overview. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP editors. Gene Reviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2010 Mar 16]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1359/
8. Mccredie J, Reid IS. Congenital diaphragmatic hernia associated with homolateral upper limb malformation. J Pediatr 1978; 92:762-5.

9. Lerone M, Soliani M, Corea D, Romeo G, Martucciello G, Silengo MC. Congenital diaphragmatic hernia associated with ipsilateral upper limb reduction defects: report of a case with thumb hypoplasia. Am J Med Genet 1992; 44:827-9.
10. Wallestein R, Wallerstein DF, Trauffer P, Desposto F. Congenital diaphragmatic hernia and ipsilateral limb reduction defect: a new case, longterm follow-up and review of the literature. Clin Dysmorphol 1997; 6:257-61.

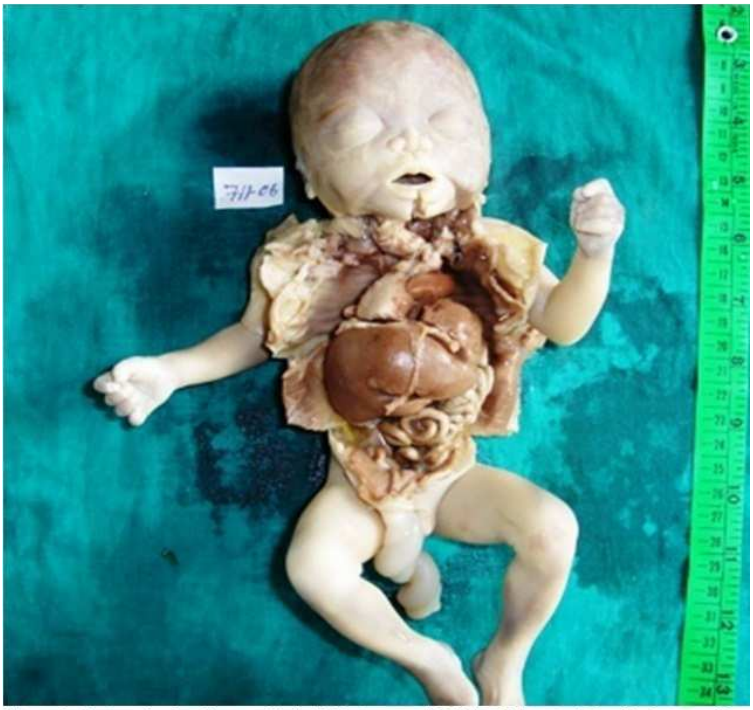

Figure1: Foetus showing dysmorphic facial features and deficient left part of the diaphragm with protrusion of left lobe of liver, spleen and coils of intestine in thoracic cavity.

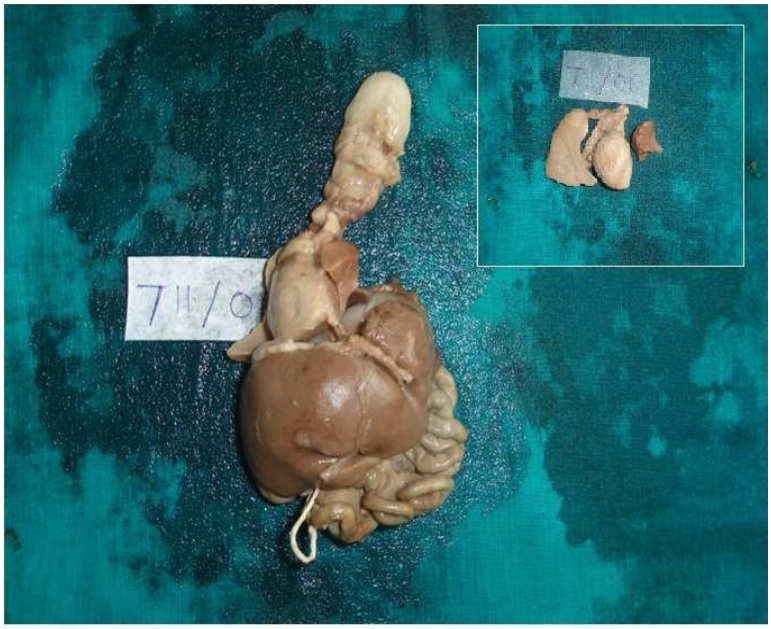

Figure 2: En-block removal of organs. Inset: Right lung, heart & hypoplastic left lung

A CORRELATIVE STUDY OF ADENOSINE DEAMINASE ACTIVITY & T.B. IgG IN SERUM IN CASES OF TUBERCULOSIS.

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ABSTRACT: INTRODUCTION: Tuberculosis is major cause of morbidity and mortality in India as well in other parts of world. It is caused by mycobacterium tuberculosis which primarily affects lung and cause pulmonary tuberculosis. Diagnosis of tuberculosis rests upon a positive history of contact, clinical symptoms, x-ray chest, sputum positivity and AFB culture. Adenosine deaminase (ADA) is an enzyme which catalyzes the deamination of adenosine into inosine and ammonia. ADA level is found to be elevated in tuberculosis and typhoid fever where cell mediated immunity is elevated. The ADA level is significantly elevated in tuberculosis and helps to differentiate between tubercular and non tubercular diseases. The ADA level is also found to be elevated in serum and pleural fluid in patients of tubercular pleural effusion, tubercular ascitis and tubercular pericardial effusion. **METHODS**: Routine hemogram, Montoux test, X-ray chest, FNAC of lymph nodes, biopsy of lymph node whenever required, estimation of serum ADA level and T.B.IgG studies were performed in each case. **RESULTS:** In the present study a total of 45 cases were selected for the study. There are 30 cases of pulmonary tuberculosis and 15 controls. The values of serum ADA and tubercular IgG in pulmonary tubercular group are significantly higher as compared to those of controls. None of the control for ADA showed significant ratio of positivity (≥1.7). One of the 15 cases showed remarkable ratio of positivity (>1.2-1.6) and 14 (93.3%) cases showed insignificant ratio of positivity. Only 2 (13.33%) of the 15 cases showed positivity for TB IgG and rest 13 (86.66%) were regarded negative. **CONCLUSIONS:** Thus it can be concluded that determination of serum adenosine deaminase levels can effectively diagnose tuberculosis with sensitivity of 96.66% and specificity of 93.33% as compared to TBIgG showing sensitivity of 90% and specificity of 86.6%. Also cost of ADA estimation is remarkably less than that of tubercular IgG
KEY WORDS: Serum ADA, T.B.IgG levels, Tuberculosis

INTRODUCTION: Tuberculosis is major cause of morbidity and mortality in India as well in other parts of world. It is caused by mycobacterium tuberculosis which primarily affects lung and cause pulmonary tuberculosis. It can also affect intestine, bones, joints, lymph nodes, genitourinary system, skin and virtually every organ of the body.

Diagnosis of tuberculosis rests upon a positive history of contact, classical symptoms, lymphocytosis on differential count, lesions in x-ray chest, sputum positivity for AFB and culture of AFB on L.J. media. But none of these except demonstration of AFB is a sure shot evidence of diagnosis. Besides this several other tests such as demonstration of tubercular antigen by Polymerase chain reaction[1,2] , rapid culture of tubercular bacilli by Bactec system have come forward but are costly and not available everywhere except at except at specialised centres.[3]

Adenosine deaminase (ADA) is an enzyme which catalyzes the deamination of adenosine into inosine and ammonia. It helps in maturation and proliferation of T cells. ADA level is found to be elevated in tuberculosis and typhoid fever where cell mediated immunity is elevated [4, 5]. The ADA level is significantly elevated in tuberculosis and helps to differentiate between tubercular and non tubercular diseases. The ADA level is also found to be elevated in serum and pleural fluid in patients of tubercular pleural effusion. [6, 7,8,9,10,11], tubercular ascitis [12, 13] and tubercular pericardial effusion.[8]

Besides this there are several other tests which detect the antibody load against tubercular antigen in patient's serum. Enzyme linked immunosorbant assay is most frequently used and it detects the quantity and quality of circulating antibodies against tubercular antigen. Usually three antibodies are detected which include-

1. Tubercular IgA (TbIgA)
2. Tubercular IgM (TbIgM)
3. Tubercular IgG (TbIgG)

Tubercular IgA antibodies are detected in serum of some apparently healthy individuals at risk, but being the secretary antibody its detection in body fluid is of more value in patients having tubercular effusion.

Tubercular IgM is detected in initial phase of infection i.e. 1-2 months.[14]

Tubercular IgG is detected in patient's serum in slightly late stages when disease is properly settled (after 2 months). Its high titre is more specific as compared to tubercular IgA and IgM in patient's serum for diagnosis of active tuberculosis.[15]

Single detection of these antibodies alone cannot confirm the diagnosis of tuberculosis unless matched or correlated with other clinical Koch's parameters. Thus the rising antibody titres are more significant than a single high titre, but because of the cost this is practically impossible.

So the present study was undertaken to evaluate serum adenosine deaminase activity in tubercular patients.

MATERIAL AND METHODS: The present study was carried out in the department of pathology, Biochemistry, Tubercular and chest disease and Medicine Rama Medical College and Research Centre Mandhana, Kanpur.

The clinical features and detailed history were recorded in a standard proforma. Routine hemogram, Mantoux test, X-ray chest, FNAC of lymph nodes, biopsy of lymph node whenever required, estimation of serum ADA level and T.B.IgG studies were performed in each case.

Estimation of ADA activity-

We have utilized the method of GLUSEPPE GIUSTI and BRUNO GALANTI for ADA estimation. The instrument used for ADA estimation were spectrophotometer or simple

photometer for accurate measurement between 620 and 650 nm, water bath 37°C, centrifuge, test tube and auto pipette.

Evaluation of T.B. IgG-

For evaluation of T.B.IgG specimen collected was patient's blood from which sera was separated and stored in a refrigerator until performance of test. Reagents used were supplied by TRANSASIA biomedical Ltd, under the name of ERBA ELISA test tuberculosis.

OBSERVATIONS: In the present study a total of 45 cases were selected for the study. There are 30 cases of pulmonary tuberculosis and 15 controls. Normal healthy individuals or patients, who were suffering from non tubercular pulmonary disease, were taken as control. The serum ADA levels and tubercular IgG of controls are shown in table I and II. Serum ADA activity and tubercular IgG of pulmonary tubercular group are shown in table III and IV.

The values in pulmonary tubercular group are significantly higher as compared to those of controls. Table V shows the comparative ratio of positivity of serum ADA and Tubercular IgG.

Table I- ADA levels in control (Mean = 25.83 U/L).

S.No.	ADA levels (U/L)	Ratio of positivity
1.	26.65	1.03
2.	31.10	1.20
3.	30.0	1.16
4.	21.65	0.83
5.	26.65	1.03
6.	16.65	0.64
7.	18.30	0.70
8.	31.65	1.20
9.	20.0	0.77
10.	26.65	1.03
11.	28.30	1.09
12.	36.0	1.39
13.	33.30	1.20
14.	16.65	0.64
15.	23.38	0.90

None of the control showed significant ratio of positivity (≥1.7). One of the 15 cases showed remarkable ratio of positivity (>1.2-1.6) and 14 (93.3%) cases showed insignificant ratio of positivity.

Table – II TB IgG in controls –

S.No.	Observed value (O.D.)	Positive cut off (O.D.)	Ratio of positivity	Interpretation
1.	113	250	0.45	-
2.	226	250	0.90	-
3.	142	250	0.56	-
4.	157	250	0.67	-

5.	440	250	1.76	+
6.	410	650	0.63	-
7.	398	650	0.61	-
8.	357	650	0.54	-
9.	401	650	0.61	-
10.	312	650	0.48	-
11.	475	450	1.05	+
12.	210	450	0.46	-
13.	317	500	0.7	-
14.	416	500	0.83	-
15.	217	500	0.43	-

Only 2 (13.33%) of the 15 cases showed positivity for TB IgG and rest 13 (86.66%) were regarded negative.

Table III- ADA and ratio of positivity in patients of pulmonary tuberculosis.

S.No.	ADA activity in serum	Cut off level	Ratio of positivity
1.	58.33	35	1.66
2.	56.66	35	1.61
3.	68.33	35	1.95
4.	35.00	35	1.00
5.	70.00	35	2.00
6.	91.66	35	2.61
7.	68.33	35	1.95
8.	73.33	35	2.09
9.	65.00	35	1.85
10.	60.00	35	1.71
11.	63.33	35	1.80
12.	60.00	35	1.71
13.	61.66	35	1.76
14.	63.33	35	1.80
15.	56.66	35	1.61
16.	53.33	35	1.52
17.	68.33	35	1.95
18.	60.00	35	1.71
19.	68.33	35	1.95
20.	61.66	35	1.76
21.	60.00	35	1.71
22.	56.66	35	1.61
23.	58.33	35	1.66
24.	63.33	35	1.80
25.	61.66	35	1.76
26.	66.66	35	1.90
27.	68.33	35	1.95

28.	63.33	35	1.80
29.	55.00	35	1.57
30.	70	35	2.00

22 (73.3%) cases showed significant ratio of positivity (≥1.7).
7 (23.3%) cases were out of remarkable ratio of positivity (>1.2-1.6).
1 (3.3%) case showed insignificant ratio of positivity (<1.2).

Table IV- TB IgG in serum of patients of pulmonary tuberculosis

S.No.	Observed value (O.D.)	Positive cut off (O.D.)	Ratio of positivity	Interpretation
1.	383	250	1.53	++
2.	940	250	3.76	+++
3.	516	400	1.29	++
4.	207	400	0.51	-
5.	1096	600	1.82	++
6.	661	600	1.10	+
7.	2001	600	3.33	+++
8.	623	600	1.03	+
9.	666	600	1.11	+
10.	696	650	1.07	+
11.	744	650	1.14	+
12.	535	500	1.07	+
13.	2046	500	4.09	+++
14.	677	500	1.35	++
15.	923	500	1.84	++
16.	616	500	1.23	++
17.	814	500	1.62	++
18.	490	500	0.98	-
19.	894	500	1.78	++
20.	774	500	1.54	++
21.	575	500	1.15	+
22.	750	500	1.50	++
23.	2136	500	4.23	+++
24.	558	500	1.11	+
25.	1231	500	2.46	+++
26.	525	500	1.05	+
27.	878	500	1.75	++
28.	632	500	1.22	++
29.	319	500	0.63	-
30.	714	400	1.78	++

Of these total 30 cases, 2 were clearly negative (false negative) and one was borderline negative (+++->2) times positive cut off, ++ ->1.2-2.0 times the positive cut off, + - >1.0-1.2 times the positive cut off, -<1 times the positive cut off.

Table V- Comparison of ratio of positivity of ADA and Tubercular IgG in pulmonary tuberculosis.

S.No.	Ratio of positivity of ADA	Ratio of positivity of IgG	Interpretation
1.	1.66	1.53	++
2.	1.61	3.76	++
3.	1.95	1.29	++
4.	1.07	0.51	+-
5.	2.00	1.82	++
6.	2.61	1.10	++
7.	1.79	3.33	++
8.	2.09	1.03	++
9.	1.85	1.11	++
10.	1.71	1.07	++
11.	1.80	1.14	++
12.	1.71	1.07	++
13.	1.76	4.09	++
14.	1.80	1.35	++
15.	1.61	1.84	++
16.	1.52	1.23	++
17.	1.95	1.62	++
18.	1.71	0.98	+-
19.	1.95	1.78	++
20.	1.76	1.54	++
21.	1.71	1.15	++
22.	1.61	1.50	++
23.	1.66	4.23	++
24.	1.80	1.11	++
25.	1.76	2.46	++
26.	1.90	1.05	++
27.	1.95	1.75	++
28.	1.80	1.22	++
29.	1.57	0.63	+-
30.	2.00	1.78	++

(++) - Parallel rise of both the parameters.
(+-) - Rise of only one parameter.

DISCUSSION: Tuberculosis continues to be a major cause of mortality and morbidity in developing countries. Although lung is the most frequent organ to be involved, inflammation of serous membranes is also very common. The definitive diagnosis is established when typical

histological features can be demonstrated or mycobacteria can be isolated from the body fluids or sputum or on gastric lavage and various other methods such as gel electrophoresis, radiometric assay and polymerase chain reaction. It is well documented that isolation of mycobacteria and culture is very difficult and time consuming (shah et al 1990).

Recently most simple techniques which are most feasible less costly and giving quick results are now available and include demonstration of antibodies by ELISA method (humoral immune response)and ADA activity (cell mediated immune response) in serum and body fluids.

So the present study was undertaken to evaluate adenosine deaminase enzyme activity and tubercular IgG together in patients of pulmonary tuberculosis. Normal healthy individuals or patients, who were suffering from non tubercular pulmonary disease, were taken as control.

In this study the overall mean serum ADA in control was 25.83 U/L (16.65 – 36 U/L). There was no significant variation in serum ADA levels in relation to age and sex in controls[16]. Only one healthy control showed increase activity of ADA in serum and may possibly be resulting from any disease involving increase cell mediated immunity e.g. typhoid [4,5].

Mean serum ADA activity in patients of pulmonary tuberculosis in this study was 58.40 U/L (Table III) which was significantly elevated as compared to that of controls (Mean 25.83 U/L). Cut off values of serum ADA >35 U/L was diagnostic of tuberculosis with 100% sensitivity. Similar observations were given other researchers who reported higher levels of serum ADA in patients with pulmonary tuberculosis as compared to healthy controls[16.] The level was also significantly higher in pulmonary tuberculosis as compared to non tubercular pulmonary diseases (suppurative and malignant) and using a cut off of 33 U/L. Sensitivity and specificity was claimed to be 98% and 100%. Significantly higher ($p<0.0005$) serum ADA in tubercular group (29.8±10 U/L) was reported than in neoplastic group (14.5±4.0 U/L) [17]. Similar data was provided by other observers who reported ADA activity of 27.38 U/L in tuberculosis as compared to malignancy (7.29) and non tubercular pulmonary diseases (12.71)[18].

Tubercular immunoglobulin IgG depicting humoral immune response was also evaluated in pulmonary tubercular patients by using ELISA method against antigen A60. In control group 2 cases (13.33%) out of total 15 cases showed a false positivity and may be because of previous exposure to disease[15]**.** The value of optical density obtained in pulmonary tuberculosis patients are show in table IV. Three (10%) of the total 30 cases studied showed false negative values. This could be attributed to ANERGY because of initial tubercular toxaemia or because of immunocompromised status[19]. The sensitivity and specificity of the test came out to be 90% and 86.6% respectively. Similar were the observations formulated by Gupta S et al 1995, who reported a specificity of 92.3% and sensitivity of 80%.

Thus it can be concluded that determination of serum adenosine deaminase levels can effectively diagnose tuberculosis with sensitivity of 96.66% and specificity of 93.33% as compared to TBIgG showing sensitivity of 90% and specificity of 86.6%. Also cost of ADA estimation is remarkably less than that of tubercular IgG [9]. However using both tests in combination increases the specificity and sensitivity for diagnosis of tuberculosis.

BIBLIOGRAPHY:

1. 1.Brisson-Noël A, Gicquel B, Lecossier D, Lévy-Frébault V, Nassif X, Hance AJ Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet. 1989 Nov 4;2(8671):1069-71

2. Tiwari V, Jain A, Verma RK. Application of enzyme amplified mycobacterial DNA detection in the diagnosis of pulmonary & extra-pulmonary tuberculosis. Indian J Med Res. 2003 Dec;118:224-8.
3. Middlebrook G, Reggiardo Z, Tigertt WD. Automatable radiometric detection of growth of Mycobacterium tuberculosis in selective media. Am Rev Respir Dis. 1977 Jun;115(6):1066-9.
4. Galanti B, Nardiello S, Russo M, Fiorentino F. Increased lymphocyte adenosine deaminase in typhoid fever. Scand J Infect Dis. 1981;13(1):47-50
5. Mishra OP, Gupta BL, Ali Z, Nath G, Chandra L. Adenosine deaminase activity in typhoid fever. Indian Pediatr. 1994 Nov;31(11):1379-84.
6. Piras MA, Gakis C, Budroni M, Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br Med J. 1978 Dec 23-30;2(6154):1751-2.
7. Nagaraja MV, Ashokan PK, Hande HM. Adenosine deaminase in pleural effusions. J Assoc Physicians India. 1992 Mar;40(3):157-9
8. 8 Segura RM, Pascual C, Ocaña I, Martínez-Vázquez JM, Ribera E, Ruiz I, Pelegrí MD. Adenosine deaminase in body fluids: a useful diagnostic tool in tuberculosis. Clin Biochem. 1989 Apr;22(2):141-9. Gaki C.The low cost of the adenosine deaminase assay. Chest. 1996 Nov;110(5):1376-7.
9. Sharma SK, Mohan A. Adenosine deaminase in the diagnosis of tuberculosis pleural effusion. Indian J Chest Dis Allied Sci. 1996 Apr-Jun;38(2):69-71.
10. Shibagaki T, Hasegawa Y, Saito H, Yamori S, Shimokata K. Adenosine deaminase isozymes in tuberculous pleural effusion. J Lab Clin Med. 1996 Apr;127(4):348-52.
11. Gupta BK, Bharat V, Bandyopadhyay D. Sensitivity, specificity, negative and positive predictive values of adenosine deaminase in patients of tubercular and non-tubercular serosal effusion in India. J Clin Med Res. 2010 May 19;2(3):121-6.
12. 13Sathar MA, Simjee AE, Coovadia YM, Soni PN, Moola SA, Insam B, Makumbi F. Ascitic fluid gamma interferon concentrations and adenosine deaminase activity in tuberculous peritonitis. Gut. 1995 Mar;36(3):419-21.
13. Gevaudan MJ, Bollet C, Charpin D, Mallet MN, De Micco P. Serological response of tuberculosis patients to antigen 60 of BCG. Eur J Epidemiol. 1992 Sep;8(5):666-76.
14. Gupta S, Kumari S, Banwalikar JN, Gupta SK. Diagnostic utility of the estimation of mycobacterial Antigen A60 specific immunoglobulins IgM, IgA and IgG in the sera of cases of adult human tuberculosis. Tuber Lung Dis. 1995 Oct;76(5):418-24.
15. jhamaria JP, Jenaw RK, Ludhada SK, Mathur Dk, Parihar HL, Sharma SK. Serum adenosine deaminase in the differential diagnosis of pulmonary tuberculosis and common non-tubercular respiratory diseases. Ind J Tub. 1988;35:25-7.
16. Baganha MF, Pêgo A, Lima MA, Gaspar EV, Cordeiro AR. Serum and pleural adenosine deaminase. Correlation with lymphocytic populations. Chest. 1990 Mar;97(3):605-10.
17. 18 Bansal SK, Singh RP, Narang RK, Joshi LD, Bansal A, Agrawal AK. Serum adenosine deaminase in pulmonary tuberculosis, malignancy and non-tubercular respiratory diseases. Indian J Chest Dis Allied Sci. 1991 Oct-Dec;33(4):189-93.
18. Fadda G, Grillo R, Ginesu F, Santoru L, Zanetti S, Dettori G. Serodiagnosis and follow up of patients with pulmonary tuberculosis by enzyme-linked immunosorbent assay. Eur J Epidemiol. 1992 Jan;8(1):81-7.

EFFICACY OF AIR DISINFECTION DEVICES IN CONTROLLING ATMOSPHERIC MICROFLORA IN ENCLOSED HEALTH CARE SETTINGS.

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ABSTRACT: INTRODUCTION: There is increasing concern with the prevalence of hospital infection. This is causing a significant economic and logistical burden on the health services. **OBJECTIVES:** To assess the efficacy of hydroxyl radical based air disinfection devices in reducing the atmospheric pathogen count in enclosed health care settings. **MATERIALS AND METHODS:** Sequential trial with repeated readings was designed before and after installation of the Air Disinfection devices. Study was conducted at 52 points in different locations which included sections from out Patient Department, Intensive Care Units, Operation Theatre, Surgical Ward, Radiology Department and cubicles in routine microbiology. The samples collected with air sampler were processed in Neuromicrobiology Laboratory. **RESULT:** It was observed that the mean (±SE) colony forming units (CFU/m^3) count after 24 and 48 hours before and after use of air disinfectant devices at the above locations were 159.75 (±35.42), 210.31 (±65.15) and 44.04 (±14.15), 55.31 (±15.06) respectively. The differences in the mean CFU concentrations were found to be statistically significant (p= 0.006). Additionally, a high degree of variance in the CFU counts was noted before using the devices as compared to that after using the same. **CONCLUSION:** The results indicate that there is considerable reduction in the number of colony forming units subsequent to the introduction of the Air Disinfection devices. These devices supplement and compliment the general hygienic practices. However owing to a lot of compounding factors affecting the colony forming units in a given location, sufficient care must be taken for ascribing the reduction in the counts only to the device.

KEYWORDS: Air Disinfection, Environmental Flora, Disinfection Devices, Air Sampling, Hospital Infection.

INTRODUCTION: Hospitals and associated areas are high risk areas for acquiring infections[1]. Therefore, it is imperative that strict policies be implemented and maintained so as to achieve the goal of infection control. One of the modes to achieve the same is by air disinfection process wherein devices are used to reduce the number of microorganisms in the air. This concept in disease prevention is pivotal in certain high risk areas within the hospital like Intensive care units, operation theatres, and other sites where virulent and resistant organisms, high risk patients and invasive interventions are present. Prevention of infections by means of air disinfection will enhance and greatly synergise with other infection control measures and

reduce the incidence of nosocomial infections(hospital acquired infections), suspected to arise by the air borne route.

There has been increasing concern with the prevalence of superbugs and hospital related infections in the past decade. Environmental dissemination of pathogens is an important issue associated with hospital and community acquired infections and therefore their control plays a vital role in the formulation of hospital infection surveillance system and antibiotic policies[2]. Different technologies are coming up for atmospheric disinfection which employs various principles for disinfection. The use of hydroxyl radicals is a very efficient mode of decontamination. The hydroxyl radical is a transient piece of energy. These radicals are highly reactive and consequently short lived. These are also called the detergent of the troposphere as these are often the first step to removal of pollutants. The attack process essentially leads to a cascade of reactions that may induce damage to nucleic acids, structural and functional changes in proteins, as well as oxidation of lipids. For example, lipids in a bacterial cell wall react with hydroxyl radicals by losing hydrogen and forming a lipid radical. Oxidation of proteins are far more complicated where a protein radical will lead many possible reactions, one of which resulting in a non-protein radical that can be a further hydroxyl radical[3,4]. In a microbial cell, due to interactions between proteins and lipids, the oxidative reactions can transfer from lipids to proteins and so on, causing destruction to main cellular structures which will render the bacteria non viable. Another study found that hydroxyl radicals are involved in cell killing by the bacterial Topoisomerase I Cleavage Complex[5]. The reactions between these hydroxyl radicals and bacteria are non-strain specific, therefore resistant and non-resistant bacterial strains will be equally susceptible to hydroxyl radicals' attack[6].

The AD unit used in the study takes the surrounding air within an enclosed space, processes it and combines it with olefin – the natural scents of flowers and plants – creating hydroxyl radicals that react with the molecules in airborne pathogens and removes them[7,8]. Through this process the AD unit disinfects the air continuously.

OBJECTIVES: To assess the efficacy of hydroxyl radical based air disinfectant devices in reducing the atmospheric pathogen count in enclosed health care settings.

METHODOLOGY: Sequential air sampling before and after installation of air disinfectant devices were carried out. The air sampling device used was of the model LA030 Air Petri from Hi- Media. The volume of air sampled was 1,000L ($1m^3$). Time taken for sampling was 3 minutes with a flow rate of 300 litres/min. The machine uses a sieve impaction particle capture mechanism.

The sampled air was cultured on culture media namely sheep blood agar for bacterial isolation and Sabouraud's dextrose agar for fungal isolation. The plates were incubated at 37^0C for 24-48 hours. First reading was taken after 24hours of incubation, and the second reading after 48 hours of incubation. Quantitative evaluation of CFU/m^3 was performed by observing the culture and counting the colonies.

Study was conducted at 52 points in different locations of the hospital which included sections from outpatient department, intensive care units, operation theatre, surgical ward, radiology department and cubicles in the department of microbiology. Further processing was performed in the Neuromicrobiology laboratory as per standard guidelines. The air disinfectant device used was from Inov8 Air Disinfection Company, United Kingdom. Specific identification of the isolates was not performed during the study.

STATISTICAL ANALYSIS: Continuous data were summarized by mean (± SD) and Categorical data as proportions and percentages. Paired t-test was used to compare the statistical significance of differences in mean CFU counts before and after use of AD devices.

RESULTS: It was observed that the mean (±SE) colony forming units (CFU)count after 24 and 48 hours before and after use of air disinfectant devices were 159.75 (±35.42), 210.31 (±65.15) and 44.04 (±14.15), 55.31 (±15.06) for Digital Subtraction Procedure centre, Neurology OPD, Neurosurgery OT and Spiral CT centre, respectively. Refer Table 1 to 4.

DISCUSSION: Pre AD CFU counts showed considerable variance and high counts viz a viz the post AD CFU counts. Subsequent to introduction of the AD device the counts reduced significantly.

The Hydroxyl radicals travel short distances and when it encounters oxidizable substrates this triggers a free radical cascade leading to cell injury at sites distant from where the initial free radical reaction occurred[9]. Previous studies[10] show that the device significantly reduces the concentration of airborne microbes like S. epidermidis and MS-2 Coliphage by a factor of 5 log10 within 1 hr. Independent tests have shown a kill rate of 99.999% in a typical space in less than 60 minutes. It has a slower effect on spores.

The air sampling showed a wide variation in the microbial counts in all the locations. The overall differences in the mean CFU concentrations were found to be statistically significant (p= 0.006). These devices supplement and compliment the general hygienic practices. However, owing to a lot of confounding factors affecting the colony forming units in a given location, sufficient care must be taken for ascribing the reduction in the counts only to the device.

The hydroxyl radical AD units may have a role in reducing the environmental burden of microorganisms in a high risk clinical setting. However, more data is required to demonstrate the efficacy of the system.

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REFERENCES:

1. World Health Organization. Guidelines on Prevention and Control of Hospital Associated Infections. WHO, Geneva, 2002.
2. Wong V, Staniforth K, Boswell TC. Environmental contamination and airborne microbial counts: a role for hydroxyl radical disinfection units? J. Hosp. Infect. 2011;1-6.
3. Garrison WM. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. Chem Rev 1987;87:381-98
4. Singh J & Thornton J M. Atlas of Protein Side-Chain Interactions, Vols. I & II, 1992. IRL press, Oxford.
5. Liu IF, Annamalai T, Sutherland JH, Tse-Dinh YC. Hydroxyl radicals are involved in cell killing by the bacterial topoisomerase I cleavage complex. J. Bacteriol. 2001; 191:5315-19.

6. Inov8 Air Disinfection Combats Antibiotic Resistant Bacteria. In . Air disinfection solutions: effective against antibiotic resistant microorganisms. Aug 2010.
7. W e s c h l e r CJ, S h i e l d s HS. Production of the hydroxyl radical in indoor air. Environ. Sci. Technol. 1996; 30:3250-58.
8. Donahue NM, Kroll JH, Anderson JG, Demerjian KL. Direct observation of OH production from the ozonolysis of olefins. Geophysical Research Letters, 1998; 25: 59-62.
9. Miller RA, Britigan BE. Role of oxidants in microbial pathophysiology. Clin Microbiol Rev 1997;10:1-18.
10. 10 Ezbiri A, Elen R. Review of current evidence on the reduction of infection rates in NHS hospitals using the Inov8 AD (air disinfection) technology. Hospital Infection Society Conference; 2010: Liverpool, UK.

TABLE 1: CFU count after 24 and 48 hours before and after use of air disinfectant devices at location 1- Digital Subtraction Procedure centre.

Location = Digital Subtraction Procedure					
	Status	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
24_CFU	**Without AD**	4	66.25	55.13	27.57
	With AD	16	50.94	123.32	30.83
48_CFU	**Without AD**	8	70.62	66.63	23.56
	With AD	16	58.75	122.37	30.59
a. Location = Digital Subtraction Procedure, P>0.05					

TABLE 2: CFU count after 24 and 48 hours before and after use of air disinfectant devices at location 2- Neurology OPD

Location = NEURO OPD					
	Status	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
24_CFU	**Without AD**	8	300.00	155.86	55.11
	With AD	8	76.88	71.71	25.35
48_CFU	**Without AD**	8	641.25	548.67	193.99
	With AD	8	110.00	94.30	33.34
b. Location = NEURO OPD, P>0.05					

TABLE 3: CFU count after 24 and 48 hours before and after use of air disinfectant devices at location 3- Neurosurgery OT.

Location = NEURO OT					
	Status	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
	Without AD	4	93.75	89.57	44.79

24_CFU	**With AD**	14	6.79	15.64	4.18
48_CFU	**Without AD**	8	62.50	75.02	26.53
	With AD	14	8.93	15.21	4.07
c. Location = NEURO OT, P>0.05					

TABLE 4: CFU count after 24 and 48 hours before and after use of air disinfectant devices at location 4- Spiral CT centre

Location = SPIRAL CT					
	Status	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
24_CFU	**Without AD**	**4**	**38.75**	**41.11**	**20.55**
	With AD	**14**	**54.64**	**134.06**	**35.83**
48_CFU	**Without AD**	**8**	**66.88**	**85.90**	**30.37**
	With AD	**14**	**66.50**	**140.23**	**37.48**
d. Location = SPIRAL CT, P>0.05					

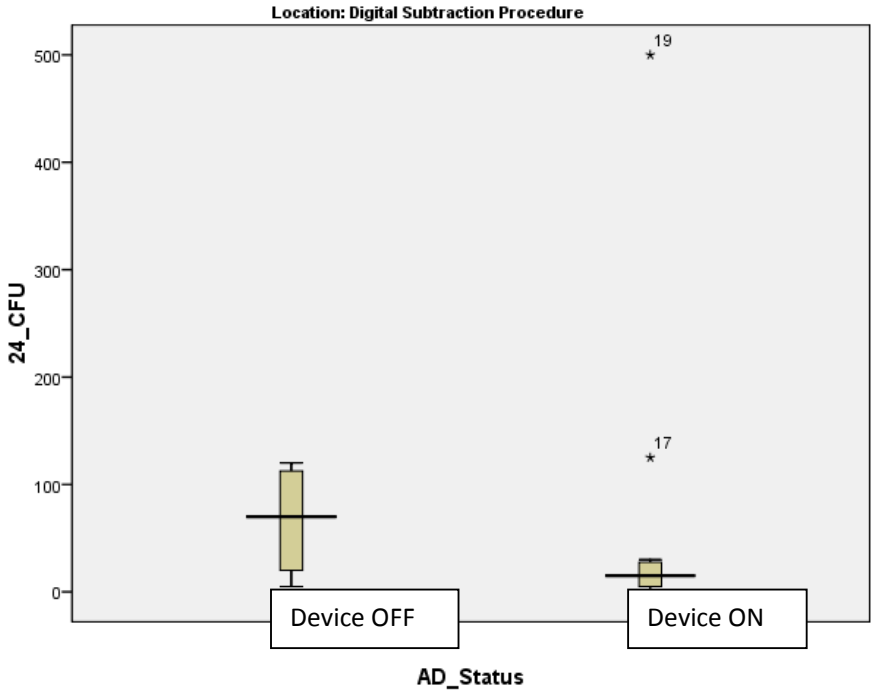

FIGURE 1: Box plot depicting the Mean (SD) CFU counts at DSP Room

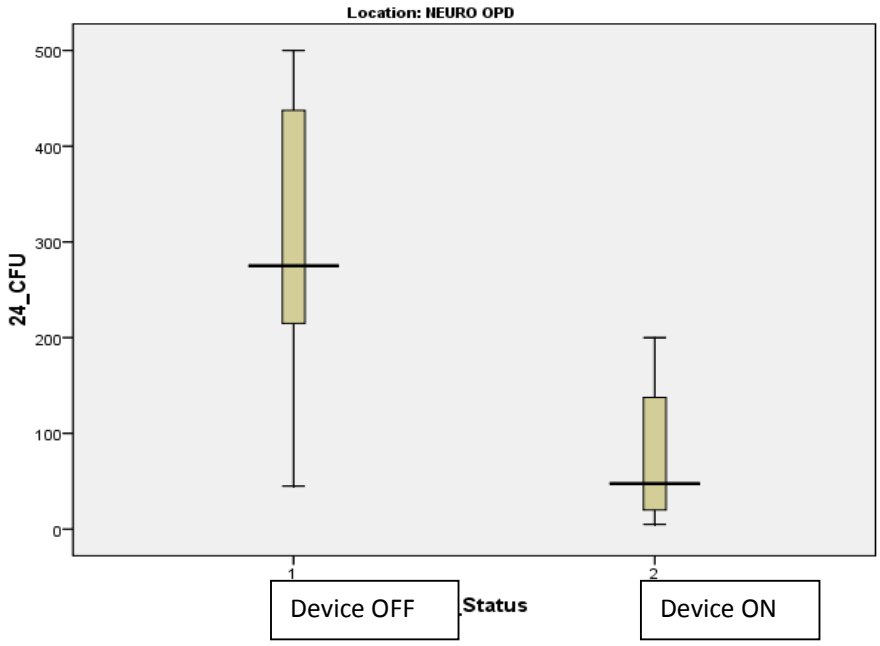

FIGURE 2: Box plot depicting the Mean (SD) CFU counts at Neuro OPD

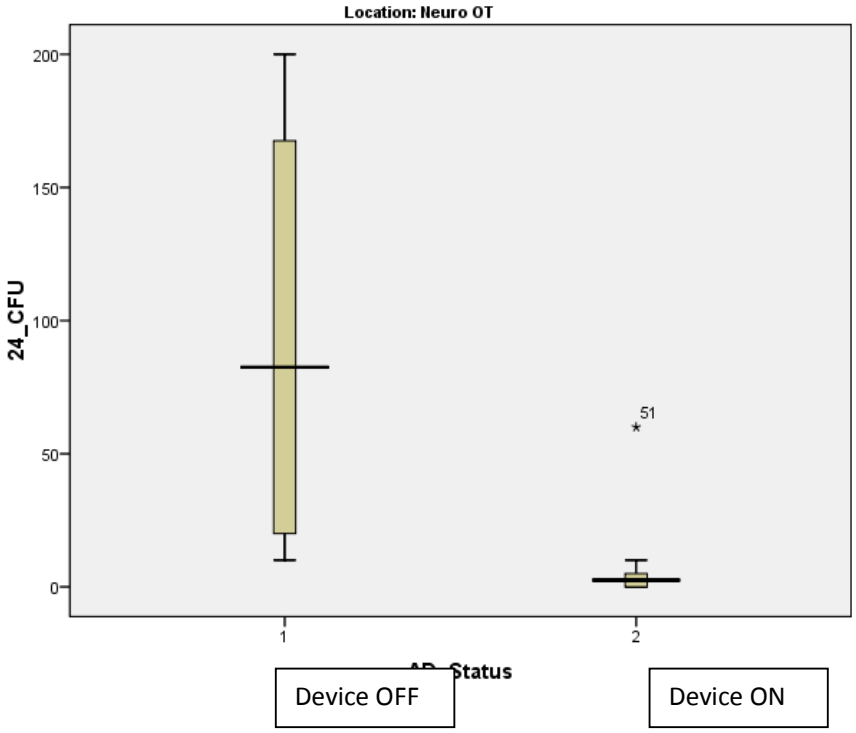

FIGURE 3 : Box plot depicting the Mean (SD) CFU counts at Neuro OT

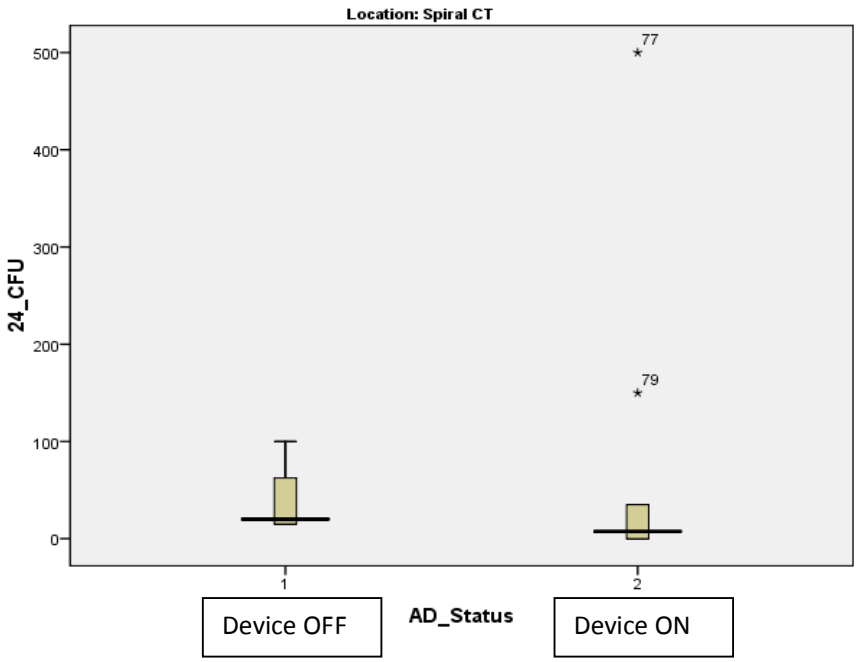

FIGURE 4: Box plot depicting the Mean (SD) CFU counts at Spiral CT center

PREVALENCE OF PERINATAL INFECTION IN SOUTHERN ODISHA, INDIA.

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ABSTRACT: AIM: This study was undertaken to evaluate the prevalence of perinatal bacterial infection in southern part of Odisha. **MATERIALS AND METHODS:** Pregnant women presenting with premature rupture of membrane (PROM), preterm labour, fever, vaginal discharge (VD), urinary tract infections and previous bad obstetric history were included in the study groups. Besides newborns < 7 days old admitted to Paediatrics Department were also included. High vaginal swab and blood sample were collected from mother whereas from neonate cord blood and umbilical swab were taken. **METHODS:** Microscopy, culture for aerobic bacteria and serology for C. trachomatis were done. **RESULT:** Total number of cases proven to have microbiological infections based upon laboratory results. Maximum percentage of PNI cases were primigravida belonging to the age group of 20-30years and most common bacterial infections were caused by S. aureus & C. trachomatis. Ampicillin, Cefuroxime & Gentamicin were found to be the most effective antibiotics for bacterial isolates.
KEYWORDS: PROM – premature rupture of membrane, VD – vaginal discharge, PNI – Perinatal infection

INTRODUCTON: Perinatal infection (PNI) means the infection transferred from the mother to the baby after 28th week of gestation and up to 7 days after delivery. [1, 2] These infection leads to suffering, incapacitation, occasional permanent disability of child and also maternal and neonatal morbidity and mortality. Still it is one of the most neglected aspects of women and child health care. The global incidence of neonatal sepsis is 10-20 per 1000 live births. [3] In India the incidences of sepsis is 2-10 cases per 1000 live births and have a high risk of mortality approximately 25-30% i.e. about up to 7, 50,000 deaths every year. [3] Data from national neonatal and perinatal data base 2000 suggests that Escherichia coli, Klebsiella spp. and Staphylococcus aureus are the common causes of neonatal sepsis in India. The perinatal infection affects neonates widely in various ways. Infection is the most important cause of premature rupture of membrane, preterm labour and pPROM all of which lead to low birth weight baby. Due to low immune status, a newborn is highly vulnerable to infections. The PNMR

(Perinatal mortality rate) reflects the quality of medical care. The PNMR in developing countries is three fold to five fold higher than in the developed countries.

MATERIALS & METHODS: The prospective study was carried out, in the Department of Microbiology, MKCG Medical College Hospital, Southern Odisha, India over a period of 22 months, in collaboration with departments of Obstetrics/Gynaecology & Paediatrics.

CASE SELECTION: 150 pregnant women admitted to the labour room, belonging to the age group of 15 to 40 years. The study group included females with premature rupture of membrane, preterm labour, fever, vaginal discharge, UTI and previous bad obstetric history. A matching group of 100 patients without such complaints admitted to labour room for safe confinement were included as controls. The newborns (<7 days) born to the pregnant women & subsequently admitted to Paediatrics Dept. and NICU were also included in the study.

SPECIMEN COLLECTION AND PROCESSING: MOTHER: Three high vaginal swabs/endocervical swabs were collected from the patients at the time of admission to labour room. One swab was used for direct microscopic examination, another swab was put in 0.9% saline for direct wet mount microscopy and yet another swab was used for aerobic isolation of micro-organism. A blood sample of 3 ml was collected from patients for serological study of C. trachomatis, and 5 ml blood for culture in puerperal sepsis.

NEONATE: Two umbilical swabs were collected for direct microscopy and aerobic isolation of organisms by culture after 24 hrs of birth. 2ml of blood collected for culture in neonatal septicaemia.

ANTIBIOTIC SUSCEPTIBILITY TESTING: Antibiotic susceptibility testing was performed by Kirby and Bauers standard disc diffusion method.

SEROLOGY: Out of 150 cases 60 cases with bad obstetric history were subjected for IgM ELISA test (By Novatec C. trachomatis IgM ELISA test kit) for C. trachomatis.

RESULT: Maximum cases were primigravida belonging to the age group 26 – 30 years followed by 21-25 years. Least number of cases was primigravida belonging to the age group 36-40 years. Maximum numbers of neonates were born from primigravida belonging to the age group 26-30 years (TABLE-I).

PROM, 43 (28.6%) was the single most common presentation in these patients (Fig: 1). Out of 150 cases, gram's staining revealed gram positive cocci in 50 (33.3%), gram negative bacilli in 35 (23.3%), clue cells in 28 (18.6%), and gram positive budding yeast cell in 10 (6.6%). Among 100 controls, gram positive cocci, gram negative bacilli, clue cells and gram positive budding yeast cell were found to be 18%,5%, 5% & 2% cases respectively (Fig: 2).

S. aureus (28%) was found to be the most common pathogen on culture isolation, followed by E. coli (20%) in the cases. In control group S. epidermidis (25%) was the most commonly isolated bacteria (TABLE-II).

Gentamicin is the most sensitive drug for gram positive isolates in new born where as ampicillin was the most effective drug followed by azithromycin & penicillin in mother (TABLE-III).

All gram-negative bacilli showed 100% resistance to ampicillin in mother. Cefuroxime was the most sensitive drug followed by cephalexin and cefepime. Gentamicin is the most sensitive drug for gram negative isolates in new born (TABLE-IV).

16 (26.6%) out of 60 cases were positive for C. trachomatis by Ig M ELISA test and only 1 (5%) out of 20 controls were showing seropositivity (Fig.3).

DISCUSSION: Microbial flora of the female genital tract presents as an extreme and diversified spectrum of pathogenic and non-pathogenic organisms. While Chlamydia trachomatis and Group B β- hemolytic Streptococcus are associated with PNI, bacterial vaginosis caused by various other pathogens, has emerged as another risk factor for PNI.[4] In this study association of selected bacterial pathogens present in the mother and transferred to baby in the causation of PNI has been studied. In our study, the proportion of antenatal mothers attending labour room were high in the primigravida belong to the age of group 26-30 years (Table-1). This is comparable with the study of I. Jehan et al. where participants were predominantly in the age group of 19-24 years. The age distribution of control was the same as that in antenatal mother with PNI.[5]

Lower age incurs an increased risk of PNI because of biological and behavioral risk factors. Adolescents tend to have cervical ectopy, which provides large zones of columnar epithelium for the targeted attachment of C. trachomatis and N. gonorrchea [4]. The present study showed the comparable observation (Table-1).

As in our study maximum no. of cases and controls were primigravida, the numbers of neonates were born maximum from primigravida belong to age group 26-30 years (Table-1).
Premature rupture of membrane was the most common symptom in cases (28.6%) found in our study (Fig-1). The other symptoms and signs were preterm labour, pPROM, vaginal discharge, puerperal sepsis etc.

In our study, direct microscopic observation by Gram's stain revealed gram positive cocci in cases 50 (33.3%), gram negative bacilli 35 (23.3%) & gram negative cocci 4 (2.6%) (Fig-2). As per Amsel's criteria for the diagnosis of G. vaginalis, clue cell were found in 28 (18.6%) cases out of 150 (Fig-2). [5, 6] They found that sexually transmitted cofactor may strengthen, the relation between bacterial vaginosis associated microorganisms and development of PNI. In some other studies the prevalence of bacterial vaginosis was reported to be 6-32%.[7] In our study bacterial vaginosis associated bacteria G. vaginalis was identified as gram variable pleomorphic coccobacilli mostly in clumps. Deborah Money [8] also used gram stain of vaginal fluid for the diagnosis of bacterial vaginosis in lieu of clinical criteria, given its greater reliability for diagnosis of bacterial vaginosis. A number of studies proved that apart from C. trachomatis, bacterial vaginosis has been a risk factor for preterm labour, premature rupture of membrane, preterm premature of membrane. [5, 6]

Gram – negative diplococci were found to be 4 (2.6%). They were diagnosed to be N. gonorrhoeae morphologically by gram stain, as the culture was very cumbersome and not done.
Bacterial culture findings of this study revealed isolation of S. aureus (28%) followed by E. coli (20%), Group B β- hemolytic streptococci (2.6%) and Enterococcus spp. (2.6%), klebsiella sp. (1.3%) and Pseudomonas aeruginosa (1.3%). Least was Proteus mirabilis (0.6%), but in control group maximum isolates were S. epidermidis (25%) (Table–2). As compare in neonates the isolates were of S aureus (17%), Group B β- hemolytic streptococci (1.3%), E. coli (8.8%), Klebsiella spp. (1.3%) and Pseudomonas aeruginosa (0.6%) (Table-2).

Serological tests were generally not useful in the diagnosis of genital tract infection caused by C. trachomatis and that the presence of immunoglobulin M (Ig M) antibodies was an unreliable marker of acute infection in adolescents and adults. Numazaki K et al reported that the rates of Ig M seropositivity for C. trachomatis during pregnancy were significantly higher in mothers who had given birth to infants with complications than in matched controls.[9] The frequency of chorioamnionitis and meconium stained amniotic fluid was also higher in the anti C. trachomatis Ig M antibody-positive pregnant women. Several investigations have reported that 2-20% of pregnant women harbour C. trachomatis in the endocervix. Ig M antibodies on the other hand may be short-lived. ELISA tests of patients suspected with C. trachomatis infection have recently been used as a diagnostic tool. Hence, we used this test to demonstrate Ig M antibodies in cases of clinically diagnosed PNI. Out of 60 cases, 16 cases (26.6%) were found to be seropositive for C. trachomatis (Fig-3). Only 1 (5%) seropositivity was found in control group (Fig-3).

Our data confirms the relationship between maternal infection and perinatal outcome and support the hypothesis that infections might be the cause of these conditions and not simply an association. For preventing perinatal infections, antenatal mothers should be screened and treated for these infections. From our study most of the gram positive cocci isolated were found sensitive to ampicillin, penicillin and azithromycin (Table-III). All the gram negative bacilli show 100% resistance to ampicillin. Most sensitive drug for gram negative bacilli is cefuroxime (Table- IV). Gentamicin was the most effective antibiotic for both gram positive cocci and gram negative bacilli in neonates (Table-III & IV).

CONCLUSION: The study was carried out over a period of 22 months in the Depart. Of Microbiology, M.K.C.G. Medical College, Southern Odisha, India. All the samples collected from both the cases and controls were subjected for bacteriological & serological analysis. In view of the above finding available from results in our study we could derive the following conclusions: incidence of PNI attending labour room was highest in primigravida belonging to the age group 26 – 30 years. Staphylococcus aureus and C. trachomatis were the most common aetiological agent of PNI. Other bacterial pathogens isolated in the culture were Gr. B β hemolytic Streptococci, Enterococci, E. coli and Klebsiella spp. Ampicillin, azithromycin, cefuroxime and gentamicin were found to be effective on the bacterial isolates.

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REFFERENCES:

1. K. Park, "Textbook of Preventive and social medicine", 19th edition, 1167, Prem nagar, Jabalpur, M.P. India, m/s Banarsidas bhanot Publisher, 2007.
2. D.C. Dutta, "Text Book of Obstetrics"; 4th Edn, 8/1 Chintamoni Das Lane, Kolkata, New Central book agency (P) Ltd., 2000.
3. Altha Roterti Edgren, Teresa G. Odle et al, Gale, Detrait, Gale Encyclopedia of Children's Health, 2006.
4. Basavaraj M. Kerur, B Vishnu Bhat, B.N. Harish, S. Habeebullah and C. Uday Kumar, "Maternal Genital Bacteria and Surface Colonization in Early Neonatal Sepsis", Indian J Pediatr, 2006; 73 (1): 29-32.

5. I. Jehan, H. Harris, S. Salat, A. Zeb, N. Mobeen, O. Pasha, E.M. McClure, J. Moore, L.L wright & R.L Goldenberg, "Neonatal mortality, risk factors and causes: a prosepective population based cohort study in urban Pakistan", bulletin of the WHO Volume 87, Number 2, February 2009, 130-138.
6. Gibbs R.S., "Severe infections in pregnancy", Med Clin North Am. 1989; 73:713-21.
7. Amsel R, "Non specific vaginitis, Diagnostic criteria and microbial and epidemiological associations", Am J Med 1983 Jan; 74 (1):14-22.
8. Deborah Money, "The laboratory diagnosis of bacterial vaginosis", Can J Infect Dis Med Microbial, 2005, Mar-Apr; 16 (2): 77-79.
9. Numazaki K, Wainberg MA, Mc Donald J, "Chalmydia trachomatis infections in infants", CMAJ 1989, 140: 615-22.

TABLE-I: Age distribution of antenatal mother and neonate of study group

Age (years)	Cases			
	Mother		Neonate	
	Primigravida	Multigravida	Primigravida	Multigravida
15-20	14	6	14	7
21-25	20	10	18	10
26-30	28	16	27	16
31-35	16	22	16	23
36-40	4	12	4	12

TABLE-II: Culture isolation of different bacteria in antenatal mother and neonate.

Bacteria	**Mother**		**Neonate**
	Cases	**Control**	
GPC			
S. aureus	42(28%)	15(15%)	25(17%)
S. epidermidis	12(8%)	25(25%)	4(2.6%)
GBS	4(2.6%)	0	2(1.3%)
Enterococci spp.	4(2.6%)	0	0
GNB			
E. coli	29(20%)	5(5%)	13(8.8%)
Klebsiella spp.	2(1.3%)	0	2(1.3%)
Pseudomonas aueroginosa	2(1.3%)	1(1%)	1(0.6%)
Proteus spp.	1(0.6%)	0	0

TABLE-III: Sensitivity pattern of GPC to various antimicrobials.

BACTERIA	MOTHR									NEONATE								
	Total no	P	A	Ox	Ch	Cp	Cu	At	Cd	Total no	A	Ak	G	Cf	Co	E	Cu	Ci
S. aureus	42	14	25	12	2	16	13	26	13	25	5	3	15	7	8	2	11	10
GBS	4	3	4	3	0	1	0	0	0	2	2	0	0	0	2	2	1	0

Enterococcus	4	2	1	0	2	0	2	3	2	1	0	0	0	0	1	0	1	1
Total no	50	19	30	15	4	17	15	29	15	28	7	3	15	7	11	4	13	11

(P – Penicillin, A – Ampicillin, Ox – Oxacillin, Ch – Cephalothin, Cp – Cephalexin, Cu- Cefuroxime, At – Azithromycin, Cd – Clindamycin, Ak – Amikacin, G – Gentamicin, Cf – Ciprofloxacin, Co – Cotrimoxazole, E – Erythromycin, Ci – Ceftriaxone)

TABLE-IV: Sensitivity pattern of GNB to various antimicrobials

BACTERIA	MOTHER						NEONATE								
	Total no	A	Cp	Cu	Cpm	At	Total no	A	Ak	G	Cf	Co	Ce	Cu	Ci
E. coli	29	0	22	13	11	11	13	2	8	9	3	4	2	5	1
Klebsiella	2	0	1	2	1	0	2	0	1	2	1	2	1	0	0
P.aeuroginosa	2	0	0	2	1	1	1	0	0	1	0	0	1	1	0
P.mirabilis	1	0	1	1	0	o	0	2	0	0	0	0	0	0	0
Total no.	34	0	24	18	13	12	16	2	9	12	4	6	4	6	1

(A – Ampicillin, Cp – Cephalexin, Cu – cefuroxime, Cpm – Cefepime, At – Azithromycin, Ak – Amikacin, G – Gentamicin, Cf – Ciprofloxacin, Co – Cotrimoxazole, Ce - Ceftazidime, Ci – Ceftriaxone)

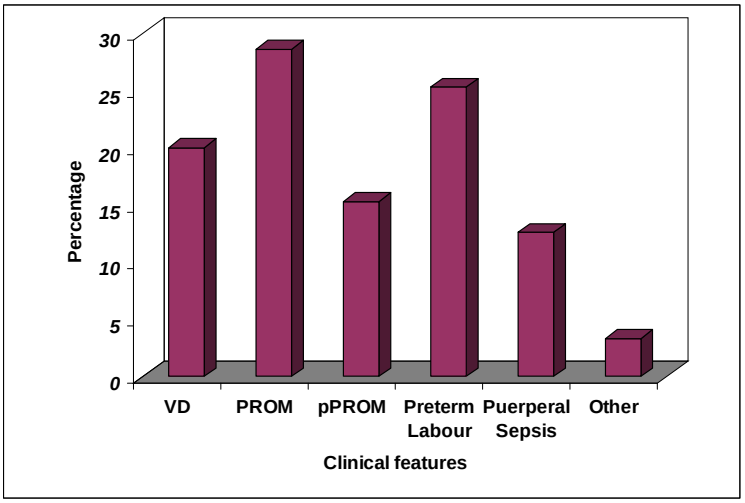

Fig-1: clinical features of the patients

(*VD: Vaginal discharge, **PROM: Premature rupture of membrane, ***pPROM: Preterm premature rupture of membrane.)

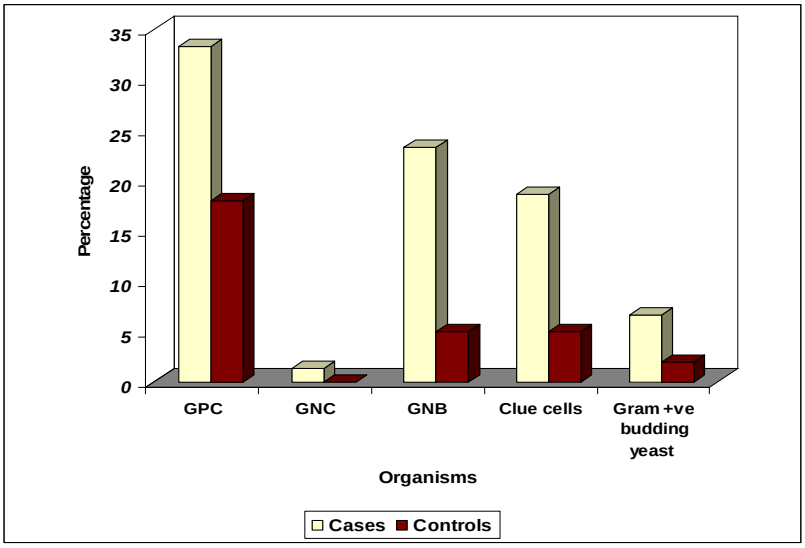

Fig-2: Microscopic observation by gram's stain method.

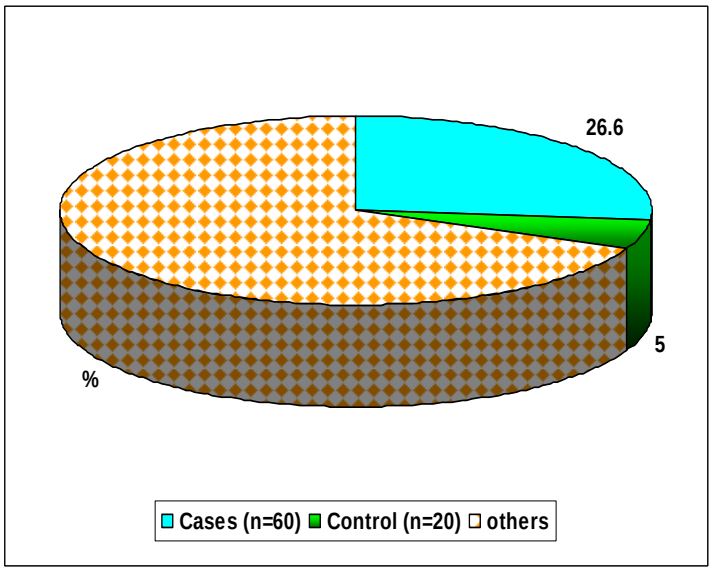

Fig. 3: Ig M ELISA test for Chlamydia trachomatis

MALIGNANCY IN AN AMPUTATED LEG STUMP – A RARE CASE

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ABSTRACT: Amputation of lower extremity followed by use of leg prosthesis is very common. However, malignancy arising in the amputation stump is extremely rare. We are reporting such a case in a 52-year-old man. Review of the English literature reveals only five additional cases occurring in men with an average age of 65 years and after a mean lag period of 40 years between the amputation and development of a low-grade squamous cell carcinoma.
KEYWORDS: Malignancy in amputation stump; Squamous cell carcinoma; Leg prosthesis.
MESH TERMS: Amputation Stumps; Carcinoma, squamous cell; Artificial Limbs

INTRODUCTION: Amputation of the leg followed by the use of artificial leg is very common. However, a malignant tumor arising in an amputation stump remains a very rare occurrence. The purpose of this paper is to report a case of squamous cell carcinoma arising in an amputation stump with a review of all other 5cases of malignancy arising in stumps published in the English literature.

CASE REPORT: A 52year old man sustained crush injury to left lower limb in a train accident 25 years back.

He underwent left above knee amputation and was fitted with an artificial limb. He had continued difficulty with proper fitting of the prosthesis over the years. Almost 25 years later, the patient started experiencing increased pain at the amputation site and noted foul smelling discharge from the area. The skin over the distal stump was macerated, ulcerated with purulent foul smelling exudate. He was managed in the wound care clinic with wound cleaning and antibiotics over the last 6months. During that period, the distal stump area developed a fungating tissue that bleeds on touch. He was unable to use the leg prosthesis. He is a known case of diabetes, taking treatment.

The amputated stump showed an irregular ulcer of 10cmx10cm with an exophytic growth of 6cm in its largest dimension. Ulcer had raised, everted edges that bleed easily on touch and an indurated base with foul smelling discharge (Fig.1). No inguinal lymphadenopathy, no evidence of metastatic spread seen.

Patient underwent a left above knee revision amputation with wide local excision of tumour margins. Specimen was sent for histopathological examination. Postoperative period was uneventful and patient was discharged on 10th postop day after suture removal.

Microscopically, well formed keratin pearls, stromal infiltration with lymphocytes and resected margins free of tumour cells were noted (Fig.2). Hence histopathology report notified features suggestive of well-differentiated type of squamous cell carcinoma.

DISCUSSION: Cutaneous carcinomata of the lower extremities seem to be never a primary condition [1]. About 60,000 leg amputations are performed each year in the United States. These patients are usually fitted with leg prosthesis. The distal aspect of the stump has the scar from surgical closure. Whereas malignant tumor, commonly a squamous cell carcinoma is known to occur in scarred tissue from burn, chronic ulcers, wounds, sinus and fistulous tracts [2,3], the occurrence of cancer in amputated leg stump is very rare.

The earliest report of a stump carcinoma that we could find in the English literature was in 1965 describing a 75-year –old white man who had undergone a left above the knee amputation following a railroad accident [4]. He was fitted with leg prosthesis. Forty-seven years later, the patient developed a well-differentiated squamous cell carcinoma at the amputation stump scar. He underwent a high thigh amputation and inguinal lymph node dissection. There were no metastases.

In 1985, a case of a 60-year-old man who developed a low-grade squamous cell carcinoma on the below-the-knee amputation stump was reported [5]. The patient had undergone amputation of his leg 30 years earlier because of traumatic injury and burn suffered in a dynamite explosion. He used an artificial leg since then. The patient developed the cancer in the burn scar rather than in the scar of stump closure. He was treated with above-knee amputation. There was no evidence of metastatic spread.

In 1991, there were two reports of carcinoma arising in leg amputation stump [6,7] In one report [6], a 56 –year –old man developed a rapidly growing squamous cell carcinoma at the end of his right below-knee amputation stump 27 years after his leg was amputated for gangrene from a snake-bite injury. He was using a poorly constructed prosthesis. The tumor was widely excised. The carcinoma arose in the scar tissue of the stump. There was no distant spread. The other case [7] was that of a 65-year- old man who developed a verrucous squamous cell carcinoma at the stump about 45 years after he underwent a traumatic below-the-knee amputation followed by use of leg prosthesis. The tumor was locally excised with a revision of the amputation.

The latest report of a carcinoma arising in an amputation stump was that of a 62-year-old man who developed a well-differentiated squamous cell carcinoma on his right thigh amputation stump 52 years after the procedure [8]. The amputation was performed because of a land mine injury. He was using a leg prosthesis ever since. The patient underwent a surgical revision of the amputation stump and inguinal lymph node dissection. There was no metastatic spread.

Malignancy occurring in leg amputation stump remains a rare event as evidenced by only six such reported cases (including the present case) in the English literature since 1965.All the patients were male, aged 56 to 75 years with a mean age of 63 years. Amputation was done mostly for trauma [4,5,7,8]. In two cases [5,6], burn from dynamite and mine explosion was an additional factor. The time between the amputation and the development of malignancy at the stump ranged from 27 to 52 years with a mean lag period of 40 years. All patients developed low-grade squamous cell carcinoma, of which two patients showed a verrucous type of squamous cell carcinoma.

The factors that may lead to malignancy at a stump may include long-standing scar tissue and burn injury. Patients who underwent amputation at an older age may not live long enough to develop stump carcinoma because the average lag period of developing such a lesion is 40 years. The patients who have undergone amputation at a younger age may have a small risk of developing malignancy at the stump over their lifetime.

Neoplasms associated with scars and chronic ulcers are well documented for humans. These lesions comprise approximately 2% of all human epidermal neoplasms. Squamous cell carcinoma is the most common tumor and typically occurs 20 to 40 y after the initial injury. Rarely, the original wound does not heal, and a neoplasm appears within a relatively short time (3 to 12 mo). These neoplasms are termed "acute wound cancers," since they appear relatively rapidly compared with other scar-associated neoplasms [9].

Carcinoma arising from scar, accounts for 0.78% of all inpatients with scars and 17% of all skin carcinoma patients. Scar carcinoma is a highly differentiated squamous cell carcinoma in most of the cases. Because of scar fibrosis and thrombosis of blood vessel and lymphatic vessel, the growth of scar carcinoma is slow. The metastasis is restricted. If the carcinoma can be diagnosed and treated early, the prognosis is favourable. The principle of operation is wide excision of the lesion [10].

The clinical appearance of skin metastases varies over a wide morphologic spectrum, cutaneous metastases mimicking herpes zoster being rare. zosteriform cutaneous metastases secondary to a squamous cell carcinoma (SCC) which developed in the stump of an amputated arm is reported. The pathogenesis is speculative, but the zosteriform distribution might well be explained by perineural lymphatic invasion and spread [11].

Marjolin's ulcers are uncommon malignancies arising from previously traumatized, chronically inflamed or scarred skin. They are usually squamous cell carcinomas and they may present decades after the original insult. Marjolin's ulcers may occur on stumps in patients using prostheses, in this situation the diagnosis may be delayed because of the false assumption that the ulcer is caused by an ill fitting prosthesis [12]. A high index of suspicion is required in the management of chronic non-healing ulcers and all suspected lesions should be biopsied [13]. The squamous cell carcinoma of Marjolin's ulcer has the worst prognosis in comparison with other squamous cell carcinomas and it requires an aggressive treatment [14].

Epithelioma cuniculatum (EC) belongs to the category of verrucous carcinomas. Invasiveness and rate of metastasis are low, but there is a high risk of local recurrence. In cases of long-standing processes with formation of exophytic, malodorous tumors with jagged edges that do not respond to conventional therapy, consideration should already be given to EC upon visual inspection. The diagnosis is always established by histological examination. The standard treatment of EC is extensive excision of the tumor with micrographic margin control [15].

REFERENCES:

1. De Asis C. CUTANEOUS CARCINOMA OF THE LOWER EXTREMITIES: A STUDY OF CASES AT THE BARNES AND THE BARNARD FREE SKIN AND CANCER HOSPITALS OF ST. LOUIS, MO. Ann Surg. 1926 May;83(5):663-81.
2. Arons MS, Lynch JB, Lewis SR, Blocker TG. Scar tissue carcinoma. Part 1. A clinical study with special reference to burn scar carcinoma. Ann Surg 1965; 161:170-188.
3. Bartle EJ, Sun JH, Wang XW, Schneider BK. Cancers arising from burn areas. A literature review of twenty-one cases. J Burn Care Rehab 1990; 11:46-49.
4. Wilhite JL. Carcinoma developing in a pre-existing scar. A case report of carcinoma of amputation stump. Va Med Mon 1964; 91: 292-294.
5. Sarma D, Weilbaecher TG. Carcinoma arising in burn scar. J Surg Oncol 1985; 29:89-90.
6. Mahaisavariya B, Mahaisavariya. Marjolin's ulcer complicating a poorly fabricated prosthesis. Injury 1991; 22: 423-424.

7. Schwartz RA, Bagley MP, Janniger CK, Lambert WC. Verrucous carcinoma of a leg amputation stump. Dermatologica 1991; 182: 193-195.
8. Karakozis S, Stamou SC, He P, Smookler B, Caceres M. Carcinoma arising in an amputation stump. Am Surg 2001; 67: 495-497.
9. Kathleen Rogers, George M. Barrington, Steven M. Parish. The Canadian Veterinary Journal. 1997 Oct; 38(10)643-644
10. Ji ZL. [Skin carcinoma arising from scar (a report of 6 cases)]. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi. 1993 Nov;9(6):428-30, 478. Chinese. PubMed PMID: 8149290.
11. Cuq-Viguier L, Viraben R. Zosteriform cutaneous metastases from squamous cell carcinoma of the stump of an amputated arm. Clin Exp Dermatol. 1998 May;23(3):116-8. PubMed PMID: 9861739.
12. Bloemsma GC, Lapid O. Marjolin's ulcer in an amputation stump. J Burn Care Res. 2008 Nov-Dec;29(6):1001-3. PubMed PMID: 18849841.
13. Chalya PL, Mabula JB, Rambau P, Mchembe MD, Kahima KJ, Chandika AB, Giiti G, Masalu N, Ssentongo R, Gilyoma JM. Marjolin's ulcers at a university teaching hospital in Northwestern Tanzania: a retrospective review of 56 cases. World J Surg Oncol. 2012 Feb 15;10:38. PubMed PMID: 22336561; PubMed Central PMCID: PMC3292918.
14. Shahla A. An overview of heel Marjolin's ulcers in the Orthopedic Department of Urmia University of Medical Sciences. Arch Iran Med. 2009 Jul;12(4):405-8. PubMed PMID: 19566359.
15. Weberschock T, Flux K, Gholam P, Hartschuh W, Hartmann M. [Verrucous tumor of the amputation stump]. Hautarzt. 2010 Mar;61(3):250-4. German. PubMed PMID: 20127299.

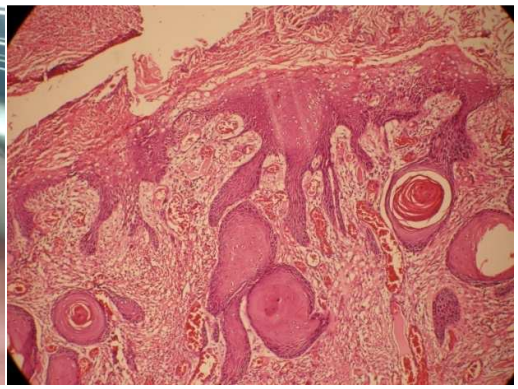

Fig.1 Irregular ulcer with exophytic growth Fig.2 Well-differentiated squamous cell carcinoma

JUVENILE IDIOPATHIC ARTHRITIS – A CASE REPORT

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ABSTRACT: The prevalence of Juvenile idiopathic arthritis (JIA) is 0.86 per 1000 children. Subcutaneous nodules have been reported in 5% to 10% of children with JIA. Approximately 90% of patients with RA and subcutaneous nodules test positive for rheumatoid factor (RF), and approximately 40% of all RF-seropositive patients with RA have subcutaneous nodules, whereas only 6% involvement is seen in seronegative cases. We hereby report a case of atypical Juvenile idiopathic arthritis (JIA) in a 6 year old, female child with joint pain & myalgia along with subcutaneous nodules over the dorsum of feet, hands and elbows. Joint pain initially involving the left ankle, slowly progressed to involve the knee, shoulder, wrist, metacarpophalangeal and interphalangeal joints over a period of one year. Joint involvement was not symmetric. RF was Negative. Fundoscopy examination was normal. Histopathological examination revealed a central zone of Fibrinoid necrosis surrounded by epithelioid histiocytes and occasional lymphocytes. Differential diagnosis of Rheumatoid Nodule (RN) or Subcutaneous Granuloma Annulare (SGA) or Necrobiosis Lipoidica Diabeticorum was made. In light of clinicopathological findings, both SGA and NLD were ruled out and the diagnosis of Juvenile idiopathic arthritis presenting as RF-negative polyarthritis was made.

KEYWORDS: juvenile idiopathic arthritis, rheumatoid arthritis, rheumatoid nodule.
MESH TERMS: Nil

INTRODUCTION: Juvenile idiopathic arthritis (JIA), previously known as juvenile rheumatoid arthritis encompasses all forms of arthritis that develop before 16 years of age and persist for a minimum of 6 weeks. Rheumatoid nodules (RN) are the most common cutaneous lesion in adult cases of rheumatoid arthritis (RA) and are present in 25% cases (both oligoarticular & polyarticular) [1]. A much greater incidence (75%) is observed in those with RA-associated Felty syndrome. [2] Approximately 90% of patients with RA and subcutaneous nodules test positive for rheumatoid factor (RF), and approximately 40% of all RF-seropositive patients with RA have subcutaneous nodules, whereas only 6% involvement is seen in seronegative cases. [3] Rheumatoid nodules are clinical predictors of more severe arthritis, seropositivity, joint erosions, and rheumatoid vasculitis. It has been suggested that the presence of RNs often requires more aggressive treatment of the underlying RA to prevent sequelae. However, in some cases disease

progression is independent of nodular disease activity. The prevalence of JIA is 0.86 per 1000 children. Subcutaneous nodules have been reported in 5% to 10% of children with JIA. [4] Rheumatoid nodules are almost always confined to those children with polyarticular arthritis i.e. involving ≥5 joints in first 6 months. [5] Genetics seems to play a role in the appearance of RNs. The HLA-DR4 haplotype (including the heterogeneous group of DRB1 alleles) is predictive of the risk of subcutaneous nodules in RA. [6]

CASE HISTORY: We hereby report a case of an atypical Juvenile idiopathic arthritis in a 6 year old, female child with joint pain & myalgia. Joint pain initially involved the left ankle, slowly progressing to involve the knee, shoulder, wrist, metacarpophalangeal and interphalangeal joints over a period of one year. Joint involvement was not symmetric. There was presence of subcutaneous nodules over the dorsum of feet [Figure 1], hands and elbows. Clinical diagnosis of Rheumatoid arthritis was made.

On investigating, rheumatoid factor was negative. Serum ANA and anti-CCP (cyclic citrullinated peptide) antibody tests were advised but not performed. Fundoscopy examination was normal. A skin biopsy was taken from the site of the subcutaneous nodule from the dorsum of the feet. Histopathological examination of the subcutaneous nodule revealed a central zone of fibrinoid necrosis surrounded by epithelioid histiocytes and occasional lymphocytes. [Figures 2 & 3] Differential diagnosis of Rheumatoid Nodule or Subcutaneous Granuloma Annulare (SGA) or Necrobiosis Lipoidica Diabeticorum (NLD) was made.

DISCUSSION: A diagnosis of rheumatoid nodules is made in the clinical context of the disease. Although biopsies of subcutaneous nodules are occasionally done, they are not useful for diagnosis. [7] The 2010 ACR-EULAR criteria of rheumatoid arthritis has not taken into consideration the presence of rheumatoid nodules or radiographic joint damage because these findings occur rarely in early RA. [8] Many different types of subcutaneous nodules are histologically identical to rheumatoid nodules; mainly NLD and SGA, which are often misdiagnosed as RNs. [9]

In general, RNs tend to be larger and to be confined to the deep dermis or subcutis, at the bony prominences, commonly located at the ulnar aspect of the forearm, elbows, occiput, and lumbosacral area. In our case, the nodules were present on bony prominences of the dorsum of feet and hands. Microscopically they have a central zone of fibrinoid necrosis surrounded by a prominent rim of epithelioid histiocytes and numerous lymphocytes and plasma cells. [1] Fibrin may be deposited in the centre of the granulomas, as opposed to the mucin deposition of SGA. [10] However, RNs with abundant mucin may be present. [11] Therefore histopathological differentiation between RNs and SGA is difficult.

Serum IgM RF has been found in 75–80% of patients with RA [8]; therefore, a negative result does not exclude the presence of this RA in our patient.

Subcutaneous granuloma annulare (SGA) is one of the most common dermatoses with the involvement of skin and/or subcutis, usually seen in adults and children, but the aetiology and pathogenesis are unclear. [12] It most often manifests as a large, asymptomatic soft tissue mass. Although nodules are usually stable for months, they may rapidly enlarge over the course of weeks. The typical lesions of SGA are single or multiple, small, pinkish, nonulcerated nodules in the deep subcutaneous tissue. The most

common lesion location is lower extremity, especially the peri-tibial area, followed by the hands. On histological evaluation, these nodules are similar to the nodules seen in adults with rheumatoid arthritis and to the lesion recognized in adult diabetic patients as necrobiosis lipoidica diabeticorum. [13] Characteristically, the necrobiotic focus is not so large or deeply situated as those of rheumatoid nodules or as broad and diffuse as those of necrobiosis lipoidica (diabeticorum). [9] In our case, one year history of joint pain and migratory polyarthritis along with subcutaneous nodules ruled out SGA and NLD.

Necrobiosis Lipoidica is a well-recognized dermatologic complication of diabetes mellitus and may develop in both juvenile (Type I) and adult-onset (Type II) diabetes mellitus. Positive family history of diabetes mellitus has also been one of the risk factors. [13] The sclerodermatous plaque is round to oval, red-brown with an elevated rim. Like SGA, the peri-tibial region is the most common site. Clinical correlation must be taken into consideration before making the diagnosis of NLD. [11] Our patient was not diabetic nor gave any positive family history for diabetes mellitus. The patient was successfully treated with steroids and has not shown sustained disease or diabetes mellitus over a one year follow up.

In light of clinicopathological findings, the diagnosis of Juvenile idiopathic arthritis presenting as RF-negative polyarthritis was made. We thus believe that histopathology of rheumatoid nodule presents a special challenge to a pathologist. Relevant clinicopathological details are essential for diagnosis and appropriate management.

REFERENCES:

1. Kumar V, Abbas A K, Fausto N, Aster J C. Robbins & Cotran Pathologic Basis of Disease. 8 th Edition. Philadelphia: Saunders Elsevier; 2010.
2. Sayah A, English JC 3rd: Rheumatoid arthritis: A review of the cutaneous manifestations. J Am Acad Dermatol 53:91-209, 2005
3. Kaye BR, Kaye RL, Bobrove A: Rheumatoid nodules. Review of the spectrum of associated conditions and proposal of a new classification, with a report of four seronegative cases. Am J Med 76:279-292, 1984
4. Vicente García-Patos. Rheumatoid Nodule. Semin Cutan Med Surg 26:100-107 © 2007 Elsevier Inc
5. Sampath Prahalad and David N Glass. Is juvenile rheumatoid arthritis/juvenile idiopathic arthritis different from rheumatoid arthritis?
6. Ahmed SS, Arnett FC, Smith CA, et al: The HLA-DRB1*0401 allele and the development of methotrexate-induced accelerated rheumatoid nodulosis: A follow-up study of 79 Caucasian patients with rheumatoid arthritis. Medicine 80:271-278, 2001
7. Patricia T. Ting and Benjamin Barankin. Dermacase. VOL 5. JANUARY 2005 Canadian Family Physician.
8. Longo DL, Fauci AS, Kasper DL et al. Harrison's Principles of Internal Medicine. Volume 2. 18th edition, New York. McGraw Hill Publications. 2012.
9. Hakan Sabuncuoglu et al. Subcutaneous Granuloma Annulare of the Scalp in Childhood: A Case Report and Review of the Literature. Turkish Neurosurgery 2007, Vol: 17, No: 1, 19-22
10. Mills SE, Carter D, Greenson JK et al. Sternberg's Diagnostic surgical pathology. 5 th Edition. Philadelphia. Lippicott Williams & Wilkins. 2010.

11. Mckee P H, Calonje E, Granter S R. Pathology of the skin with clinical correlations. Volume 1. 3 rd edition. Philadelphia. Elsevier Mosby. 2005.
12. Requena L, Fernandez- Figueras MT. Subcutaneous granuloma annulare. Semin Cutan Med Surg 2007; 26: 96-9
13. Grogg KL, Nascimento AG. Subcutaneous granuloma annulare in childhood: clinicopathologic features in 34 cases. Pediatrics: 107(3):42, 2001

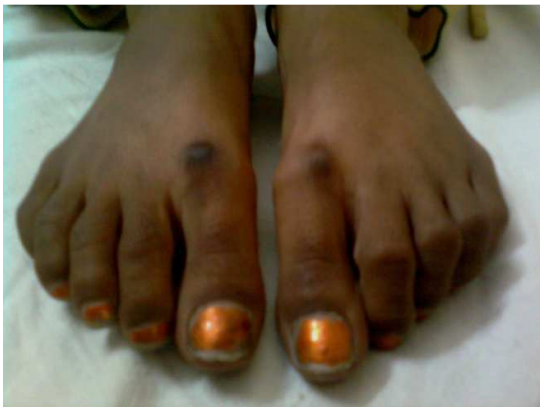

Figure 1. Subcutaneous nodules present on the dorsum of foot.

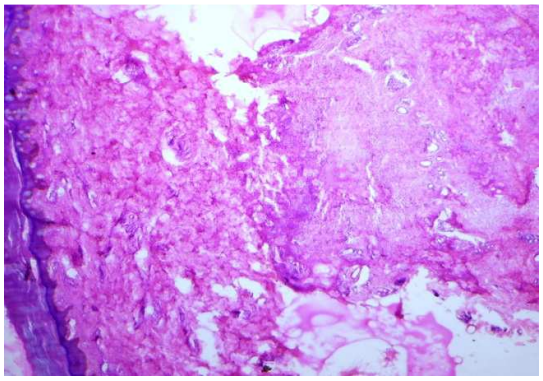

Figure 2. Low power view through subcutaneous nodule showing a necrobiotic focus situated in the deeper dermis. (10x; H&E)

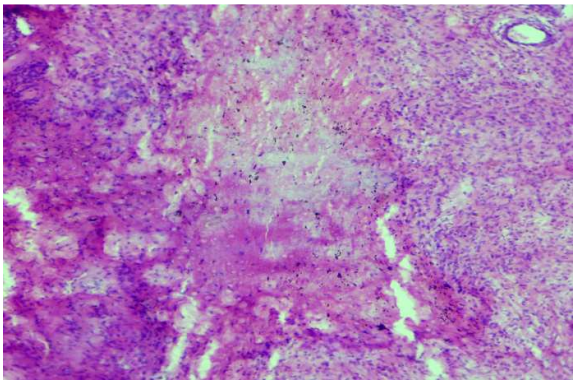

Figure 3. Necrobiotic focus showing central fibrinoid necrosis surrounded by histiocytes and lymphocytes (40x; H&E)

ANTIBACTERIAL EFFECT OF CALCIUM HYDROXIDE IN DIFFERENT VEHICLES

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ABSTRACT: AIM: This study evaluated the antibacterial effect of calcium hydroxide in different vehicles in an in vitro model. **MATERIAL AND METHODS:** Calcium hydroxide paste prepared with two conventionally used vehicles namely, camphorated monochlophenol, distilled water and also propylene glycol. The antibacterial activity of these paste were tested against five micro- organisms that can commonly occur in the infected root canals. **RESULTS AND CONCLUSIONS:** The results of the study indicate that a paste of calcium hydroxide made with propylene glycol exerts significant antibacterial action. Hence, it can be recommended for use as an intracanal medicament in preference to a paste prepared with a tissue toxic phenolic compound like camphorated mono chlorophenol.
KEY WORDS: Calcium hydroxide, camphorated monochlorophenol, propylene glycol.

INTRODUCTION: The endodontist today, concentrates more upon instrumentation, irrigation thorough cleaning and shaping of the root canal for elimination of responsible microbial flora from the root canal. [12], [15],[17],[21]

With due respect to all norms laid down in the field of endodontics, the review of literature is suggestive that establishing the complete debridement and disinfection of the root canal contributes to maximum therapeutic success.

In routine clinical practice, disinfection of the root canal is established by comprehensive effect of biomechanical preparation, use of intra canal irrigation and intra canal medicament. [1], [23],[14]

Calcium hydroxide is known to endodontists for its application from many years since its introduction by Herman in 1930.

In present day, it has become more popular as intra canal medicament. Its bactericidal or bacteriostatic action is related to its dissociation into calcium and hydroxyl ions thereby creating alkaline environment in its vicinity, not allowing the growth of acidophilic microorganisms. In addition to this it also inhibits the enzymatic activity that is essential for microbial growth. [8],[9]

To facilitate its application in the field of endodontics, it is generally mixed with vehicles like camphorated mono chloro phenol, distilled water, glycerin and propylene glycol. Various studies are carried out to identify or to understand the most suitable vehicle and also to find out the effect of vehicle on the action of the calcium hydroxide as an intra canal medicament. Observations followed to such study suggest that calcium hydroxide when mixed with water as

a vehicle promotes the dissociation and initiates the meditational action of the calcium hydroxide.

Calcium hydroxide when mixed with camphorated mono chlorophenol has dual effect vehicle as well as additional antibacterial action.

Calcium hydroxide when mixed with propylene glycol also has dual effect like camphorated mono chlorophenol. However, it exhibits no tissue irritating effect and being alcoholic in nature, it remains in paste form for longer time.

However there are very few study related to propylene glycol that serves as better vehicle when mixed with calcium hydroxide to form a paste for the purpose of intracanal medicament.

It was purposed to comparatively evaluate the antibacterial effect of calcium hydroxide when mixed with distilled water, camphorated mono chlorophenol and propylene glycol.

MATERIAL AND METHODS: Infected Root Canal Flora Commonly Consist of gram positive cocci like streptococci, staphylococci, gram positive bacilli like acidophillus, lactobacilus, actinomycotis fungi like candida albicans, gram negative organism like E-coli, pseudomonas.

More common and more resistant organisms in root canals are streptococci and E. Faecalis respectively.

Considering infected microbial flora of infected root canal it was purposed to obtained the five micro organisms samples from the stock culture of the department of microbiology, grand medical college, Sir. J.J. Group of Hospital Mumbai, namely

1) H. Streptococcus,
2) Enterococcus Faecalis
3) Staphylococcus aureus
4) Pseudomonas aeruginosa
5) Candida albicans

Calcium hydroxide powder was pre-weighed (1 gm.) and stored in autoclaved vials.
Calcium hydroxide paste preparations used in the study are:

1. Calcium Hydroxide and distilled water
2. Calcium Hydroxide and propylene glycol 90%
3. Calcium Hydroxide and propylene glycol 100%
4. Calcium Hydroxide and camphorated monochlorophenol

The ratio used in this study to prepare paste was 1:1 that 1gm calcium hydroxide powder with 1 CC of appropriate liquid. Sterile instrument were used for each preparation. Such prepared sample pastes were divided in to 3 different groups namely

Group 1

Calcium hydroxide & distill water (control group), 1gm of calcium hydroxide powder mixed with 1cc of distilled water.

Group 2

Calcium hydroxide & propylene glycol (Experimental group).
Group two was experimental group, which was further subdivided in to two sub groups

Group 2 A: - Calcium hydroxide & propylene glycol 100%, 1gm. of calcium hydroxide powder mixed with 1 cc 100% propylene glycol.

Group 2 B: - Calcium hydroxide & propylene glycol 90%, 1gm. of calcium hydroxide powder mixed with 1 cc 100% propylene glycol.

Group 3 :- Calcium hydroxide & Camphorated monochlorophenol 1gm. of calcium hydroxide powder mixed with 1 cc camphorated monochlorophenol.

Solid media were used for propagating the organisms, which were tested for sensitivity by ditch plate. Glucose broth, peptone water, nutrient broth for aerobes and Sabouraud's dextrose broth for fungal organisms.

Four wells of 5 mm diameter were cut on each solid media using a sterile metallic punch fitted with bulbed teat. A standard loop with an internal diameter of 4 mm, which could deliver 0.01 ml of the suspension of culture of test organism, then spread a cross the plate using sterile cotton swab.

The wells were then filled with the 4 different preparation of calcium hydroxide and the culture plates were then incubated.

The plates of aerobic organisms were incubated aerobically at 37oC and the zone of inhibition measured after 24, 48 and 72 hours accordingly.

The antibacterial sensitivity pattern represented as the zone of inhibition at its maximum diameter was measured around each well using a caliper and results observed were tabulated.

RESULTS: In the present study, in group one (1) was control group where one gram of calcium hydroxide powder was mixed with one cc of distilled water to form a paste. The result showed that calcium hydroxide distilled water paste produced zone of inhibition that was significantly smaller than other groups, only Candida albicans exhibited a large zone of inhibition. It requires more incubation period. It is, well-established fact that calcium hydroxide has antibacterial property. When calcium hydroxide paste prepared with distilled water, water acts as a vehicle only. It does not possess antibacterial property.

In-group 2 where 1 gram of calcium hydroxide powder was mixed with 1cc of propylene glycol to form paste

Group 2 further divided into two sub groups

Group 2A, where 1gm. of calcium hydroxide powder mixed with 1cc of 100% of propylene glycol.

Group 2 B, where 1 gm. of calcium hydroxide powder mixed with 1cc of 90% propylene glycol.

It was observed in the present study that among all the calcium hydroxide paste, only calcium hydroxide propylene glycol paste remain in the paste form for longer period of time which indicate his good handling qualities and exerts significant antibacterial action. The average diameter of the zone of inhibition for both groups calcium hydroxide and 90 % propylene glycol and calcium hydroxide and 100% propylene glycol combination were similar.

In group-III, where 1gm of calcium hydroxide powder was mixed with 1cc of camphorated Mono Chloro Phenol (CMCP) to form a paste. These pastes gave the best results as a maximum zone of inhibition.

Different preparation of calcium hydroxide used in this study produced some degree of inhibition of growth of the test organisms including enterococci. The average diameter of the zones of inhibition for aerobic organisms is shown in table 1. The inhibition of growth was

exhibited as clear zone, around the well. It had the appearance of a fresh agar surface and was smooth and clear. Besides the zone of inhibition, there was another distinct zone, which was white in color. The zones sometimes extended even beyond the inhibition zone and appeared to be the diffusion of Calcium hydroxide through the agar medium. There was also a slight brownish discoloration around the immediate periphery of the phosphates due to the increased alkaline PH in this area of the agar.

Among the various preparation of Calcium hydroxide, Calcium hydroxide camphorated monochlorophenol combination demonstrated the highest degree of growth inhibition; the entire organisms exhibited a fairly high sensitive to this paste.

The average diameter of the zone of inhibition for both Experimental group Calcium hydroxide with 90% propylene glycol & Calcium hydroxide with 100% propylene glycol combinations were equal. In case of Calcium hydroxide and distilled water paste, as a control group, only Candida albicans exhibited a large zone of inhibition.

There was distinct growth inhibition observed with enterococcus on 3 days of incubation with Calcium hydroxide camphorated monochlorophenol paste. Candida albicans showed no growth on the first day of incubation in all specimens. It required more incubation period.

Antibacterial Activity of Calcium Hydroxide with Various Vehicles

Sr.No.	ORGANISM (Fungal and Aerobic)	Zone Of Inhibition In MM				
		Days Of Incubation	CH+ Distilled Water	CH+ 90% PG	CH+ 100% PG	CH+ CMCP
	Candida Albicans	1st(no growth) Candida required more incubation period				
		2nd	22	24	24	34
		3rd	22	25	24	36
	Haemolytic treptococcus	1st	15	18	18	30
		2nd	16	19	19	32
		3rd	16	19	19	32
	Enterococcus	1st	R	R	R	16
		2nd	R	R	R	16
		3rd	R	R	R	16
	Staphylococcus Aureus	1st	13	13	14	30
		2nd	13	14	14	30
		3rd	13.5	16	16	32
	Pseudomonas Aeroginosa	1st	12	12	14	26
		2nd	12	12	14	29
		3rd	12	12	14	29

DISCUSSION: It is, well-known fact that success in endodontic therapy depends upon adhering strictly to various norms laid down namely diagnostic phase, preparatory phase and phase of obturation. [7], [13],[24]

The endodontist today concentrates more upon instrumentation, irrigation thorough cleaning and shaping of the root canal for elimination of responsible microbial flora from the infected root canal.

Calcium hydroxide is effective in eliminating microorganisms from the root canal space. [11]

Calcium hydroxide has bactericidal effect and its capacity to neutralize the bacterial endotoxins.[20]

In the present study, in group one as control group where one gram of calcium hydroxide powder was mixed with one cc of distilled water to form a paste. The result showed that calcium hydroxide distilled water paste produced zone of inhibition that was significantly smaller than other groups, only Candida albicans exhibited a large zone of inhibition. It requires more incubation period. It is, well-established fact that calcium hydroxide has antibacterial property. When calcium hydroxide paste prepared with distilled water, water acts as a vehicle only. It does not possess antibacterial property. [4], [6],[10],[18]

Paul K. evaluated the antibacterial efficacy of calcium hydroxide with different vehicles like distilled water, camphorated monochloro phenol, and propylene glycol. The results showed that calcium hydroxide paste with camphorated monochloro phenol, and propylene glycol was more effective as compare to the calcium hydroxide distilled water paste. [16]

Antony B studied the antibacterial property of calcium hydroxide with four different vehicles like distilled water, glycerin, camphorated monochloro phenol and propylene glycol. The result of the study showed that all the different preparation of calcium hydroxide paste with glycerin, camphorated monochloro phenol and propylene glycol was more effective in controlling the microorganisms as compared to calcium hydroxide distilled water paste. [3]

In-group II where 1 gram of calcium hydroxide powder was mixed with 1cc of propylene glycol to form paste

Group II further divided into two sub groups

Group II A, where 1gm. of Calcium Hydroxide powder mixed with 1cc of 100% of propylene glycol.

Group II B, where 1 gm. Of Calcium Hydroxide powder mixed with 1cc of 90% propylene glycol.

It was observed in the present study that among all the Calcium Hydroxide paste, only Calcium Hydroxide propylene glycol paste remain in the paste form for longer period of time which indicate his good handling qualities and exerts significant antibacterial action. The average diameter of the zone of inhibition for both groups Calcium Hydroxide + 90 % propylene glycol and Calcium Hydroxide + 100% propylene glycol combination were similar.

Antibacterial property of Calcium Hydroxide in different vehicles an in vitro evaluated by the Paulk 100% and 90% propylene glycol as vehicle used to make the paste with Calcium Hydroxide. The result of the study showed that the paste of the Calcium Hydroxide made with propylene glycol exert significant antibacterial action and it can be considered for use as an intra canal medicament in preference to the paste prepared with tissue irritating phenolic compound like camphorated monochloro phenol. [2]

Propylene glycol which was suggested by Laws in (1962) has been evaluated for its possible use as a vehicle in the field of endodontics. [5] It permits the release of Calcium and

Hydroxyl ions essential for the therapeutic action. It has been found to be antibacterial, not irritating to the periapical tissue. Both 90 and 100% propylene glycol Calcium Hydroxide paste showed antibacterial properties, which might be due to the slow diffusion of the Calcium and Hydroxyl ions as compared to the paste prepared with distilled water. The addition of 10% water was done in group II B with view of facilitating immediate ionization of the Calcium Hydroxide which is necessary for its action. The addition of water did not facilitate the enhancement of antibacterial action.

In group-III, where 1gm of calcium hydroxide powder was mixed with 1cc of camphorated Mono Chloro Phenol (CMCP) to form a paste. These pastes gave the best results as a maximum zone of inhibition. Its clinical use is objectionable due to its tissue irritating property. The size of the zone of bacterial inhibition does not necessarily reflect the strength of antibacterial agent. The zone size may be influenced by the molecular size of the chemical and its diffusion. An agent that diffuses more easily will exhibit a large zone and camphorated monochloro phenol has been shown to diffuse readily through blood agar medium. Since dis-infection of the root canal is established by comprehensive effect of Biomechanical Preparation, irrigation and Intra canal Medicament.

The loss of long-term activity can be attributed to the insoluble weak salt formed in combination with the hydroxyl ions dissociation, thus affecting the biological activity of calcium hydroxide. [19]

Calcium hydroxide Camphorated Mono Chloro Phenol paste has a tendency to become sticky and set fast.

Besides, camphorated monochloro phenol in volatile liquid can be lost from the paste rapidly.

The toxicity of phenol, camphorated phenol and camphorate mono chloro phenol was evaluated by Saekonto and confirmed the cytotoxicity of these antiseptic. They are irritants to the Periapical tissue. [22]

Even camphorated monochloro phenol alone or with calcium hydroxide is effective more than other two pastes but it is not prefer as it causes irritation to the periapical tissue.

Considering the result of the study and revive of literature, it can be said that: -

1. Both 90% and 100% propylene glycol paste of calcium hydroxide are bactericidal agents against microorganisms commonly found in infected root canals.
2. Camphorated monochlorophenol calcium hydroxide paste is most effective bactericidal agent as compared to other two pastes.
3. The routine use of propylene glycol in place of camphorated monochlorophenol can be recommended as vehicle for calcium hydroxide in view of its efficacy and non-irritating property to the periapical tissues.

CONCLUSIONS:

The results of this study are suggestive of: -

1. Both 90% and 100% propylene glycol paste of calcium hydroxide are bactericidal agents against microorganisms commonly found in root canals.
2. Calcium hydroxide camphorated monochlorophenol paste is more effective bactericidal agent as compared to the other two pastes.

3. The routine use of propylene glycol in place of camphorated monochlorophenol can be recommended as vehicle for calcium hydroxide in view of its efficacy and better handling properties.
4. The diffusion of calcium hydroxide propylene glycol paste along with tissue irritation and its efficacy in vivo, require further investigation.

REFERENCES:

1. Amanda Law, An evidenced based analysis of the anti bacterial effectiveness of intracanal medicaments. JOE,2004;30; 689-93.
2. Angky S. Shonei K., Shiro M., Kerichi O, Hideaki O. Toxicity of camphorated phenol and camphorated parachlorophenol in dental pulp cell culture. J. Endod 1996; 6 : 284-86.
3. Antony B, Comparitive evaluation of antibacterial efficacy of calcium hydroxide with four different vehicles. Ind JE 1997 : 9; 50 -55.
4. Behnen, Antimicrobial activity of several calcium hydroxide preparation in root canal dentin. J Endod 2000;27(12):765-67.
5. Bhat KS, Walvekar SV. Evalution of bactericidal property of propylene glycol for its possible use in endodontics. Arogya J. Health Sci 1975; 1:54-0.
6. Bystrom, The antimicrobial effect of camphorated monochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol1985;1:170-75
7. Cohen, Cleaning and shaping of root canal,Textbook of Pathways of pulp.
8. Estrela C. Control of micro-organisms invitro by calcium hydroxide paste. Int Endodon J 2001;34:341-345.
9. Estrela C, In vitro determination of direct antimicrobial effect of calcium hydroxide JOE,1998;24; 15-17.
10. Estrela C, Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Brazil Dent J. 1995;6:85-19.
11. Evans M, Sundqvist G, Mechanism in the resistance of E. faecalis to calcium hydroxide Int Endodont J, 2002;35:221-8.
12. Gianuck G, Shaping and cleaning the root canal system SEM study evaluation of new instrumentation and irrigation technique. JOE 1999;25; 800-03.
13. Grossman LI, Endodontic practice. 10th Ed. Philadelphia Lea and Febiger 1982;279(Ingle).
14. Gomes, In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of E. faecalis. JOE 2000;34; 424 – 28.
15. Keniolime, Chemomechanical reduction of the bacterial population in root canal after instrumentation and irrigation. JOE 2000.
16. Paul K, Antibacterial property of calcium hydroxide using different vehicles. Ind EJ 1997; 9 ; 43-49.
17. Peters LB, Effect of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical lesion. Int Endod 2002;35:13-21.
18. Roy HS, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament J. Endod 1983; 9 :372-74.
19. Safavi K, Influence of mixing vehicles on dissociation of calcium hydroxide in solution. J Endodon 2000;26:649-51.

20. Safavi and Nichols, Alteration of biological properties of bacterial lipopolysaccharides by calcium hydroxide treatment. J Endodon 1994;20:127-29.
21. Siqueira JF, Mechanical reduction of bacterial population in the root canal by in strumentation technique. JOE 1999;25; 332-35.
22. Soekanto , Toxicity of camphorated phenol and camphorated monochlorophenol in dental pulp cell culture. JOE 1996;22; 284-86.
23. Tu Chawchany, Effect of camphorated monochlorophenol on human periodontal cells in vitro. JOE 1999;25.
24. Wein, Basis of successful endodontic therapy, Textbook of endodontics. 1996 ; 05 ; 01-27.

A COMPARATIVE STUDY OF PRELOADING VERSUS COLOADING OF CRYSTALLOID TO PREVENT SPINAL ANAESTHESIA INDUCED HYPOTENSION

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ABSTRACT: CONTEXT: Preloading of crystalloid is a traditional practice to prevent spinal anaesthesia induced hypotension. But coloading seems to be more physiological and rational approach as effect was achieved during the time of spinal anaesthesia. **AIMS:** To compare crystalloid preload and coload for the prevention of spinal block induced hypotension in lower limb surgeries. Secondary outcomes included no. of dose of mephenteramine & atropine, bradycardia, nausea, vomiting, total volume of infusion, blood loss & urine output. **SETTINGS AND DESIGN:** Tertiary level, teaching hospital. Prospective, randomized study. **MATERIALS AND METHODS:** Total sixty patients of either sex, aged 20 to 60 years, scheduled for lower limb surgery under spinal anaesthesia were randomized into preload and coload group, 30 patients in each group. In preload group, 20 ml/kg of Ringer lactate was preloaded 20 minutes before commencement of spinal anaesthesia. In group Coload, 20 ml/kg of Ringer lactate was coloaded in 20 minutes just after lumbar puncture. **STATISTICAL ANALYSIS USED:** Quantitative data were compared with student's t test and qualitative data with chi-square or fisher exact test. $P<0.05$ was considered significant. **RESULTS:** patient characteristics were comparable in both groups ($p>0.05$). Mean baseline value and trends at various time intervals of heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure were comparable in both groups. Total incidence of hypotension in group Preload and group Coload were 13.33% and 10% respectively ($p=0.463$). Total incidence of bradycardia in group Preload and group Coload were 6.67% and 10% respectively ($p=0.394$). No. of dose of atropine to treat bradycardia was 2 out of 30 patients (6.67%) and 3 out of 30 patients (10%) for group P and C respectively ($p=0.394$). No. of dose of mephenteramine to treat hypotension was 4 out of 30 patients (13.33%) for each group ($p=1$). Incidence of nausea was 6.67% for group Preload and 10% for group Coload ($p=0.394$). Incidence of vomiting was 3.33% for group Preload and 6.67 % for group Coload ($p=0.278$). Total volume used for preloading was about 1474 ± 206 ml and for coloading was 1386 ± 176 ml ($p=0.0805$). Urine output in preloading and coloading groups was 223 ± 100 and 173 ± 89 ml respectively ($p=0.0453$). **CONCLUSIONS:** Coloading with 20 ml/kg of Ringer's lactate was as effective as preloading of same amount 20 minute before lumber puncture to prevent spinal induced hypotension.

KEY WORDS: preload, coload, ringer lactate, hypotension, spinal anaesthesia.

INTRODUCTION: Spinal anaesthesia is a popular and well-accepted technique for surgery below umbilicus in adult patients[1].

Hypotension following spinal anaesthesia (SA) is a common and troublesome complication[2]. Fluid administration before SA (preload) for the prevention of SA induced hypotension is a common and traditional practice in anaesthesia.

But, preloading of crystalloid is rapidly redistributed, and may induce atrial natriuretic peptide secretion, resulting in peripheral vasodilatation followed by an increased rate of excretion of the preloaded fluid. A more rational approach might be to apply fluid loading at the time that SA is starting to take effect. This might maximize intravascular volume expansion during vasodilatation from the sympathetic blockade and limit fluid redistribution and excretion[3].

The present study was undertaken to compare the efficacy of crystalloid (Ringer lactate) preload versus coload for prevention of spinal induced hypotension in patients scheduled for lower limb surgeries under SA.

MATERIALS AND METHODS: The prospective, randomized study was conducted in tertiary level hospital and teaching institute. Approval of institutional ethical committee and informed written consent of patients was obtained. Total sixty patients of either sex, aged 18 to 60 years, American Society of Anaesthesiologist (ASA) physical status 1 or 2, with weight < 90 kg and height >150 cm recruited for lower limb surgery under spinal anaesthesia. Exclusion criteria included obstetrical case, anatomically abnormal spine, hypertension, known cardiovascular disease, Hb less than 8gm/dl, bradycardia and any contraindications of SA. The patients were randomized using computer generated random numbers into group P (preload) and group C (coload) including 30 patients each.

All patients were fasted for 6 hours before surgery. On arrival to operation theatre, standard monitoring was instituted, including ECG, non-invasive blood pressure, pulse oximetry and baseline value were recorded. Baseline vitals of patient were recorded. 18 Gauge intravenous cannula was inserted in forearm.

In group P, 20 ml/kg Ringer lactate (RL) fluid was preloaded 20 minutes before commencement of surgery. In group C, 20 ml/kg RL fluid was coloaded in 20 minutes just after lumbar puncture (LP). Thereafter, all patients were infused 2 ml/kg of RL intraoperatively.

All patients received 3 ml of 0.5% hyperbaric bupivacaine intrathecally, in L3-L4 inter-vertebral space with 23 Gauge spinal needle. After spinal injection, the patients were put in supine position. A sensory level T8 was achieved using pin prick to 25 Gauge needle. Urinary catheterisation was done. Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), Mean blood pressure (MBP) and pulse oximetry (SPO2) were recorded every 5 minutes. Observations were recorded 50 minutes after administration of SA and the patients were handed over to the respective anaesthesia team for further management.

Hypotension was defined as fall in systolic blood pressure to less than 80% of the baseline value or systolic blood pressure less than 90 mm of Hg. One dose of Mephenteramine 3 mg was injected intravenously to treat each incidence of hypotension along with 200 ml ringer lactate bolus. Bradycardia was defined as HR less than 55 beats per minutes and each incidence was treated with one dose of atropine 0.6 mg intravenously. Incidence of hypotension, bradycardia, nausea and vomiting were noted. The number of doses atropine and mephenteramine drug was recorded. Total intravenous fluid, urine output (UO) and surgical blood loss were also noted.

Data were analysed using Graphpad prism software version 5.1. We found that a sample size of 25 patients per group will be required (by a priori power analysis) to provide an 80% power of detecting an absolute difference of 25% in the incidence of spinal induced hypotension between the treatment groups, with and $\alpha = 0.05$. accounting for possible dropouts, 60 patients were included in study. Student's t test was applied for quantitative data and Chi square for qualitative data. P value < 0.05 was taken as significant.

RESULTS: Patients in both groups were well matched in terms of age, weight and sex. No statistical difference was seen in both groups.

The mean baseline of HR, SBP, DBP, MBP were comparable in both groups. The trends of HR, SBP, DBP, MBP at various time intervals were also comparable in both groups. Total volume used for preloading was about 1474 ± 206 ml and for coloading was 1386 ± 176 ml (p=0.0805). Urine output in preloading and coloading groups was 223 ± 100 and 173 ± 89 ml respectively (p=0.0453).

Total incidence of hypotension in group Preload and group Coload were 13.33% and 10% respectively (p=0.463). Total incidence of bradycardia in group Preload and group Coload were 6.67% and 10% respectively (p=0.394). No. of dose of atropine to treat bradycardia was 2 out of 30 patients (6.67%) and 3 out of 30 patients (10%) for group P and C respectively (p=0.394). No. of dose of mephenteramine to treat hypotension was 4 out of 30 patients (13.33%) for each group (p=1). Incidence of nausea was 6.67% for group Preload and 10% for group Coload (p=0.394). Incidence of vomiting was 3.33% for group Preload and 6.67 % for group Coload (p=0.278).

DISCUSSION: Ever since its introduction by august Bier[4] in 1898, the popularity of SA has seen periods of waxing and waning in anaesthetist practice. With the introduction of safe local anaesthetic drugs and consequent reduction in the incidence of neurological complications, SA is still widely used in clinical practice.

Due to its advantage such as rapid onset of action, uniformly distributed analgesia, profound muscle relaxation, maintenance of clear mentation intraoperatively, blunting of stress response, good post-operative recovery, SA has replaced GA for lower abdominal and lower limb surgeries. SA has proved to be extremely safe when managed well; however, there is still a risk of complications. Some of the complications of SA are hypotension, bradycardia, total spinal anaesthesia and accidental intravascular injection. Hypotension is an important complication which may be preventable or avoidable.

Various techniques have been used to prevent SA induced hypotension. Some of these are preloading with IV fluids, low dose local anaesthetics in SA with or without additives and use of vasopressors prophylactically. Of these preloading with IV fluids has been considered safe and effective method. But, studies did not consistently prove the efficacy of preloading[5] and preloading before commencement of SA is time consuming. It is not possible to deliver preload in all the time with heavy routine work schedules and large no. of emergency surgeries in the second most populous country India. Attempts were done to use fluids after LP (coloading) instead of previous practice of preloading by various investigators with different success rate. In the present study, we have attempted to the study the efficacy of preloading versus coloading with RL in SA induced hypotension in lower limb surgeries.

In present study, we have used RL for preloading and coloading for SA as RL is the most commonly used fluid as a crystalloid in anaesthetic practice[7]. However, the best method of

preloading, rate of administration, total volume of fluid remained controversial. Patients' characteristics were comparable in both groups. Mean baseline value and trends of HR, SBP, MBP, DBP were also comparable in both groups. Incidence of hypotension and bradycardia were comparable in both groups and thereby no. of dose of atropine and mephenteramine were also comparable in both groups. Incidence of nausea and vomiting were similar. Even though total IV fluids used were comparable in both groups but urine output were not comparable. Urine output was more in preload group which was suggestive of more redistribution and excretion associated with preloading. Nausea and vomiting were less in the preload group in our study, even though, the difference is not statistically significant.

Concept of coloading can be explained by the timing of hemodynamic events after SA. Sympathetic nerve blockade is completed within the first 10 minutes after administration of bupivacaine in subarachnoid space. There are high chance of hemodynamic changes like hypotension and bradycardia in this period. Preloading before commencement of SA may be effective but with considerable risk of volume overload. But, coloading makes available extra fluids in intravascular space during period of the highest risk of hemodynamic changes due to SA. So, it leads to timely compensatory changes in cardiovascular system and limits fluid redistribution and excretion with reduced risk of fluid overload. So, Coloading is physiologically more appropriate and rational approach.

Few studies are available in literature, which compares the effects of preloading and coloading in SA induced hypotension.

Kamenik M et al[6] studied the effects of RL solution coload compared with preload, or no load on cardiac output after SA. They found that a sustained rise above baseline in coload group, whereas it returned to baseline in preload group and decreased in the group that received no fluid in general surgical population.

Ewaldsson CA et al[7] found in their kinetic analysis of an IV infusion of RL as preload that a rapid fluid administration over two minutes after induction of spinal or general anaesthesia for non-obstetric surgery might prevent hypotension caused by central hypovolaemia.

Jose L. Mojica et al[8] conducted a randomized clinical trial to evaluate the efficacy of crystalloids in preventing spinal-induced hypotension and cardiovascular side effects (CVSE) in total 404 surgical patients. Crystalloid administration at the time of spinal block resulted in an incidence of SIH almost identical to that seen in the Placebo group, but led to a significant reduction in the risk of CVSE as compared with placebo (RR, 0.23; P =0.019; number needed to treat,13) or with crystalloids administered before spinal block (RR, 0.26; P = 0.014; number needed to treat, 14). Administering crystalloids at the time of spinal block had a beneficial effect in preventing CVSE in general and specialty surgery patients undergoing spinal anaesthesia as compared with administering crystalloids before spinal block or administering no crystalloids. This study favours the results of our study. But, we did not include any placebo or control group for ethical reasons in our study. So, the efficacy of crystalloid preloading in preventing SA induced hypotension could not be evaluated per se.

Our study confirms the finding of Manu Bose, et al[9]. They conducted a randomized study to compare the effect of preloading against coloading with 15 ml/kg ringer lactate in preventing hypotension and bradycardia following spinal anaesthesia in total 54 patients undergoing arthroscopies of lower limb. They found that trend of HR and MBP at various time intervals was comparable for both preloading and coloading groups. No. of incidence of bradycardia was 48.15% for both groups (p value = 1). No. of incidence of hypotension was 18.52% for

preloading group and 11.11% for coloading group (p value =0.140). Incidence of nausea and giddiness were comparable between two groups (p=0.239 and 0.491 respectively).

Jacob, et al[10] Conducted a study of crystalloid preload versus coload for hypotension in 100 parturient scheduled for caesarean section under SA and found that incidence of hypotension was 30 in preload and 23 in coload group. High incidence of nausea (19 versus 10, p=0.0473) and vomiting (14 versus 6, p=0.0455) in preloading group as compared to coloading group. The number of doses of ephedrine required (p=0) and the total dose of ephedrine used (p=0.1528) in the groups were comparable. They concluded that both preloading and coloading with 15 ml/kg of RL were ineffective for SA induced hypotension. Frequent monitoring and prompt treatment with vasopressors were recommended. This study differs to our study by three ways: (1) They used 15 ml/kg RL solution (2) They included parturient (3) The patients were scheduled for caesarean section.

The drawbacks of our study are lack of a control group (no load) and non-blinding method. It is concluded that preloading before commencement of SA is not essential and coloading is equally effective for the prevention of SA induced hypotension. So, we believe that one should not spend valuable time to deliver preload before SA to prevent hypotension specifically in patients with ASA I & II risk and unnecessarily delay surgery. Though care must be taken for parturient and patients with ASA >III risk.

CONCLUSION : Coloading with 20 ml/kg of ringer lactate is as effective as preloading with same volume over 20 minutes in the context of prevention of spinal induced hypotension in lower limb surgery. So, we believe that it is unnecessary to spend time to deliver preload and delay surgery for the prevention of SA induced hypotension.

REFERENCES:

1. Spinal anaesthesia - a practical guide http://update.anaesthesiologists.org/wp-content/uploads/2009/10/Spinal-Anaesthesia-a-Practical-Guide.pdf
2. Bernd Hartmann, Axel Junger, Joachim Klasen, Matthias Benson, Andreas Jost, Anne Banzhaf, et al. The incidence and risk factors for hypotension after spinal anesthesia induction: an analysis with automated data collection. Anesth Analg 2002;94:1521–9.
3. RA Dyer, Z Farina, IA Joubert, P du Toit, M Meyer, G Torr, et al. Crystalloid preload versus rapid crystalloid administration after induction of spinal anaesthesia (coload) for elective caesarean section. Anaesth Intensive Care 2004; 32: 351-7.
4. K. Drasner. Spinal anaesthesia: a century of refinement, and failure is still an option. Br J Anaesth 2009;102 (6):729-730.
5. Singh J, Ranjit S, Shrestha S, Sharma R, Marahatta SB. Effect of preloading on haemodynamic of the patient undergoing surgery under spinal anaesthesia. Kathmandu university medical journal 2010;8(2): 216-221.
6. Kamenik M, Paver-Erzen V. The effect of lactated Ringers solution infusion on cardiac output changes after spinal anesthesia. Anesth Analg 2001; 92: 710-14.
7. Ewaldsson CA, Hahn RG. Volume kinetics of Ringers solution during induction of spinal and general anaesthesia. Br J Anaesth 2001; 87:406-14.

8. Mojica JL, Melendez HJ, Bautista LE. The timing of intravenous crystalloid administration and incidence of cardiovascular side effects during spinal anesthesia: the results from a randomized controlled trial. Anesth Analg 2002; 94: 432-37.
9. Manu Bose, Gurudas Kini, Krishna H.M. Crystalloid preload versus rapid crystalloid administration after induction of spinal anaesthesia (coload) for elective caesarean section J Anaaesth Clin P
10. Jevel J. Jacob, Aparna williams, Mary verghese, Lalita Afzal. Crystalloid preload versus crystalloid coload for parturients undergoing cesarean section under spinal anesthesia. Journal of Obstetric Anaesthesia and Critical Care 2012; 2(1): 10-15.

Table : 1 Patients's characteristics

Patient's characteristics	Group P (Mean ± SD)	Group C (Mean ± SD)	P value (student"s t test)
Age (years)	32.63±10.73	34.63±10.80	0.4747
weight (kg)	54.4±8.20	57.83±7.78	0.1019
Sex (ratio)	1:1	1:1	-
Total IV fluid (ml)	1205±187	1214±163	0.8432
Urine output (ml)	235±57	203±46	0.0166

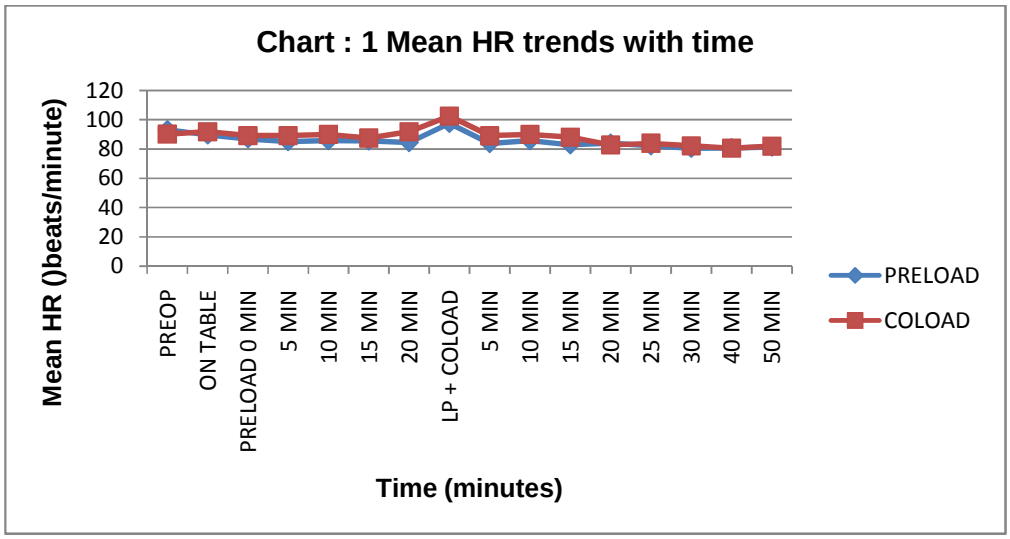
Table : 2 Perioperative adverse effects and use of drug.

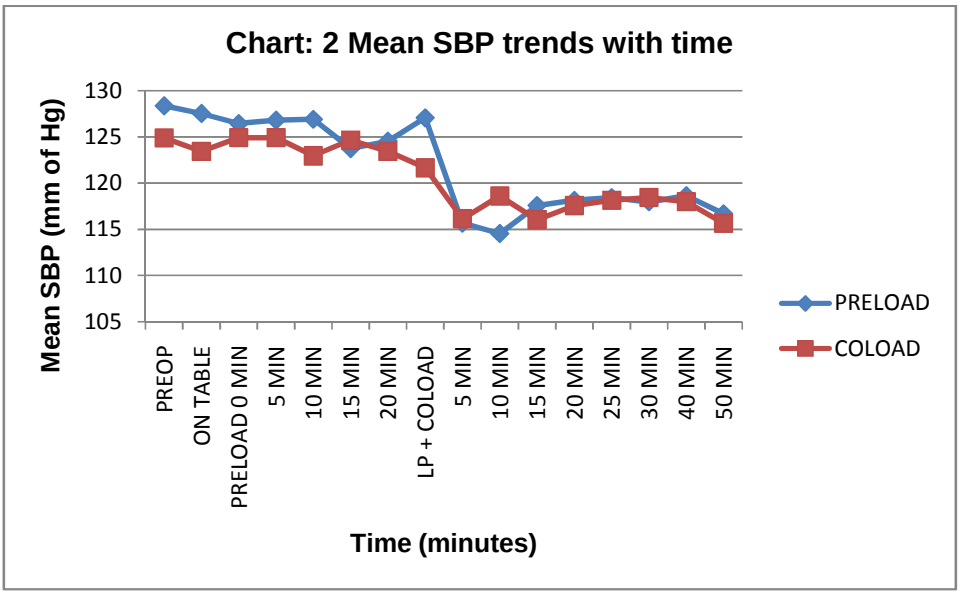
Parameters	Group P		Group C		P Value
	No.	%	No.	%	
Hypotension	4	13.33	3	10	0.463
Bradycardia	2	6.67	3	10	0.394
Atropin dose	2	6.67	3	10	0.394
Mephenteramine dose	4	13.33	4	13.33	1
Nausea	2	6.67	3	10	0.394
Vomiting	1	3.33	2	6.67	0.278

Table : 3 Trends of HR and SBP among study groups.

TIME	HR				SBP			
	PRELOAD		COLOAD		PRELOAD		COLOAD	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
PREOP	93.13	14.19	90.20	13.23	128.37	10.48	124.87	12.52
ON TABLE	89.60	12.41	91.87	12.32	127.53	10.80	123.40	12.43
PRELOAD 0 MIN	86.93	12.94	89.20	11.74	126.43	11.11	124.90	14.17
5 MIN	84.97	12.27	89.20	11.74	126.80	13.17	124.90	14.17
10 MIN	85.63	12.67	89.93	13.54	126.90	12.55	122.93	13.55
15 MIN	85.53	12.20	87.53	14.32	123.73	10.31	124.63	14.67
20 MIN	84.43	12.77	91.87	12.32	124.53	10.16	123.40	12.43
LP + COLOAD	97.43	12.73	102.43	12.70	127.07	9.99	121.63	12.65
5 MIN	83.77	15.42	89.07	12.10	115.67	12.39	116.13	12.40

10 MIN	85.63	12.67	89.93	13.54	114.53	10.84	118.60	9.40
15 MIN	82.83	13.75	88.17	13.41	117.57	10.12	116.03	10.62
20 MIN	83.93	12.04	82.83	13.75	118.13	9.28	117.57	10.12
25 MIN	82.20	12.05	83.93	12.04	118.43	7.62	118.13	9.28
30 MIN	80.57	11.57	82.20	12.05	117.97	8.65	118.43	7.62
40 MIN	80.83	9.99	80.57	11.57	118.63	8.19	117.97	8.65
50 MIN	81.37	9.58	81.93	11.86	116.67	9.17	115.63	10.52





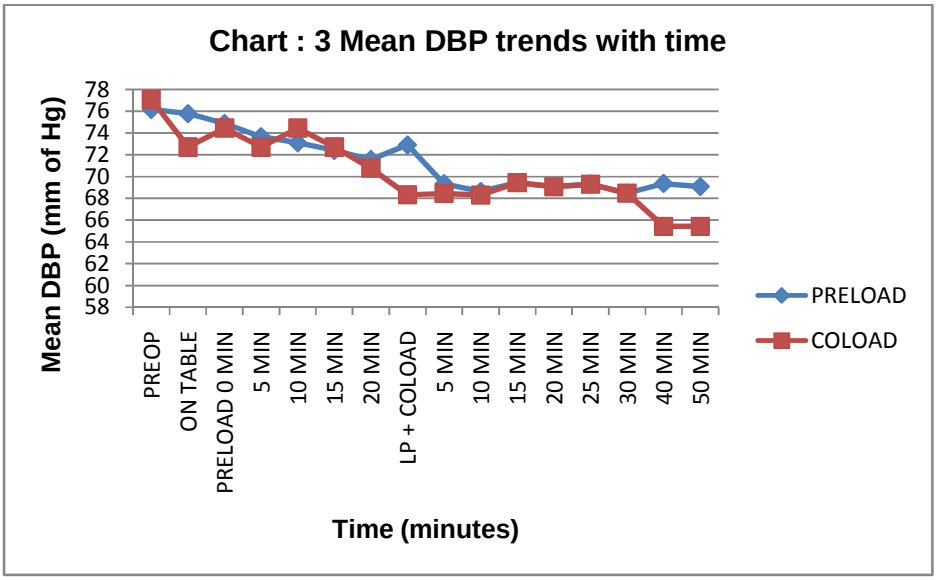


Chart : 3 Mean DBP trends with time
Mean DBP (mm of Hg)
78
76
74
72
70
68
66
64
62
60
58
PREOP
ON TABLE
PRELOAD 0 MIN
5 MIN
10 MIN
15 MIN
20 MIN
LP + COLOAD
5 MIN
10 MIN
15 MIN
20 MIN
25 MIN
30 MIN
40 MIN
50 MIN
PRELOAD
COLOAD
Time (minutes)

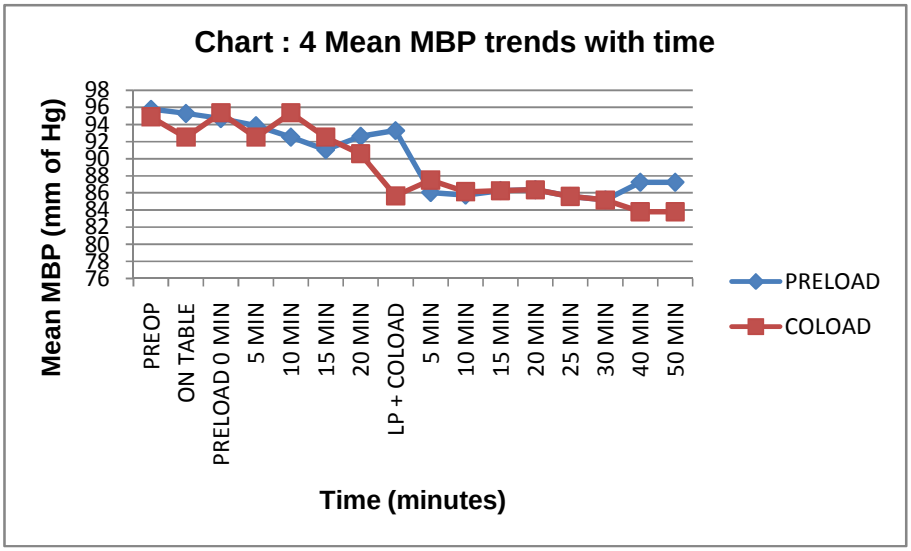


Chart : 4 Mean MBP trends with time
Mean MBP (mm of Hg)
98
96
94
92
90
88
86
84
82
80
78
76
PREOP
ON TABLE
PRELOAD 0 MIN
5 MIN
10 MIN
15 MIN
20 MIN
LP + COLOAD
5 MIN
10 MIN
15 MIN
20 MIN
25 MIN
30 MIN
40 MIN
50 MIN
PRELOAD
COLOAD
Time (minutes)

EARLY DIAGNOSIS OF TUBERCULOUS PERITONITIS BY NESTED PCR AND AUTOMATED CULTURE TECHNIQUE

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ABSTRACT: INTRODUCTION: Early laboratory diagnosis of tuberculous peritonitis (TBP) is crucial to start antitubercular chemotherapy and to prevent its complications. However conventional methods are either less sensitive or time consuming. Hence the diagnostic potential of BacT/ALERT and polymerase chain reaction (PCR) was evaluated in this study. **MATERIAL AND METHOD**: The study group comprised of 21 cases and nine controls. The cases were divided into confirmed sub group (seven cases) – smear or culture or histopathologically proven and probable subgroup (fourteen cases) - clinically suspected cases. Ziehl Neelsen (ZN), Auramine Phenol (AP) staining, Lowenstein Jensen (LJ) culture, BacT/ALERT and nested Polymerase chain reaction (PCR) targeting IS6110 were carried out on all the patients. **RESULTS:** Sensitivity of ZN, AP staining and LJ culture were found to be 23.8%, 28.5% and 57.1% respectively. Whereas BacT/ALERT and nested PCR showed a sensitivity of 76.1% and 90.4% respectively. The mean detection time of growth by LJ culture was 32.10 days where as that of BacT/ALERT was 21.28 days. The contamination rates in LJ culture and BacT/ALERT were 5.0%% and 10.0% respectively. **CONCLUSION:** Nested PCR and BacT/ALERT found to be more sensitive compared to LJ culture and smear microscopy. BacT/ALERT detects mycobacterial growth at a faster rate with less contamination rate compared to LJ culture. As both false negative and false positive results are reported on nested PCR, so alone it should not be used as a criterion for initiating or terminating the therapy but should be supported by clinical, radiological, cytological and other microbiological finding.
KEY WORDS – Tuberculous peritonitis, nested PCR, BacT/ALERT

INTRODUCTION: Tuberculous peritonitis (TBP) accounts for 10-12 % of all cases of extra-pulmonary tuberculosis[(1)]. Although it is uncommon in western world, it remains a serious problem in Asia especially in HIV positive patients. Laboratory methods play a crucial role in establishing the diagnosis, early starting and monitoring the chemotherapy, preventing the transmission, identifying the changing pattern of epidemiology and detection of resistance to drug. Diagnosis of TBP is often difficult due to paucibacillary nature of the disease. Though ZN staining is rapid and cheap but it is neither sensitive nor specific requiring a minimum bacillary load of 10^4/ml[(2)]. Culture is more sensitive than ZN staining with detection limit of 10-100

bacilli/ml but requires prolonged incubation time of 6-8 weeks because of the long generation time of tubercle bacilli[3]. Even histology is also at times not conclusive.

Many newer diagnostic modalities like automated microbial detecting systems and molecular methods have come up with increase isolation rate and early detection of the organism. BacT/ALERT Microbial Detecting System is based upon colorimetric detection of pH change which occurs due to CO_2 production by Mycobacteria [3]. Not much studies are there evaluating diagnostic potential of BacT/ALERT for tuberculosis (TB) especially for TBP. Polymerase chain reaction (PCR) is another alternate for the early diagnosis of TBP. IS6110 is a transposone present as multiple repetitive elements in the genome of M. tuberculosis complex with variable copy numbers ranging from 0 to 26 [3]. Due its repetitive nature, tests using IS6110 as primer yield higher sensitivity. Nested polymerase chain reaction (PCR) using IS6110 though has been widely evaluated in the diagnosis of TB but fewer studies are only available for the diagnosis of TBP. Hence we have evaluated the potential of BacT/ALERT and nested PCR for the diagnosis of TBP.

MATERIAL AND METHODS: The present study was carried out in the department of Microbiology of a tertiary care centre and was approved by the institute ethical committee.

CASES: Samples were collected from 21 cases of TBP admitted to the hospital, during July 2010 to September 2012 and divided to the following subgroups.

a) Confirmed TBP subgroup: comprised of seven case confirmed by smear, culture or histopathology.

b) Probable TBP subgroup: comprised of fourteen clinically suspected cases having features like pyrexia of unknown origin, abdominal tenderness, weight loss, night sweat and / or positive Mantoux test and / or elevated ESR and / or positive peritoneal fluid cytology like pleocytosis, elevated protein level and reduced sugar level and / or evidence of pulmonary or any other organ tuberculosis and patient improving on antitubercular drug (ATT) (Table 1).

CONTROLS: Nine peritoneal fluids were collected from known cases of bacterial peritonitis. Approximately 2ml of sample was collected aseptically. One ml of the sample was used for both smear and culture, 0.5 ml was used each for BacT/ALERT & PCR. Smears were made from the samples and were stained by both Ziehl–Neelsen technique and auramine phenol method as per the standard methods [3]. Cultures were carried out on Lowenstein – Jensen (L-J) egg based medium as per standard method [4].

BACT/ALERT: The processing of peritoneal fluid was done as per as the manufacturer's instruction [5]. Inoculated BacT/ALERT MP bottles were loaded in MB/BacT instrument for the incubation and the growth was monitored. BacT/ALERT MP bottle with mycobacterial growth produced a color change of sensor from dark green to yellow, changing the screen color to yellow. The positive BacT/ALERT MP bottles were unloaded, vortexed heavily to make the large clumps to break and suspend uniformly. The mycobacterial growth was confirmed by performing ZN staining from it.

POLYMERASE CHAIN REACTION: The PCR was carried out as per the method described elsewhere [6]**.** The peritoneal fluid was centrifuged at 3000 rpm for 15 minutes, and then the supernatant was discarded. To the sediment, 50 µl lysozyme was added followed by incubation at 37^0C in water bath overnight. 70 µl 14% SDS and 6 µl proteinase K were added and the

mixture was incubated at 65^{0}C for 15 minutes. Subsequently, 10 µl of 5M NaCl and 80 µl of 10% CTAB (activated at 55^{0}C) were added and the final mixture was incubated at 65^{0}C for 10 minutes. Then, 800 µl of phenol, chloroform and iso amyl alcohol was added in ratio of 25: 24: 1. The mixture was spun at 10000 RPM for 10 minutes at 4^{0}C. Supernatant was collected, 600 µl of ice cold isopropanol was added and was incubated at -20^{0}C overnight. Next day it was spun for 10 minutes at 12000 RPM at 4^{0}C. After the fluid was drained out, it was kept in the incubator for drying. Finally, 20 µl Tris buffer was added and it was stored at -20^{0}C.

Primers were validated by blasting the primer sequence used for detection of M.tuberculosis in the genome database of all the organisms in the web site (http://www.ncbi.nlm.nih.gov/blast/) and were found to be specific for the organism. The sequence of the TB PCR Primers were: first set conventional PCR round- forward primer (FL): 5' CTC AAG TGA AGG AGG CAA CC – 3' and reverse primer (FR): 5' TGG GCT AGG GTG TTG ATC TC – 3' where as for the nested PCR: forward primer (NFL): 5' CGT CTG GAG CGT GAC CTA CT – 3' and reverse primer (NFR): 5' GAC ATC TCG ACG GTC AGT CA – 3' respectively.

PCR mix consisted of 4µl of extracted DNA, 10 µl of 2X readymade master mix, 2µl of 10µM Primer (FL and FR for 1st round PCR and NFL and NFR for nested PCR) and 10µl of milliQ water. The settings of the thermocycler programmed were similar for both first round and nested PCR except for the primers used. Total 30 cycles were carried out in each round, each cycle comprised of initial denaturation at 94°C for 5 minutes, denaturation occurred at 94°C for 60s, annealing occurred at 56 °C for 60s, amplification occurred at 72 °C for 60s followed by final extension at 72 °C for 7 min . Agar gel electrophoresis was carried out and bands are visualized under UV rays. The MTB specific nested PCR product size is 219 bp length. The bands were separated in the agarose gel according to the molecular weight which then checked by comparing the bands with standard molecular weight marker.

RESULT: The microbiological findings of various tests were depicted in table 1. ZN staining and AP staining have shown 23.8% (5 out of 21) and 28.5% (6 out of 21) sensitivity respectively whereas LJ culture was found to be 57.1% sensitive (12 out of 21 cases). BacT/ALERT microbial detecting system detected sixteen out of 21 cases (76.1%). There was one case (in probable group) which was detected by BacT/ALERT but was negative by nested PCR. The contamination rate of BacT/ALERT and LJ culture in the present study was found to be 5.0% and 10.0% respectively. Mean detection time of BacT/ALERT in all the isolates was 21.28 days compared to 32.10 days taken by LJ culture. Gel image after nested PCR targeting IS6110 of M.tuberculosis was shown in figure 1. Nested PCR could detect nineteen out of 21 (90.4%) cases of TBP where as one out of nine (11.1%) in control group was also detected positive by nested PCR. There were four cases detected by nested PCR but was negative by BacT/ALERT. Both the cases and controls were age and gender matched.

DISCUSSION: Tuberculous peritonitis (TBP) is the sixth most frequent site of extrapulmonary involvement [7]. Although an infrequent disease, TBP with its non-specific symptoms and sinister clinical course can be easily confused with other intraabdominal diseases. Both the incidence and severity of TBP are expected to increase with increasing incidence of HIV infection. Hence, early and prompt diagnosis of the condition contributes to early start of anti tubercular therapy, thus preventing thereby the complications due to the condition. However, accurate laboratory diagnosis of TBP still continues to be a challenge.

In the present study, acid fast microscopy by ZN and AP staining showed low sensitivity which can be explained because of the paucibacillary nature of peritoneal fluid. A high bacterial load (>10^4 – 10^5 bacilli / ml) is needed in the specimen to render an AFB microscopy result positive[2]. Various studies documented similar sensitivities of smear microscopy for TBP of < 20 % [6]. AP staining is found to be more sensitive than ZN staining as the smears can be examined by AP staining at 250x or 450x, covering larger area in the same time [12].

Culture by LJ media showed an overall sensitivity of 57.1%. Similar sensitivities had been documented in different studies ranging from 20-75% [6]. Low sensitivity of LJ culture could be due paucibacillary nature of the peritoneal fluid as minimum detection threshold being 100-1000 bacilli per ml [3]. Other reasons could be due to presence of dead bacilli, intake of other broad spectrum antibiotics inhibiting Mycobacteria and prior starting of ATT.

The BacT/ALERT microbial detecting system showed an overall sensitivity higher than the LJ culture and specificity of 100%. In confirmed & probable sub group the sensitivity was 100% and 64.2% respectively. Out of sixteen mycobacterial isolates were recovered in our study, all (100%) were recovered by BacT/ALERT compared to 75.0 % (twelve isolates) recovery rate of LJ culture. Other studies also have shown recovery rate of 91% to 94% by BacT/ALERT [7,8]. The contamination rate of BacT/ALERT and LJ culture in our study was found to be 5.0% and 10.0% respectively. Similar reports were documented in different studies[7]. Mean detection time of BacT/ALERT in all seven isolates was 21.28 days compared to 32.10 days taken by LJ. The mean detection time of BacT/ALERT in smear positive specimen was 17days where as that in smear negative specimens were 22.8 days. Various studies documented mean detection time of 15-16 days by BacT/ALERT [7-8]. Early detection of the bacilli in these studies could be due to inclusion of both pulmonary and extrapulmonary specimens in these studies.

In the present study, nested PCR showed an overall sensitivity of 90.4 % which was higher than culture but differed in the confirmed (100%) and probable (85.7%) subgroup. In an European study by T.zoanopoulos et al, all the three suspected cases of TBP were found positive by PCR targeting IS6110 [9]. As the sample size was very less and response to ATT was considered as final inclusion criterion for TBP, so the sensitivity was found higher in this study which might be misleading.

In our study, there were two cases which could not be detected by nested PCR (false negative results). The reasons of lower sensitivity might be due to many reasons: First, presence of PCR inhibitors which are found to be more associated with extrapulmonary specimen compared to pulmonary specimen [10-11]. A better extraction procedure like immunomagnetic separation technique should be used which could capture all M.tuberculosis DNA, but not inhibitors [10]. Various resin matrixes like 'Gene releaser preparations' which absorb inhibitors without entailing further loss of DNA may also be used [10]. Second, poor lysis of Mycobacteria in the extraction procedure due to the complexity of the cell wall [9,12]. Third, some strains of M.tuberculosis in Asia lack the IS6110 sequence [13-15]. Hence few cases might have got undetected if Mycobacteria present in the samples lack the IS6110 sequence Fourth, low number of bacilli present in the peritoneal fluid.

The overall specificity of nested PCR in our study was 88.8%. There was one out of nine controls which was detected positive (false positive) by our study where actually Escherichia coli was isolated and patient responded to the antibiotics. The reasons of false positive results could be due to cross contamination during initial handling or due to amplicon carry over

contamination which can be overcome by using a single tube nested PCR [10]. It is also found that use of dUTP/Uracil-N-glycosylase could decrease the amplicon contamination[10].

There were seven cases which were detected by nested PCR but were rendered negative by LJ culture. The gain in the sensitivity (40%) over the so called "gold standard" can be explained by low detection limit of nested PCR of as few as 10 bacilli per ml . The sensitivity gain in the etiological diagnosis by nested PCR substantiates the diagnostic utility of this method. Moreover nested PCR offers the advantages like speed in obtaining the result, option of referring the sample rather than the patient to a specialized centre.

There was one case (in probable group) which was detected by BacT/ALERT but was negative by nested PCR whereas, there were four cases which was detected by nested PCR but was negative by BacT/ALERT. So the combined sensitivity of BacT/AERT and nested PCR in the probable group (92.8%, 13 out of 14 cases) is more than individual sensitivity of BacT/AERT (64.2%) and nested PCR(85.7%).

In conclusion, diagnosis of TBP is often difficult due to the atypical clinical presentation and paucibacillary nature of the sample. Both Nested PCR and BacT/ALERT found to be more sensitive compared to conventional LJ culture and smear microscopy. BacT/ALERT takes lesser time to detect growth and associated with less contamination rate compared to conventional LJ culture. We suggest that nested PCR should deserve a place in laboratory diagnosis of TBP but careful adherence to the test protocol is mandatory. As both false negative and false positive results are reported on PCR, hence PCR alone should not be used as a criterion for initiating or terminating the therapy. This should be supported by clinical, radiological, cytological and other microbiological finding (smear microscopy, culture by conventional and automated system) to guide the clinician in decision making for appropriate therapy whenever possible.

The limitations of the present study are as follows. 1) It was based on clinical criteria which though had been validated well before but cannot be considered as gold standard. 2) Internal controls were not used for nested PCR. We believe that use of internal control could have helped in eliminating the possibility of PCR inhibitors. 3) We have not evaluated the diagnostic potential of these tests in HIV positive patients, where the sensitivity would have been more. 4) Only IS6110 is targeted in our study which might be absent in some Indian isolates of M.tuberculosis. 5) The sample size is small. Since TBP is relatively rare, it is difficult to get more patients. If the sample size can be increased then it will provide a clear picture about the parameters.

REFERENCES:

1. Basu S, Ganguly S, Chandra PK, Basu S. Clinical profile and outcome of abdominal tuberculosis in Indian children. Singapore Med J 2007 Oct;48(10):900-5.
2. Survashi B.S, Bhanu V, Seth P, Suresh N, Arora J, Rana T. Utility of polymerase chain reaction in diagnosis of tuberculosis from samples of bone marrow aspirate. Am J Trop Med Hyg 2006;75(5):960-3.
3. Murray PR. Manual of Clinical Microbiology. 9th ed. Washington DC.: Asm press; 2007.
4. Washington CW, Allen SD, Koneman EW, Procop GW, Janda WM, Wood GL. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. NewYork: Lippincott Williams & Wilkins; 2009:1232-35.
5. BioMerieux India (Pvt)Ltd. BacT/ALERT manual. 2009. Ref Type: Catalog.

6. Sharma MP, Bhatia V. Abdominal tuberculosis. Indian J Med Res 2004 Oct;120 (4):305-15.
7. Piersimoni C, Scarparo C, Callegaro A, Tosi CP, Nista D, Bornigia S, et al. Comparison of MB/Bact alert 3D system with radiometric BACTEC system and Lowenstein-Jensen medium for recovery and identification of mycobacteria from clinical specimens: a multicenter study. J Clin Microbiol 2001 Feb;39(2):651-7.
8. Mattei R, Savarino A, Fabbri M, Moneta S, Tortoli E. Use of the BacT/Alert MB mycobacterial blood culture system for detection of Mycobacteria in sterile body fluids other than blood. J Clin Microbiol 2009 Mar;47(3):711-4.
9. Tzoanopoulos D, Mimidis K, Giaglis S, Ritis K, Kartalis G: The usefulness of PCR amplification of the IS6110 insertion element of M. tuberculosis complex in ascitic fluid of patients with peritoneal tuberculosis. Eur J Intern Med 2003, 14(6):367-371.
10. Parija SC, Gireesh AR. Early diagnosis of tuberculous meningitis: A comparison of nested polymerase chain reaction and BacT/ALERT. Indian J Pathol Microbiol April-June 2011;(54):421-2.
11. Khan R, Abid S, Jafri W, Abbas Z, Hameed K, Ahmad Z: Diagnostic dilemma of abdominal tuberculosis in non-HIV patients: an ongoing challenge for physicians. World J Gastroenterol, 2006, 12(39):6371-5.
12. Honore-Bouakline S, Vincensini JP, Giacuzzo V, Lagrange PH, Herrmann JL. Rapid diagnosis of extrapulmonary tuberculosis by PCR: impact of sample preparation and DNA extraction. J Clin Microbiol 2003 Jun;41(6):2323-9.
13. Das S, Paramasivan CN, Lowrie DB, Prabhakar R, Narayanan PR. IS6110 restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, south India. Tuber Lung Dis 1995 Dec;76(6):550-4.
14. Sahadevan R, Narayanan S, Paramasivan CN, Prabhakar R, Narayanan PR. Restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, India, by use of direct-repeat probe. J Clin Microbiol 1995 Nov;33(11):3037-9.
15. Radhakrishnan I, MY K, Kumar RA, Mundayoor S. Implications of low frequency of IS6110 in fingerprinting field isolates of Mycobacterium tuberculosis from Kerala, India. J Clin Microbiol 2001 Apr;39(4):1683.
16. 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.

Table- 1 : Various clinical, radiological, cytological and histopathological findings among the cases

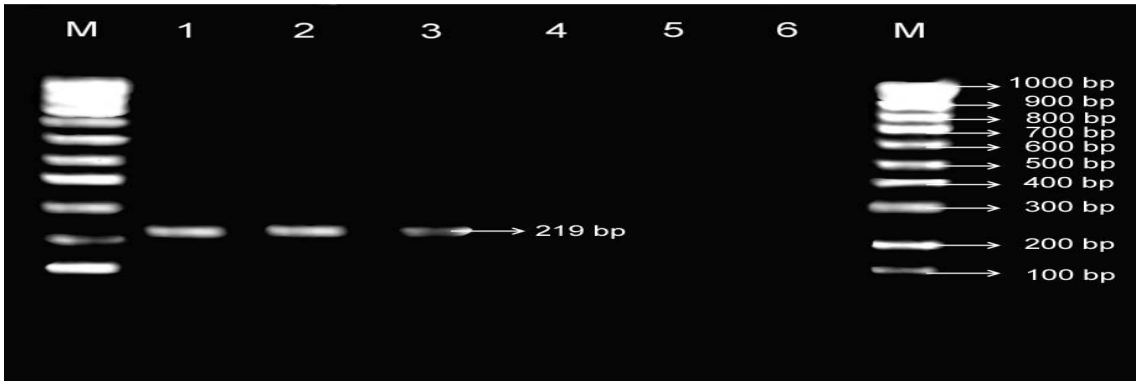
Findings	% of patients
Fever	81.5
Abdominal tenderness	67.1
Weight loss	74.6
Night sweat	65.8
Abnormal peritoneal fluid cytology	57.14
Raised ESR	53.1

Positive Mantoux test	100
Caeseating granuloma in histopathology	14.2
Past history of pulmonary tuberculosis	21.4

Table – 2: Microbiological findings among various subgroups of tuberculous peritonitis (TBP)

Sub groups	No of cases	Number of cases positive for M. tuberculosis by					
		Zn staining	AP staining	LJ culture	BacT/ ALERT	Nested PCR	Combined BacT/ALERT & Nested PCR
Total cases	21	05	06	12	16	19	20
Confirmed TBP	07	03	03	07	07	07	07
Probable TBP	14	02	03	05	09	12	13
Control(Non TBP)	09	0	0	0	0	01	0

Figure-1: Gel image after nested PCR targeting *IS6110* of *M.tuberculosis*



Lane M : 100bp DNA ladder, Lane 2 : 219bp PCR product of positive control,

Lane2&3: 219bp PCR product of clinical control,

Lane 4 to 6 : Clinical samples negative for IS6110 of M.tuberculosis

IF THE MIND KNOWS, THE EYES CAN SEE – THE FIRST CASE OF NEONATAL LISTEROSIS REPORTED FROM EASTERN INDIA

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ABSTRACT: A three day old baby delivered at B. S. Medical College Hospital, Bankura developed features of neonatal septicaemia two days after normal delivery. Patient's blood culture sample was processed in BacT/Alert 3D Automated blood culture system. Positive growth signal was obtained at 48 hrs. Subculture on solid non-selective and selective media, morphological studies including typical motilities and standard recommended bio-chemical tests revealed the isolate to be Listeria monocytogenes. Antibiogram studies further corroborated the identity of the isolate. This case report underlines the importance of Automated blood culture detection system in the identification of fastidious but clinically dangerous pathogens. This is most probably the first report of neonatal septicemia due to Listeria monocytogenes from eastern India.

KEY WORDS: Listeria monocytogenes, Neonatal Septicemia**,** Automated Blood Culture System

INTRODUCTION: Listeria monocytogenes is a relatively rare aerobic, gram positive non-sporing bacillus causing human infection most commonly in neonates. It can survive in a wide range of environmental conditions. The temperature tolerance of this bacteria ranges from 4^0 C to 37^0 C. It can withstand high salt concentration and low pH[1]. These properties indicate that they can overcome food preservation procedures and safety barriers, thus becoming an important and highly fatal food borne pathogen[2]. It has been found that 20-30% of clinical infections can result in death[3]. The first case of human listeriosis was reported in March, 1978 from South Africa[4].

CASE HISTORY: This case report is being made from the Department of Microbiology, Bankura Sammilani Medical College, Bankura which is one of the well known government run teaching hospitals in West Bengal and has a well equipped Microbiology laboratory. The patient was a three day old baby whose birth weight was 2.5 kg. Delivery was institutional (in this hospital) and full term. Two days after normal vaginal delivery the patient presented with frequent episodes of convulsions. Each episode lasted for 5-10 minutes. The baby was immediately transferred to the SNCU (Sick New Born Unit) of this hospital. Examination revealed: Anaemia, Jaundice, Oedema & Clubbing – Nil, Chest- Clear, Heart sounds - S1, S2 audible, Abdomen- Soft, Liver & Spleen- Not palpable. Patient was diagnosed provisionally as Neonatal Septicaemia.CBC

revealed Neutrophil 7,000/cc, ESR 30 mm first hour , Platelet count 2 ,10,000/cc. 0.5 cc of blood was sent for culture in BacT/ALERT 3D Select system in appropriate culture bottle. CSF sample could not be collected as the baby was suffering from repeated convulsions. Treatment was started with Inj. Cefotaxime & Amikacin, Inj. Gardenal, Inj. Vit K, I.V fluid with Isolyte P.

Culture media provided in BacT/Alert 3D Select blood culture bottle contains Soyabean Caesin Digest, Sodium Polyanethol Sulfonate, Pyridoxine, Menadione, Hemin, L-Cysteine Broth with Brain Heart infusion. The culture bottle was incubated at 37^0 C in an atmosphere of CO_2 in oxygen and nitrogen under vacuum in the above system and positive growth signal was obtained in 48 hours. Direct smear was made from Blood Culture bottle and stained by Gram stain. Smear showed small non-sporing gram positive bacilli. Arrangement was mostly single, few pairs, few short chains, V and L shaped diphtheroid arrangements were found also. There were no granules. Typical pallisades were not seen. Subculture was made in 5% Sheep Blood Agar, Nutrient Agar & MacConkey Agar. The plates were incubated at 37^0 C for 24 hrs.

CULTURE FINDINGS: After 24 hrs in 5% Sheep Blood Agar, small zone of haemolysis was seen around the discrete colonies. In Nutrient Agar, small translucent colonies and in MacConkey agar no growth occurred. Growth on selective medium, Palcam Listeria Selective Supplement Agar was also checked. Smear was made from the colonies on Blood Agar and Nutrient Agar and Listeria selective medium and stained with Gram stain. Gram positive non-sporing bacilli having the previous characteristics were detected. One inoculated peptone water tube was incubated for 2 hrs at 37^0 C and another was incubated at 24^0 C for 2 hrs. Hanging drop preparations were made from both tubes. Most of the bacilli incubated at 37^0 C were non-motile and most bacilli incubated at 24^0 C showed typical tumbling motility. Biochemical reactions: Catalase test – positive, Oxidase test – Negative, Glucose & Maltose fermented with production of acid only and aesculin hydrolysis were positive. MR and VP – Positive, CAMP Reaction was also positive.[5]

FINAL DIAGNOSIS: So the patient, a new born baby following vaginal delivery developed neonatal septicemia with convulsion whose aerobic blood culture yielded positive result showing Gram positive bacilli with characteristic arrangement. This is followed by the culture finding of growth in 5% Sheep Blood Agar with haemolysis & growth in Nutrient Agar, but no growth in Mac Conkey Agar. Tumbling motility at 24^0C and no motility at 37^0C with the characteristic biochemical test findings clearly pointing towards a diagnosis of Listeria monocytogenes. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. The organism was sensitive to Ampicillin, Co-trimoxazole and Gentamycin and it was resistant to Cephalosporin and Fluoroquinolones.

Listeriosis in man may show diverse clinical features. Meningitis is a very important outcome of L. monocytogenes infection in newborn, elderly and immunocompromised patients. Asymptomatic infection of the female reproductive canal may cause intranatal infection of the newborn, as was most probably the case in this patient. The patient was discharged early after delivery.

As neonatal listeriosis has never been reported from this region of West Bengal, this case definitely needs to be reported. Scanning of scientific literature revealed four previously reported cases of neonatal listeriosis from India (Mokta KK et al 2016[6], Gogate AA et al 1981[7], Khan Sadia et al 2011[8], and Gupta Ritu et al 19979[9]), but none of these cases were from eastern India. This should be an eye-opener to clinicians who need to be more vigilant. This case also authenticates the great diagnostic sensitivity of automated blood culture systems in the detection of fastidious blood pathogens.

BIBLIOGRAPHY:

1. Human Listeriosis caused by Listeria Ivanovi. Emerg Inf Diseases.2012; 168(1): 16-18.
2. Bortollusi R, Mailman TM: Listerosis, in Infectious Disease of the Fetus and Newborn Infant.6th ed, S Remington et al. Philadelphia.2005 .Elsevier Saunders : 465
3. Kraft Jurgen,Goebel Werner et al.Listeria, Pathogenesis and Molecular Virulence Determinants. Clinical Microbiology ReviewJuly,2001; 14(3): 584-640.
4. Adams Hilde,Toxopeus H. Neonatal Listeria monocytogenes Meningitis. South African Medical Journal.March, 1978; 53: 463 .
5. Mahy Brian W J,Murray Patricket al..Topley & Wilson's Microbiology & Microbial Infections.
   John Wiley & Sons,Inc.,UK,10th Edition. 2005. Vol 2: 959-961.
6. Mokta KK, Kanga AK, Kaushal RK. Neonatal listerosis : a case report from Sub-Himalayas. .Indian Journal of Medical Microbiology .October 2010;Vol 28. Issue 4: 385.
7. Gogate AA, Deodha LP. Meningitis due to Listeria monocytogenes
   Journal of Post Graduate Medicine. 1981. Vol 27. Issue 4 : 240-2
8. Khan Sadia, Sujatha S, Harish B N, Praharaj I, Parija S.C. Neonatal meningitis due to Listeria monocytogenes : A case report from Southern India.Journal of Clinical and Diagnostic Research .2011 June. 5(3): 608-609
9. Ramachandran V.G, Gupta Piyush.Gupta Ritu Listerosis : An Opportunistic Infection. Indian Pediactrics .August 1997;34:732-734

UTILITY OF KIRBY BAUER DISC DIFFUSION METHOD FOR VANCOMYCIN SUSCEPTIBILITY TESTING OF STAPHYLOCOCCAL ISOLATES: PRACTICABILITY, ACCEPTABILITY, AND QUALITY ASPECTS.

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ABSTRACT: Testing MIC of Vancomycin for all staphylococcal isolates is mandatory according to the current CLSI guidelines and this will considerably increase the cost of culture and sensitivity testing. This study is an attempt to re-consider the utility of the conventional disc diffusion method for cost-effective testing in resource poor settings. **MATERIALS AND METHODS:** Thirty coagulase positive and twenty eight coagulase negative staphylococci from various clinical samples have been randomly tested for minimum inhibitory concentration along with the Kirby Bauer disc diffusion method for Vancomycin over a period of five months. **RESULTS:** Susceptibility results of all the 58 isolates tested have been identical by both disc diffusion and HiComb MIC methods. Out of 58 isolates, 57 (98.26%) staphylococci have been sensitive to vancomycin by Kirby Bauer disc diffusion method as well as by the MIC testing method. The MICs of the susceptible strains have been <2 µg/mL. One isolate, a coagulase negative staphylococcus, has been tested to be resistant to vancomycin by both the methods with an MIC of 32 µg/mL **SUMMARY:** In our study of staphylococcal isolates from various clinical samples, there is overt significant concordance between disc diffusion and MIC testing methods in the routine susceptibility testing of vancomycin. **CONCLUSION:** Kirby Bauer disc diffusion method may still be of utility for routine testing of vancomycin susceptibility except for few cases especially in resource poor settings.

KEYWORDS: Staphylococci, Vancomycin, disc diffusion, minimum inhibitory concentration, quality control.

INTRODUCTION: Updating Clinical Laboratory Standards Institute (CLSI) zone diameter interpretive standards for vancomycin susceptibility testing of any staphylococcal isolate no longer recommends disc diffusion methods.[1] Testing MIC for all staphylococcal isolates is mandatory according to the current CLSI guidelines right from Jan 2010 and this will considerably increase the cost and time of culture and sensitivity testing procedures. This study is an attempt to reconsider the utility of the disc diffusion method for cost-effective testing methods in resource poor settings. This study is also an attempt to stress the need for framing indigenous performance and interpretive standards for antimicrobial susceptibility testing for validation of quality control criteria. The study has been performed in a tertiary care hospital in Bangalore, for a sample of randomly selected isolates between June and October 2011 over a period of 5 months on coagulase positive and coagulase negative staphylococci.

MATERIALS & METHODS: Randomly chosen 30 coagulase positive and 28 coagulase negative staphylococci isolated from various clinical samples have been tested by the Kirby Bauer disc diffusion method along with HiComb MIC test for minimum inhibitory concentration for Vancomycin by standard methods and the results compared and evaluated. Previous[2] CLSI guidelines for disc diffusion zone diameter interpretive standards has been used for the disc diffusion method and the current[3] minimum inhibitory concentration interpretive standards for the MIC method. Zone diameters ≥15mm has been taken as susceptible to vancomycin for the disc diffusion method and, the MICs ≤2 µg/mL as "susceptible," 4–8 µg/mL as "intermediate," and ≥16 µg/mL as "resistant" have been taken for the MIC method. All the plates were incubated for the complete 24 hr period, carefully checked for heteroresistant subpopulation. Media, Vancomycin(30µg) discs and HiComb MIC strips for vancomycin were procured from HiMedia, India.

RESULTS: Susceptibility results of all the 58 isolates tested have been identical by both disc diffusion and HiComb MIC methods. Out of 58 isolates, 57 (98.26%) staphylococci have been sensitive to vancomycin by Kirby Bauer disc diffusion method as well as by the MIC testing method. The MICs of the susceptible strains have been <2 µg/mL**.** One isolate, a coagulase negative staphylococcus, has been tested to be resistant to vancomycin by both the methods with an MIC of 32 µg/mL. Table 1, lists the randomly selected sample types and the number of isolates. Table 2, lists the vancomycin zone diameter[8] and MIC means and ranges. Table 3, lists the distribution of Staphylococci in the present study. Figure 1, shows the vancomycin Kirby Bauer disc diffusion test. Figure 2, shows the vancomycin HiComb MIC test. Fig 3: Age wise Distribution of male and female patients.

DISCUSSION: In our study of staphylococcal isolates from various clinical samples, there is significant concordance between disc diffusion and MIC testing methods in the susceptibility testing of vancomycin. The MICs tested for vancomycin in a study done in Southern India were <2 µg/ml**.**[4] Kirby-Bauer disc diffusion method for vancomycin may continue to be of routine practical utility in developing countries like India. Although vancomycin agar screen method is recommended for screening for less susceptible isolates, studies show in house vancomycin screen agar preparations are inferior to commercially prepared ones.[5] Moreover, total prevalence of hVISA according to a meta-analysis supported by National Institute of Health, USA was < 2% [6] and studies from India are sparse. Different countries have been reporting variable incidence of hVISA attributable to variable MIC interpretation breakpoint criteria.[7] So routine testing of all staphylococci for vancomycin by methods other than simple disc diffusion should not be made mandatory, in view of the increasing demand for National Accreditation Board for Testing and Calibration Laboratories (NABL) accreditation of lab testing, quoting CLSI as the performance and interpretive standards criteria for quality assurance in our country. In keeping the costs low for resource poor labs, any cost increases in routine testing practices will have negative impact on quality control practices and general acceptability of the testing itself. This also opens up a new arena where regulatory indigenous diagnostic interpretive standards for antibiotic sensitivity testing at the national level are framed in the Indian context with CLSI or any other international guideline as a possible model.

CONCLUSION: Kirby Bauer disc diffusion method may still be of utility for routine testing of vancomycin susceptibility except for few cases and there is an urgent need for national level

guidelines pertaining to antimicrobial susceptibility reporting standards in clinical microbiology labs for standard practice and sustainable development of clinical microbiology in India.

REFERENCES :

1. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. CLSI document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. CLSI document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
4. Tenover, F. C., M. V. Lancaster, B. C. Hill, C. D. Steward, S. A. Stocker, G. A. Hancock, C.M. O'Hara, S. K. McAllister, N. C. Clark, and K. Hiramatsu. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J. Clin. Microbiol. 1998, 36**:**1020-27.
5. Liu, C., and H. F. Chambers. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob. Agents Chemother. 2003, 47**:**3040-45.
6. Bhateja P, Mathur T, Pandya M, Fatma T, Rattan A. Detection of vancomycin resistant *Staphylococcus aureus*: A comparative study of three different phenotypic screening methods. Indian J Med Microbiol 2005; 23:52-55.
7. G. A. Menezes, B. N. Harish, S. Sujatha, K, Vinothini ,S. C. Parija. Emergence of vancomycin-intermediate *Staphylococcus* species in southern India. Journal of Medical Microbiology. 2008, 57(7): 911–12.
8. D Oosthuizen, TR Walsh, GF Weldhagen. Detection of Staphylococcus aureus with reduced susceptibility to vancomycin in a South African teaching hospital. The South African journal of Epidemiology and Infection. 2005, 20 (4): 127-29

Table 1: Sample wise distribution of isolates

Randomly Selected Sample Types	No. of Isolates from Various Samples
Abscess Wall	1
Blood	5
Brain Abscess Pus	1
CSF	12
Cystic Fluid	1
Ear Swab	1
EVD Tip	2
Graft Site Swab	1
Periorbital Pus	1

Pleural Fluid	1
Pus Swab	8
Shunt Tip	2
Tracheal	7
Urine	2
Wound Swab	13
Total No. of Isolates from Various Samples	58

Table 2: Vancomycin zone diameter and MIC mean and range

	Mean	Range
Disc Diffusion zone diameter in mm	20.41304	7.0-32.0
MIC in µg/mL	0.678902	0.016-32

Table 3: Distribution of staphylococci.

Isolates	Number
MSSA	6
MRSA	24
CONS	10
MRCONS	18
Total no. of Isolates	58

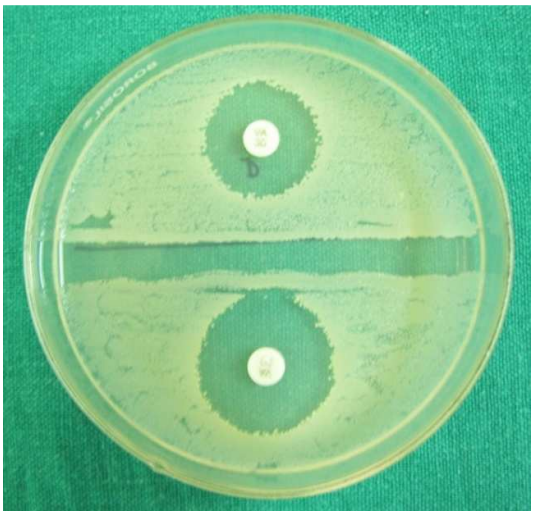

Fig. 1: Vancomycin testing by disc diffusion method

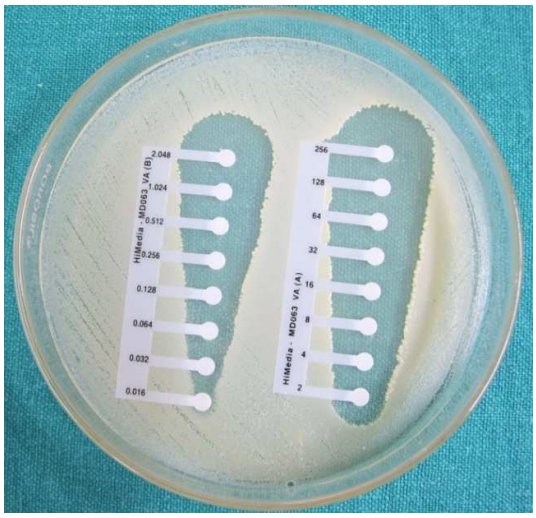

Fig. 2: Vancomycin MIC by HiComb method

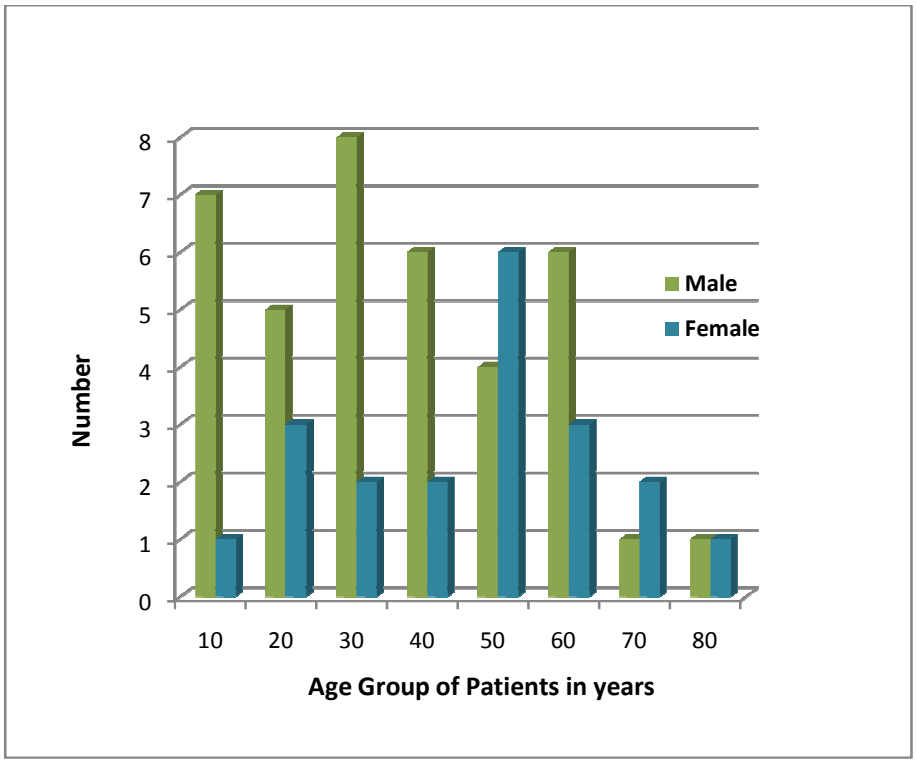

Fig 3: Age wise Distribution of male and female patients.

MARKERS OF OXIDATIVE STRESS AND SERUM LIPIDS IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT: Dyslipidemia and oxidative stress were evaluated in patients with polycystic ovarian syndrome. **MATERIALS AND METHODS:** Total cholesterol, Triglyceride, HDL cholesterol, LDL cholesterol, Malondialdehyde (MDA) and Total antioxidant capacity were measured in serum of PCOS subjects and age matched controls. **RESULTS:** Study group comprised of 31 women with PCOS and control group with 31 healthy volunteers. Mean serum levels of MDA, Cholesterol, Triglycerides and LDL cholesterol were significantly increased and TAC and HDL cholesterol were significantly decreased in PCOS subjects compared to controls.
CONCLUSION: Our results revealed that PCOS is associated with dyslipidemia and altered oxidative status.
KEY WORDS: MDA, PCOS, TAC.

INTRODUCTION: Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction that is characterized by anovulation, hyperandrogenism, and/or the presence of polycystic ovary (PCO) morphology [1].Obesity and insulin resistance occur frequently in association with this syndrome. A wide variety of risk factors have been studied in association with PCOS, including obesity, insulin resistance, dyslipidemia, endothelial dysfunction, and the presence of the metabolic syndrome [2,3,4,5].

Women with PCOS would be predicted to be at high risk for dyslipidemia because they have elevated androgen levels and are frequently obese. Moreover, since they are also often hyperinsulinemic and insulin resistant, they would also be expected to be at increased risk for the dyslipidemia associated with insulin resistance. Insulin, rather than androgen, levels correlate best with lipid abnormalities, and suppressing androgen levels does not alter lipid profiles in PCOS. Insulin resistance and hyperinsulinemia are also associated with an atherogenic plasma lipid profile. Elevated plasma insulin concentrations enhance very low density lipoprotein (VLDL) synthesis, leading to hypertriglyceridemia. Progressive elimination of lipid and apolipoproteins from the VLDL particle leads to an increased formation of intermediate-density and low-density lipoproteins, both of which are atherogenic. Last, insulin, independent of its effects on blood pressure and plasma lipids, is known to be atherogenic. The hormone enhances cholesterol transport into arteriolar smooth muscle cells and increases

endogenous lipid synthesis by these cells. Insulin also stimulates the proliferation of arteriolar smooth muscle cells, augments collagen synthesis in the vascular wall, increases the formation of and decreases the regression of lipid plaques, and stimulates the production of various growth factors[6,7].

In summary, insulin resistance appears to be a syndrome that is associated with a clustering of metabolic disorders, including non-insulin-dependent diabetes mellitus, obesity, hypertension, lipid abnormalities, and atherosclerotic cardiovascular disease. Oxidative stress, which is generally known to be present in women with PCOS regardless of whether they are lean or have metabolic abnormalities, has been documented in infertile women[8]. And it is also reported to affect IR in these patients[9].

Oxidative stress may influence not only cardiovascular system but also female reproductive system[10].

Products of lipid peroxidation reactions have been widely employed as biomarkers for oxidative stress. MDA, produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a good biomarker. Various studies have measured antioxidant markers to correlate oxidative stress and PCOS and the diverse clinical manifestations of metabolic syndrome including diabetes, obesity and cardiovascular diseases. Total antioxidant capacity is the ability of serum to quench free radical production, protecting the cell structure from molecular damage. Various detection assays for TAC assay measures the combined antioxidant capacity of all its components including vitamins, proteins, lipids, glutathione, uric acid, etc[11].

The aim of our study was to investigate the relationship between PCOS, oxidative stress status and lipid profile in patients with polycystic ovary syndrome.

MATERIAL AND METHODS: In the present study, 31 PCOS patients in the age group of 21-40 years who were admitted to Gynaecology Unit of Mamata General Hospital, Khammam were recruited for the study after obtaining written informed consent (Study group). 31 healthy persons in the corresponding age group were selected from the patient's attendants and hospital staff were recruited as controls (control group).

PCOS was diagnosed according to the Rotterdam criteria[12]. The patients having two or more of the following criteria were defined as PCOS:

1. History of oligo and/or anovulation in reproductive age.
2. Clinical and/or biochemical signs of hyperandrogenism: hirsutism score of >6 and/or high total testosterone level.
3. Typical ovarian imaging of polycystic ovaries on ultrasound: multiple follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10ml).

Fasting lipid profile [Total cholesterol, High density lipoprotein Cholesterol (HDL-cholesterol), Triglycerides] was done to all patients by using enzymatic kits on biochemistry autoanalyser [tulip diagnostics, India]. Low density lipoprotein cholesterol values have long been estimated using the Friedwald formula:

[Total HDL cholesterol] – 20% of the Triglycerides value = Estimated LDL-cholesterol [13].

Malonaldehyde (MDA) is determined as Thiobarbituric acid reactive substances (TBARS)[14,15].

Estimation of Total Antioxidant Capacity using the FRAP (Ferric Reducing Ability of Plasma) assay.TAC assay measures the combined antioxidant capacity of all its components.(16)

The subjects having Diabetes mellitus, Hypertension, Coronary heart disease, endocrine disorders, alcohol abuse, and on lipid lowering drugs are excluded from the study.

STATISTICAL ANALYSIS: Mean±S.D values of all biochemical parameters were calculated in study and control groups and the mean difference was compared by using student't' - Test.

Mean serum cholesterol, LDL-Cholesterol, triglyceride levels were significantly increased in study group when compared to controls and mean serum HDL-Cholesterol level was significantly decreased.(TABLE 1)

These observations are consistent with previously mentioned related studies T Valkenburg O et al[17], Djuro Macut et al[18], Anuradha Kalra et al[19].

Mean MDA level in study group was significantly increased when compared with controls. The Total antioxidant capacity in study group was significantly decreased when compared with controls (TABLE 2).

Present results are consistent with the previous studies Zhang D et al[20], Palacio JR et al [21] and Fenkci V et al[22].

DISCUSSION: In our study we tried to assess lipid profile and oxidative stress in PCOS cases. We observed that mean serum levels of total cholesterol, TG, LDL-C, MDA in cases of PCOS have increased significantly, when compared with controls. Mean serum HDL-C & TAC levels are decreased significantly when compared with controls.

Women with PCOS would be predicted to be at high risk for dyslipidemia because they have elevated androgen levels and are frequently obese. PCOS women are often hyperinsulinemic and insulin resistant, which can lead to dyslipidemia . Elevated plasma insulin concentrations enhance very-low-density lipoprotein (VLDL) synthesis, leading to hypertriglyceridemia. Progressive elimination of lipid and apolipoproteins from the VLDL particle leads to an increased formation of intermediate-density and low-density lipoproteins, both of which are atherogenic (6)

ApoA-1 - apolipoprotein A-1; ApoB - apolipoprotein B; CE - cholesteryl ester, CETP - cholesteryl ester transfer protein; FFA - free fatty acid; HL - hepatic lipase; LPL - lipoprotein lipase; SD LDL, - small dense LDL cholesterol; TG - triglyceride.

Insulin resistance initiates the characteristic triad of high triglyceride level, low HDL cholesterol level and high small dense LDL level. If the concentration of VLDL-transported triglyceride is high, CETP promotes the transfer of LDL cholesteryl ester or HDL cholesteryl ester in exchange for triglyceride. Triglyceride-rich HDL cholesterol or LDL cholesterol can undergo hydrolysis by hepatic lipase or lipoprotein lipase. (22)

CONCLUSION: Dyslipidemia and increased oxidative stress is observed in patients with polycystic ovary syndrome. PCOS women should be evaluated for status of serum lipids and oxidative stress, which aids in the management of these cases. Correction of dyslipidemias and antioxidant supplementation can be beneficial in treatment of PCOS cases. It also reduces the overall morbidity and enhances the prognosis of PCOS.

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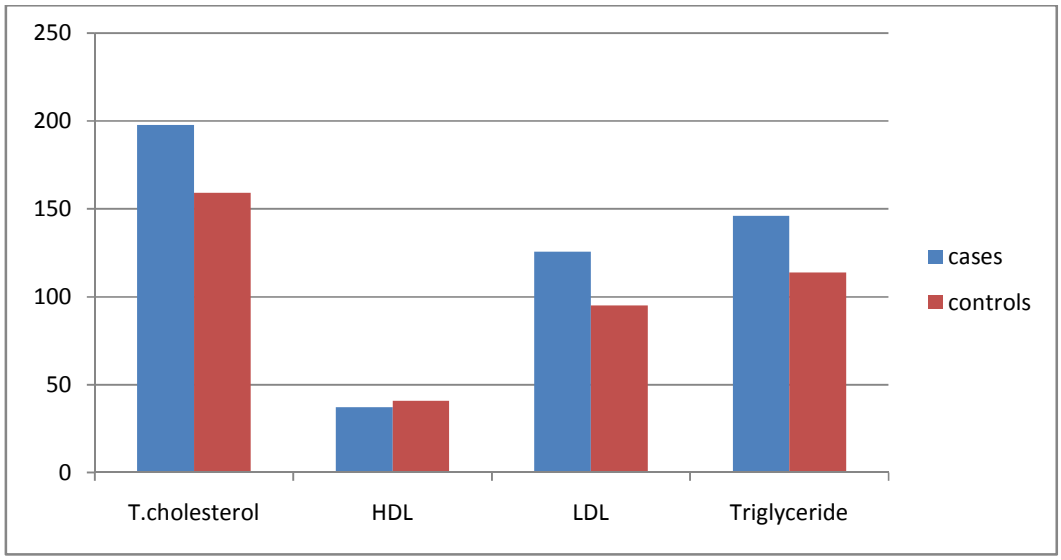
BIBLIOGRAPH:

1. Wild RA 2002 Long-term health consequences of PCOS. Hum Reprod Update 8:231–241]
2. Lorenz LB, Wild RA 2007 Polycystic ovarian syndrome: an evidence-based approach to evaluation and management of diabetes and cardiovascular risks for today's clinician. Clin Obstet Gynecol 50:226–243]
3. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, Kuller L 1995 Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol 15:821–826
4. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN 2006 Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 91:48–53
5. Wild S, Pierpoint T, McKeigue P, Jacobs H 2000 Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf) 52:595–60
6. DeFronzo RA, Ferrannini E 1991 Insulin resistance . A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care14:173–1947.
7. Reaven GM: Banting lecture 1988: role of insulin resistance in human disease. Diabetes 37:1595-607,1988
8. Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91:336-40.
9. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2005;3:28.
10. Verit FF, Erel O. Oxidative stress in non-obese women with polycystic ovary syndrome: correlations with endocrine and screening parameters. Gynecol Obstet Invest. 2008;65:233-9.
11. Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. Fertil Steril 2009; 91(3): 805-11.
12. Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81:19–25.
13. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18:499-502.
14. Sangeetha P, U.N. Das, Koratkar.R, Surya prabha.P, Increase in Free radical lipid peroxidation following chemotherapy in cancer; Free radical Biol Med 1990 ;8:15-19.
15. Iris F.F. Benzie and J.J. Strain .The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical biochemistry 1996; 239:70-76.
16. Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. Fertil Steril 2009; 91(3): 805-11.
17. T Valkenburg O ,Steegers –Theunissen RP,Smedts HP,Polycystic ovary syndrome linked to atherogenic lipid profile J Clin Endocrin Metab 2007.
18. Djuro Macut, Dimitrios Panidis, Biljana Glisic, Nikolaos Spanos, Milan Petakov,Jelica Bjekic, Ivana Bozic and Svetozar Damjanovic. Relation of

proatherogenic lipid profile and insulin resistance in PCOS Endocrine Abstracts 2008;16 P:646.
19. Anuradha Kalra, Sreekumaran Nair, Lavanya Rai . Association of obesity and insulin resistance with dyslipidemia in Indian women with polycystic ovarian syndrome Indian journal of medical sciences 2006 ; 60(11): 447-453.
20. Zhang D, Luo WY, Liao H, Wang CF, Sun Y. The effects of oxidative Stress to PCOS. Sichuan Da Xue Xue Bao Yi Xue Ban 2008;39(3): 421-3.
21. Palacio JR, Iborra A, Ulcova-Gallova Z, Badia R, Martinez P. The presence of antibodies to oxidative modified proteins in serum from polycystic ovary syndrome patients. Clin Exp Immunol 2006; 144(2): 217-22.
22. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 2003; 80(1): 123-7.
23. Arshag D Mooradian. Dyslipidemia in type 2 diabetes mellitus. Nature Clinical Practice Endocrinology & Metabolism (2009) 5, 150-159

RESULTS: TABLE 1: Mean Total Cholesterol, HDL, LDL, TG Levels In Control Group & Study Group

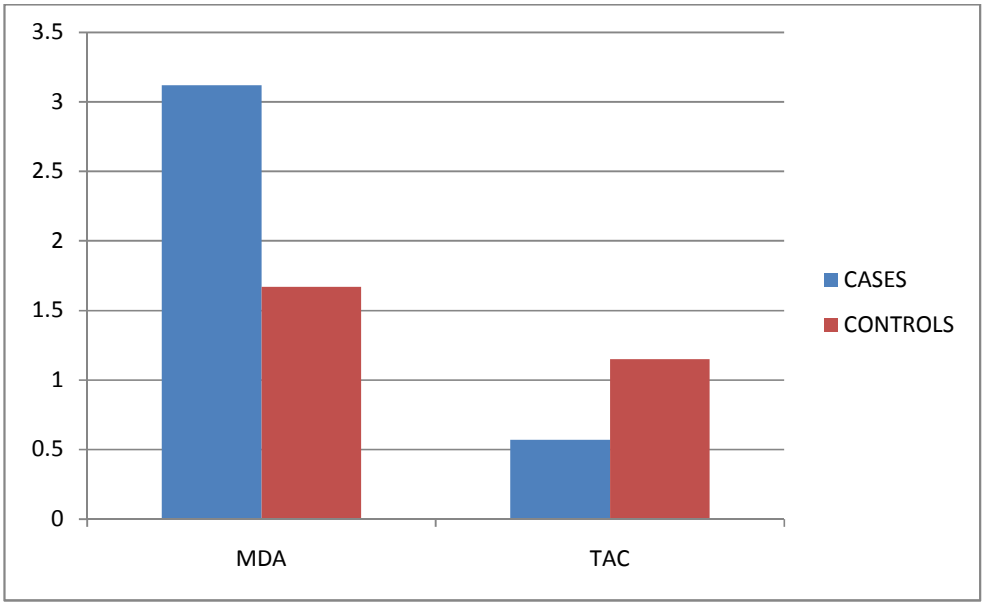
PARAMETERS (mg/dl)	Control group		Study group		P value
	Mean	Std.Dev	Mean	Std.Dev	
T.Cholesterol	159.16	12.87	197.81	37.94	<0.0003
HDL	40.87	3.58	37.13	5.02	<0.0013
LDL	95.13	14.16	125.52	41.29	<0.0003
Triglyceride	113.71	38.36	146.19	57.77	<0.0115



Graph- 1

TABLE 2: Mean MDA &TAC Levels In Control & Study Group

PARAMETERS	Control group		Study group		
	Mean	Std.Dev	Mean	Std.Dev	P value
MDA(nmol/ml)	1.67	0.53	3.12	1.16	<0.0001
TAC(umol/ml)	1.15	0.33	0.57	0.25	<0.0001



Graph -2

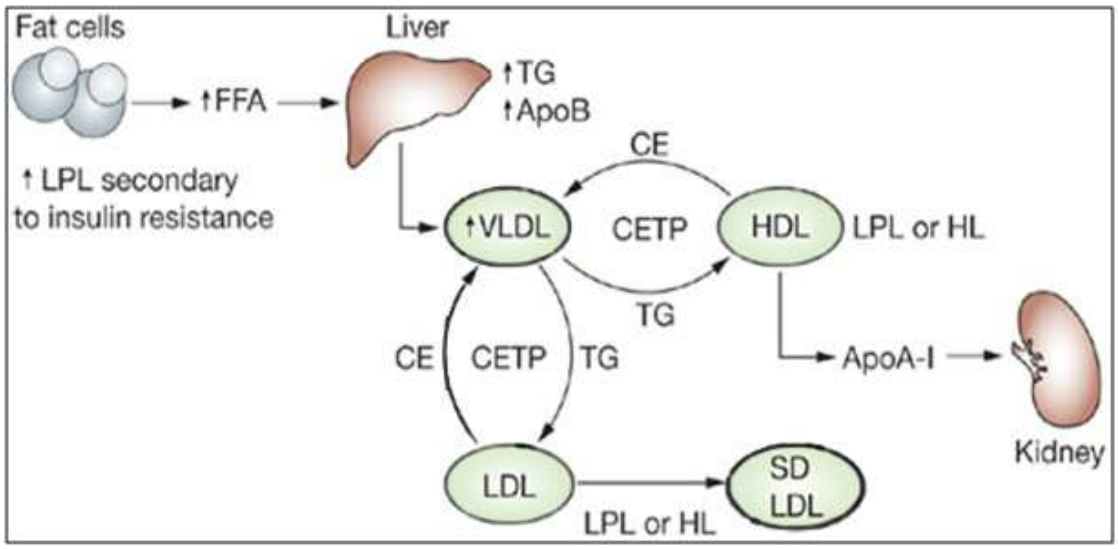


FIG.1:The role of insulin resistance in dyslipidemia

THE CORRELATION BETWEEN NT Pro-BNP LEVELS AND ECHOCARDIOGRAPHIC FINDINGS IN A PATIENT WITH ACUTE ONSET DYSPNEA.

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ABSTRACT: BACKGROUND: Cardiac failure is one of the most serious and life threatening condition which needs an early diagnosis and prompt management to avoid mortality. 2 D echo serves as a very important tool in confirming the diagnosis of cardiac failure. Many centers in our country still don't have the facility of the same and hence we studied the role of NT pro-BNP as a substitute for 2 D echo. **AIM:** The aim of this study was to compare the level of serum NT pro-BNP with the 2 D echo findings in patients with acute onset dyspnea. **METHODS:** We studied 100 patients with acute onset dyspnea. Through history and examination was done. 2 d echo was done in all the patients. We measured the baseline level of serum NT Pro BNP in all the patients and a cut off level of 1800pg/ml was kept to compensate for the caveats in the measurement of NT ProBNP. The personnel who did echo were blinded to the result of NT pro-BNP . We compared the 2 D echo findings of the patients with the levels of NT pro-BNP. **RESULTS**: The ejection fraction, regional wall motion abnormality and diastolic dysfunction had significant correlation with a positive NT-ProBNP level, where as ventricular hypertrophy and pulmonary artery systolic pressure had no correlation with the same**. CONCLUSIONS:** NT pro-BNP level can be used to confirm the diagnosis of cardiac failure state in absence of 2 D echo facilities.

KEY WORDS: Cardiac failure, NT Pro BNP, 2 D echo, diagnosis

INTRODUCTION: Heart failure is amongst the most common cause of acute onset dyspnoea. It is a clinical syndrome resulting from the inability of the heart to pump adequate amount of blood to meet the demands of the body. Main causes include coronary artery disease, hypertension, and complications of diabetes mellitus and anemia. The important precipitating factors include chest infection, accelerated hypertension, acute renal failure and other metabolic derangements. The left ventricular injury due to these causes leads to left ventricular remodeling, reduced ejection fraction, arrhythmias and sudden cardiac death (1). Hence, it is very essential to establish the diagnosis of cardiac failure early in the course of the disease to prevent mortality and morbidity from this condition. Several investigations including X-ray chest and 2D Echo serve as a useful guide to distinguish between the causes of dyspnoea.

Over a period of years, BNP–brain natriuretic peptide has gained a lot of attention due to its high negative predictive value in dyspnoea of cardiac origin. The suspicion that the heart may have an endocrine function was raised approximately 50 years ago. At that time it was shown that dilatation of cardiac atria produced natriuresis (2). Initially BNP was proposed as a simple diagnostic tool to aid in the clinical assessment of decompensated heart failure; studies have now shown that this hormone offers enormous diagnostic as well as prognostic information in a variety of settings including cardiomyopathy, congestive heart syndromes, ischemic heart disease and even pulmonary thromboembolism(3). The **PRIDE** study conducted by the Experta Medica Inc. concluded that "Increased NT-proBNP was the strongest independent predictor of a final diagnosis of acute congestive heart failure (4)".

One more study conducted by Résumés Mogelvang et al at Copenhagen Denmark concluded that -In general population with dyspnoea, plasma pro-BNP concentration are increased in left ventricular dilatation, hypertrophy, systolic dysfunction or diastolic dysfunction but are unaffected by pulmonary dysfunction(5).

The main aim of our study was to establish the correlation between the serum level of NT pro-BNP and 2 D echo findings so as to establish its role as a good substitute in the settings where 2 D echo is unavailable.

MATERIALS AND METHODS:

- This is a prospective study of 100 patients with acute onset breathlessness for three years. Indoor patients admitted to Emergency Room and/ or intensive care unit of tertiary centre were taken as a trial population. Patients with acute onset dyspnea between 18-75 yrs both male and female were included. Patients with or without prior history of cardiac failure and on anti cardiac failure measures were taken. Patients presenting with acute onset dyspnea due to traumatic causes, known renal dysfunction or on dialysis of any form and with serum Creatinine >2mg/dl on day one of presentation were excluded.

This prospective analysis involved 100 patient presented to Lilavati hospital and research centre, a tertiary care centre with acute onset dyspnea. In all the patients taken, an initial history and through physical examination was done. The patients selected for the study were those who on first presentation to the emergency department needed intensive care support. The patients underwent routine blood investigations like complete blood count, liver profile, renal profile, x-ray chest, and electrocardiogram. The personnel doing the echocardiography were blinded to the result of NT-pro BNP. An echocardiography, cardiac troponin T, and NT-pro BNP levels were measured in all the patients on presentation.

NT-PROBNP ASSAY:

- Elecsys pro BNP kit was used. It detects two polyclonal antibodies directed against NT-proBNP amino acid 1-21 and amino acid 39-50 respectively.
- NT-proBNP level was measured in heparinised serum sample of 20 microlitre.
- The test principle is Electrochemiluminescence sandwich immunoassay.
- It measures the level between 5-35,000 pg/ml
- A cross reactivity of 0.001% with ANP, BNP and CNP

The cut-off value to be considered significant in a given patient was kept 1800pg/ml to compensate for the fluctuation in the NT-proBNP level with respect to age, gender, body mass index, previous history of heart failure etc. The patients were divided into positive and negative groups based on this cut-off level.

The levels of NT pro-BNP were compared with the 2D echo findings and all the data collected was analyzed statistically using chi- square tests which includes the Pearson chi-square, continuity correction and fisher's exact test. A p value was calculated using these tests. The association between all the parameters with the positive and the negative value was made and conclusions were drawn accordingly.

OBSERVATION AND RESULTS: A total of 100 patients of acute onset breathlessness presenting to ER of Lilavati hospital were studied. Out of these, 43 were females and 57 were males. The average age was 56.64 yrs with minimum being 18 and maximum being 75 years.

Amongst the 100 patients, 87 patients had an abnormal ECG and 52 had pulmonary edema on X-ray chest. 2D-echocardiography showed ejection fraction of less than 40% in 43 patients, diastolic dysfunction in 46 patients, regional wall motion abnormality in 41 patients, PASP of >25mmhg in 39 patients and ventricular hypertrophy in 40 patients. A cardiac troponin T level of >0.01 was seen in 33 cases. (Figure: 1)

NT- Pro BNP test was performed in all patients and showed a positive result (>1800pg/ml) in 49% patients with a negative result in 51% (figure 2).

The 2 D echo findings had a significant correlation with the positive result. The ejection fraction, regional wall motion abnormality and diastolic dysfunction had significant correlation with a positive NT-ProBNP level, where as ventricular hypertrophy and pulmonary artery systolic pressure had no correlation with the same. (Table1,2,3,4,5)

DISCUSSION: Cardiac failure is a burgeoning problem worldwide, with more than 20 million people affected. It is a clinical syndrome that occurs in patients who, because of an inherited or acquired abnormality of cardiac structure and or function, develop constellation of clinical symptoms (dyspnoea and fatigue) and signs (edema and rales) that lead to frequent hospitalization, a poor quality of life and shortened life expectancy (6). When confronted with a patient of acute onset dyspnoea in the emergency setting, a quick decision has to be made regarding the diagnosis of the cause. Heart failure (HF) is one of the most common causes. Patients with this syndrome often have co morbidities that contribute to their symptoms, thereby making the diagnosis difficult (7). Delays in diagnosing HF result in increased mortality, hospital stays, and treatment costs (8,9). However, prompt evaluation of cardiac function with imaging such as nuclear scans and cardiac catheterization is not feasible in most settings. Furthermore, echocardiography, if available, can miss HF of diastolic origin(10) Given the increasing burden of HF and the increased benefits of early goal directed therapy, physicians in acute care settings require an accurate diagnostic test that will allow them to rapidly determine whether or not HF is the cause of their patients' symptoms .Various pulmonary diseases and other causes mimicking acute heart failure makes it challenging for the emergency department physician to take a call on the same .The Brain natriuretic peptides have gained a lot of interest in the recent years as a promising diagnostic and prognostic tool in the setting of acute onset dyspnoea Initially it was proposed as a simple diagnostic tool to aid in the clinical assessment of

decompensated heart failure; studies have now shown that this hormone now offers enormous diagnostic as well as prognostic information in a variety of settings including cardiomyopathy, congestive heart failure syndromes, ischemic heart disease and even pulmonary thromboembolism. The first study done was by David and colleagues who measured the levels of the natriuretic hormones ANP and BNP in 52 patients presenting with acute dyspnoea. They found that admission plasma BNP concentration more accurately reflected the final diagnosis than did left ventricular ejection fraction or ANP plasma concentration.(11)

The first use of the point-of-care BNP assay was used by Dao and colleagues who evaluated 250 patients presenting to an urgent care centre with the chief complaint of dyspnoea. The end result of the study was that BNP levels were the strongest predictor of those who had heart failure. (12) This particular finding spurred the international Breathing Not Properly study which was a large-scale prospective study using BNP levels to evaluate the cause of dyspnoea. This trail prospectively evaluated 1586 patients who presented to the ED with acute dyspnoea. A BNP cutoff value of 100pg/ml had a sensitivity of 90% and a specificity of 76% for differentiating heart failure from other causes of dyspnoea . (13) A study on the lines of Breathing Not Properly trail was conducted using the NT pro-BNP level in patients with dyspnoea. The proBNP Investigation of Dyspnoea in the Emergency Department (PRIDE) study (4) evaluated 600 patients presenting to the ED with acute onset dyspnoea. NT-proBNP levels were compared with the clinical assessment of managing physicians for identifying CHF. The authors suggested two different, age-based cut-points for diagnosing acute heart failure: NT pro BNP above 450 pg/ ml for those younger than 50 yrs old and NT-proBNP above 900 pg/ ml for those 50 yrs or older. The study found that NT-proBNP was the strongest independent predictor of a final diagnosis of acute heart failure with an odds ratio of 44. The optimal cut-point for ruling out heart failure was NT-proBNP less than 300 pg/ ml, which had a 99% negative predictive value. This study helped establish the clinical utility of NT-proBNP for diagnosing or excluding heart failure in the ED setting.

Another study done in the general population with dyspnoea concluded that plasma proBNP concentrations are increased in left ventricular dilatation, hypertrophy, systolic dysfunction, or diastolic dysfunction, but are unaffected by pulmonary dysfunction (5) The level of B-type natriuretic peptides is influenced by a many factors. These factors may play an important role in the discordance observed in the clinical picture and the given value of NT-proBNP in the blood. (14) Previous history of heart failure often gives a natriuretic peptide value above the suggested cut-off points, even though the patient is evoluemic. To minimize this error, it is worth while having a base line value in such patients in their dry weight, any significant deviation from this value could then be used for either diagnosing acute exacerbation of heart failure or much lower value might indicate that the patient is over diurised. (13) The level of NT-pro BNP is much higher in the elderly age group as compared to the general population. Defining an age based cut-point for NT-proBNP has helped to over come this short coming. (14) The levels of natriuretic peptides have found to be higher in females as compared to males of the corresponding age. Studies have shown that estrogen might have a role to play in this; however these differences are unimportant while dealing with an acutely dyspneic patient. (15) The natriuretic peptides level rise with worsening renal function though it is unclear whether the concomitant heart disease with renal failure or decreased clearance of the hormone via the kidneys is responsible for this rise.

As there are factors which give a false high value, there are factors which give a value lower than normal despite clear presence of cardiac failure. The following conditions need to be

kept in mind while dealing with a difference in the clinical scenario and the levels of NT-proBNP. Obesity is one of the conditions which give rise to a lower value even in the presence of heart failure. The pathphysiologic base for this is still debated. There is some evidence that natriuretic peptide receptors in the adipose cells lead to increased clearance of the hormone, there may also be a component of reduced natriuretic peptide secretion in obesity that may also contribute to this low level. For patients with acute dyspnoea and a body mass index of more than 35, a cut-point of BNP 54 pg/ml or higher maintains the same 90% sensitivity for diagnosing acute CHF as does the standard cut-point of BNP 100pg/ml or higher in non obese individuals. Flash pulmonary edema can give an in appropriately low level of the natriuretic peptide. This may be due to the time required for natriuretic peptide up-regulation and expression in response to ventricular wall stress. CHF resulting due o upstream from the left ventricle, such as acute mitral regurgitation or mitral stenosis, natriuretic peptide levels can be low or normal despite severe CHF. This is because the left ventricular function may be uncompromised, especially in the acute setting. On the similar lines, the patients with constrictive pericarditis may have symptoms of heart failure and elevated filling pressures, but tend to have low natriuretic peptide level due to lack of ventricular stretch.(14) A significant correlation between the higher levels of NT-proBNP and echocardiography findings makes it a good substitute for echocardiography in the setting of absence of trained personnel. The excellent correlation between the raised value and diastolic dysfunction helps in better diagnosis of the condition.

CONCLUSION: NT pro-BNP level can be used to confirm the diagnosis of cardiac failure state in absence of 2 D echo facilities.

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BIBLIOGRAPHY:

1. Girish B Ramteke, Lincy G Ramteke, Current concepts in management of congestive heart failure. Postgraduate Medicine (Emergency medicine-Emerging Discipline) 2008 197-211.
2. Henry JP, Gauer OH, Reeves JL. Evidence of the atrial location of receptors influencing urine flow. Circ Res 1956; 4:85 –90.
3. Jagat Narula, James B. Young editorial Natriuretic peptides in heart failure: Empathizing with the sobbing heart. Heart failure clinics July 2006 volume 2 xi-xiii
4. James L. Januzzi, Jr., The N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study
5. Rasmus Mogelvang, MD, Discriminating Between Cardiac and Pulmonary Dysfunction in the General Population with Dyspnoea by Plasma Pro-B-Type Natriuretic Peptide. Journal of the American College of Cardiology Vol. 50, No. 17, 2007 1694-701.
6. Douglas L. Mann Heart failure and cor pulmonale Harrison's principles of internal medicine 1433-45
7. Doust JA, Glasziou PP, Pietrzak E, Dobson AJ. A systematic review of the diagnostic accuracy of natriuretic peptides for heart failure. Arch Intern Med 2004; 164:1978–84.

8. Bales AC, Sorrentino MJ. Causes of congestive heart failure. Prompt diagnosis may affect prognosis. Post grad Med 1997; 101:44–6.
9. Wuerz RC, Meador SA. Effects of pre hospital medications on mortality and length of stay in congestive heart failure. Ann Emerg Med 1992; 21:66974
10. Wang CS, FitzGerald JM, Schulzer M, Mak E, Ayas NT. Does this dyspneic patient in the emergency department have congestive heart failure? JAMA 2005; 294:1944–56.
11. Davis M, Epiner E, Richards G, et al. Plasma brain natriuretic peptide in assessment of acute dyspnea. Lancet 1994:343(8995):440-4
12. Dao Q, Krishnaswamy P, Kazanegra R, et al. Utility of B-type natriuretic peptide in the diagnosis of congestive heart failure in an urgent care setting. J am coll Cardiol 2001;37(2):379-85
13. Maisel AS, Krihnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. New England Journal of Medicine 2002;347(3):161-7
14. Loris B. Daniels, MD, Alan S. Maisel, MD Natriuretic peptides as diagnostic test : Lessons from the first 5 years of clinical application Heart failure clinic 2 (2006) 299-309
15. Redfield MM, Rodeheffer RJ, Jacobsen SJ, et al. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002:40(5):976-82

Table no. 1: Association among the cases between- Ejection Fraction (%) & NT- Pro BNP (pg/ml)

Ejection Fraction (%)		NT- Pro BNP (pg/ml)		Total
		>= 1800	< 1800	
Low	No.	34	9	43
	%	79.1%	20.9%	100.0%
Normal	No.	15	42	57
	%	26.3%	73.7%	100.0%
Total	No.	49	51	100
	%	49.0%	51.0%	100.0%

Chi-square tests	Value	df	p-value	Association is-
Pearson Chi-Square	27.2950	1	1.75E-07	Significant
Continuity Correction	25.225	1	5.10E-07	Significant
Fisher's Exact Test			2.30E-07	Significant

Table 2: Association among the cases between- Diastolic Dysfunction & NT- Pro BNP (pg/ml)

Diastolic Dysfunction		NT- Pro BNP (pg/ml)		Total
		>= 1800	< 1800	
Yes	No.	28	18	46
	%	60.9%	39.1%	100.0%
No	No.	21	33	54
	%	38.9%	61.1%	100.0%
Total	No.	49	51	100
	%	49.0%	51.0%	100.0%

Chi-square tests	Value	df	p-value	Association is-
Pearson Chi-Square	4.8030	1	0.028	Significant
Continuity Correction	3.963	1	0.047	Significant
Fisher's Exact Test			0.044	Significant

Table 3: Association among the cases between- RVMA & NT- Pro BNP (pg/ml)

RVMA		NT- Pro BNP (pg/ml)		Total
		>= 1800	< 1800	
Yes	No.	32	9	41
	%	78.0%	22.0%	100.0%
No	No.	17	42	59
	%	28.8%	71.2%	100.0%
Total	No.	49	51	100
	%	49.0%	51.0%	100.0%

Chi-square tests	Value	df	p-value	Association is-
Pearson Chi-Square	23.4650	1	1.27E-06	Significant
Continuity Correction	21.536	1	3.47E-06	Significant
Fisher's Exact Test			1.79E-06	Significant

Table 4: Association among the cases between- Ventricular Hypertrophy &NT- Pro BNP (pg/ml)

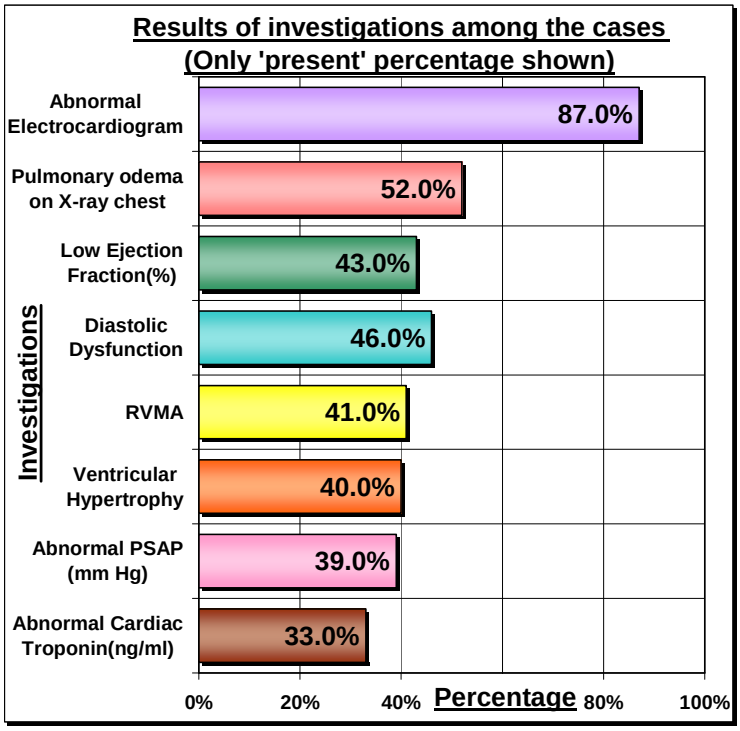
Ventricular Hypertrophy		NT- Pro BNP (pg/ml)		Total
		>= 1800	< 1800	
Yes	No.	24	16	40
	%	60.0%	40.0%	100.0%
No	No.	25	35	60
	%	41.7%	58.3%	100.0%
Total	No.	49	51	100
	%	49.0%	51.0%	100.0%

Chi-square tests	Value	df	p-value	Association is-
Pearson Chi-Square	3.2280	1	0.072	Not significant
Continuity Correction	2.536	1	0.111	Not significant
Fisher's Exact Test			0.102	Not significant

Table 5: Association among the cases between- PSAP (mm Hg) & NT- Pro BNP (pg/ml)

PSAP (mm Hg)		NT- Pro BNP (pg/ml)		Total
		>= 1800	< 1800	
Abnormal	No.	23	16	39
	%	59.0%	41.0%	100.0%
Normal	No.	26	35	61
	%	42.6%	57.4%	100.0%
Total	No.	49	51	100
	%	49.0%	51.0%	100.0%

Chi-square tests	Value	df	p-value	Association is-
Pearson Chi-Square	2.5450	1	0.111	Not significant
Continuity Correction	1.933	1	0.164	Not significant
Fisher's Exact Test			0.151	Not significant



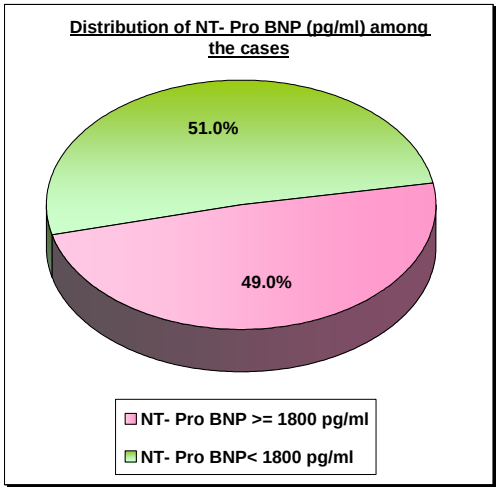


Fig: 2 NT- Pro BNP Test

ANAESTHETIC MANAGEMENT OF A CASE OF HEREDITARY SPHEROCYTOSIS FOR SPLENECTOMY AND CHOLECYSTECTOMY.

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ABSTRACT: We report successful anaesthetic management of a patient with hereditary spherocytosis who underwent laproscopic splenectomy, cholecystectomy and appendioectomy. Hereditary spherocytosis is a familial hemolytic disorder with marked heterogeneity of clinical features, ranging from asymptomatic condition to a fulminant hemolytic anaemia. Commonly recommended perioperative management in these patients includes preemptive erythrocyte transfusion, aggressive hydration and avoidance of hypoxia, aplastic crisis, hypothermia and acidosis. The management of such a case is challenging from anaesthetic point of view because of sickling oriented anaesthetic approach. Key words: Hereditary spherocytosis, splenectomy, cholecystectomy, perioperative management.

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INTRODUCTION: Hereditary spherocytosis is a genetically transmitted (autosomal dominant) form of spherocytosis. It is an auto-hemolytic anemia characterized by the production of red blood cells that are sphere-shaped rather than biconcave disc shaped and therefore more prone to hemolysis.1,2,3 An osmotic fragility test can aid in the diagnosis.2

It is characterized by anaemia jaundice and splenomegaly.1,2,3,4 The perioperative management of a patient with hereditary spherocytosis is challenging because of sickling oriented anaesthetic approach. The key to successful management of a such a patient is to avoid triggering factors, vigilant perioperative monitoring and aggressive treatment of aplastic crisis hypothermia, hypotension.

CASE REPORT: A 32 year old female presented with right upper quadrant pain and nausea. On physical examination she had pallor, icteric sclera and splenomegaly. She had past history of jaundice 9 years back for which she had received conservative management. Also patient had history of icteric sclera since childhood. Though her past medical history was typical of hereditary spherocytosis, she was diagnosed as a case of hereditary spherocytosis only on admission on basis of her history and investigations.

Her preoperative investigations were as follows: USG abdomen and pelvis was suggestive of appendicitis, splenomegaly and cholelithiasis, Hb: 6.2gm%, reticulocyte count: 18.5%, PBS : Hereditary spherocytes (+), osmotic fragility ↑ed, Coomb's test was negative.

In the liver function tests, total serum bilirubin was 5.8 mg /dl and indirect bilirubin was 5.4 mg/dl and PT/INR was 27/2 (Control 13.5 sec). Platelet count was 1 lakh / mm3. Investigations were within normal limits.

Patient received five units of packed cell volume, five units fresh frozen plasma and inj Vit K 30 mg iv preoperatively. Her post-transfusion invest were as follows.

Hb: 9.9 gm%, platelet count: 71,000/mm3 and PT/INR:18.1/1.35. Before surgery, she was given vaccine against pneumococci, H-influenza and hepatitis B.

Considering preoperative investigations and the need of the surgery, it was decided to proceed with general anaesthesia. Preoperatively 3 units fresh frozen plasma, 2 units platelet concentrates and 2 units packed cell volume were keep ready.

In the operation theatre intravenous line was secured with 18 G intracath. The patient was premedicated with intravenous glycopyrrolate 0.2 mg. Monitoring included ECG pulse oximetry, NIBP and EtCO2, temperature and urine output. Pre-induction patient was given inj midazolam 0.5 mg and fentanyl 50µg. After adequate preoxygenation patient was induced with i.v Inj. Thiopentone sodium 5 mg/kg and muscle relaxation was achieved with succinylcholine 2 mg/kg. Intubation was done gently with 6.5 no cuffed portex tube. Anaesthesia was maintained with 50% N2O in O2 and isoflurane (0.2-1%) and intermittent top-ups of vecuronium.

Intra-operatively patient received 3 units fresh frozen plasma, 2 units platelet concentrates. Patient was given 1 unit packed cell volume after clamping splenic vessels. Intra-op hypothermia was avoided using warm IV fluids, properly covering the patient and monitoring temperature.

Intra-operative hypoxia was prevented by providing 50% Fi O2, giving IPPV, monitoring SPO2. Hypoperfusion was prevented maintaining adequate intra vascular volume with crystalloids, FFP, platelet concentrates and PCV and hypotension was prevented by giving titrated dose of isoflurane and fentanyl and maintaining adequate intravascular volume.

Intraoperatively the patient was stable. The duration of surgery was 7 hours. At the end of surgery, the neuromuscular block was reversed with Inj. neostigmine 2.5 mg and Inj glycopyrrolate 0.4 mg. Patient was extubated and shifted to ICU for monitoring.

DISCUSSION: Hereditary spherocytosis is a rare inherited red cell membrane disorder that is characterized by spherically shaped red blood corpuscles on peripheral blood smear.2,3,4 It was described in initially in 1871.5 Spherocytosis results from red cell membrane defect.4 Spectin deficiency is the most common defect. Complications of hereditary spherocytosis include gallstones, aplastic, hemolytic and megaloblastic crises, poor growth and skeletal deformities.2,3,4

For practical purpose, the treatment of hereditary spherocytosis involves splenectomy, pre and post splenectomy care. Commonly recommended perioperative management includes preemptive erythrocyte transfusion, aggressive hydration, avoidance of hypoxia, hypothermia and acidosis.6,7

Anaesthesia management of patient of hereditary spherocytosis is challenging. Avoidance of hypoxaemia is the key goal. Premedication and opioid based analgesia has to be used with extreme caution because of concern about respiratory depression, hypoxia and sickling.8 Intraop hypothermia is to be avoided to minimize vasoconstriction and associated circulatory stasis.

Splenectomy is very effective in reducing hemolysis leading to significant prolongation of red cell span. The clinical manifestations and complications (anaemia and gallstones) are

much reduced in hereditary spherocytosis but at the price of life threatening sepsis from encapsulated organism particularly streptococcus pneumonia.9

For pre-splenectomy vaccination and post-splenectomy follow up, our patient was preoperatively vaccinated and also advised to repeat pneumococcal vaccination at 5 year intervals.

CONCLUSION: The patient of hereditary spherocytosis is at increased risk for developing various complications like aplastic or megaloblastic crisis, hemolytic crisis, acute chest syndrome stroke, etc. The anaesthetist should have better understanding of silent but insidious end-organ damage (brain, kidney and lungs). This allows for more accurate preoperative assessment. It also points to the development of potentially effective ways to avoid and treat complications.

REFERENCES:

1. The Lancet, volume 372, Issue 9647, 1411-26, 18 Oct. 2008.
2. Hoffbrand AV, Petit JE, Moss PAH. Hereditary hemolytic anaemias. In: Essential haemotology. 4th Ed. Massachusetts Blackwell science, 2001:60-63.
3. Gallager PG, Forget BG. Hereditary spherocytosis, elliptocytosis and related disorders. In: Williams Haemotology, 6th Ed. Chicago : McGraw Hill, 2000: 1189-1209.
4. Pallister C. Disorders of red cell survival. In: Blood Physiology and pathophysiology. Oxford : Butterworth-Heinemann, 1994: 33-52.
5. Shafqat S, Roger V, Hereditary Spherocytosis. Paediatrics in Review 2004; 25:168-172.
6. Steinberg MH. Management of sickle cell disease N Eng J. Med 1999; 340:1021-30.
7. Vichinsky EP, Haberkern CM, Neumary L, et al. A comparision of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The preoperative transfusion in Sickle Cell Disease Study Group. N Eng J. Med 1995; 333:206-13.
8. Shapiro ND, Poe MF. Sickle Cell disease; an anaesthesiological problem, Anaesthesiology 1995; 16:771-80.
9. P. H. B. Bolton – Maggs, R. F. Stevens et al. Guidelines for diagnosis and management of hereditary spherocytosis, British Journal of Haemotology, 2004;126, 455-74.

STUDY OF EPIDEMIOLOGICAL FACTORS IN CRYPTOSPORIDIOSIS IN CHILDREN WITH DIARRHOEA

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ABSTRACT: BACKGROUND: Cryptosporidium has emerged as one of the major parasitic agents as a cause of diarrhoea in children. Various epidemiological factors have been described by different workers. Aims: This study was done to determine different epidemiological factors incriminated in cryptosporidiosis. Methods: Stool samples from 240 children with diarrhoea were examined for presence of Cryptosporidium. Wet mount examination, modified Ziehl-Neelsen (Z.N.) and Safranine-methylene blue staining methods were performed. For 177 samples, ELISA was also done. Detailed history of patients regarding their socioeconomic status and various sociodemographic factors was taken. Statistical Analysis: Chi-square and z tests were used to compare differences between the groups. A p value of ≤0.05 was considered significant. Results: Majority of patients were in Class IV socioeconomic group. Top-feeding, use of insanitary wells for drinking purposes, close association with animals, field defaecation and residence in rural areas were different factors that contributed to the spread of infection. Oocysts were present in 21 children on different staining procedures and 23 were positive by ELISA. Conclusion: Different sociodemographic factors like improper sanitation practices, drinking contaminated water, early withdrawal of breast feeding and close intimacy with animals are various factors that can enhance the spread of infection in community. Preventive measures are of great importance in control of spread of infection as there is no specific therapy for cryptosporidiosis.

KEYWORDS: Cryptosporidium, children, diarrhoea, socioeconomic status, sociodemographic factors

INTRODUCTION: Diarrhoea accounts for childhood morbidity and mortality in developing countries like India. In our country, every year about one million cases of diarrhoea are reported in children[1]. Besides other causes of bacterial and parasitic infectious diarrhoeas, Cryptosporidium has emerged as one of the major causes of diarrhoea in immunocompetent and immunocompromised children[2]. Cryptosporidium is a coccidian protozoan parasite found in the brush border of enterocytes of the small intestine in many vertebrates, including humans[3]. Cryptosporidiosis can induce self-limiting diarrhoea in immunocompetent persons

but potentially life-threatening diarrhoea in immunocompromised persons, especially those with AIDS, transplant recipients, those receiving chemotherapy, institutionalized patients and patients with other immunosuppressive infectious diseases[4]. Infection by this parasite accounts for up to 6 percent of all diarrhoeal disease in immunocompetent persons. The infection is also present in up to 24 percent of persons with both AIDS and diarrhoea worldwide[5].

The best-documented routes of transmission are waterborne, food borne, and person-to-person spread. The majority of the documented outbreaks of waterborne infection in the world have been attributed to contaminated drinking water supplies, although contaminated water used for recreational activities has also been implicated[4]. Diagnosis of infection generally requires observation of infective stage (oocysts) by microscopic examination of faeces by using different types of staining methods like modified Z.N. and safranine-methylene blue and immunoflourescence with monoclonal antibodies[6,5,7].

This study was undertaken to determine the relevance of various epidemiological factors in transmission of cryptosporidial infection in children with diarrhoea. The different epidemiological factors studied in this study were age, sex, socio-economic status and sociodemographic characteristics.

MATERIAL AND METHODS: The present study was carried out in the Department of Microbiology for the detection of Cryptosporidium on stool samples from children attending the out-patient and in-patient sections of Department of Paediatrics at J. N. Medical College, AMU, Aligarh over a period of 15 months. A total of 240 children upto the age of 12 years suffering from acute, persistent and chronic diarrhoea were selected as cases. Fifty age and sex matched children attending the outpatient department of Paediatrics during the period of study with no symptoms of gastrointestinal disorder for a period of at least one month were selected as controls.

A detailed history was taken after taking informed consent from one of the parents or guardians for socioeconomic status, breast-feeding, locality, source of water supply and types of latrines used and physical examination was done before collecting the stool specimen. All the samples were subjected to various diagnostic procedures like wet mount examination[8], modified Z.N.[9] and safranine-methylene blue staining methods[10] and ELISA for the detection of Cryptosporidium.

For statistical analysis**,** Chi-square and z tests were used. A p value of ≤0.05 was considered significant.

RESULT: The wet mount examination and staining techniques were conducted on all the 240 stool samples. ELISA test was performed for detection of Cryptosporidium antigen in 177 stool specimens. Out of 240 stool specimens processed for detection of Cryptosporidium species, 16 were positive by the wet mount examination and 21 were positive by both the staining techniques. Out of 177 samples subjected to ELISA, 23 were positive. Since no 'gold standard' for the detection of Cryptosporidium oocysts in human stool specimens has yet been established [11], we considered cryptosporidiosis to be a definite diagnosis if the organisms were found in any two of the four techniques employed. There were 21 samples out of 177 for which confirmed identification was made by any two of the tests. The overall prevalence of Cryptosporidium was found to be 11.8% in this area.

The highest prevalence of Cryptosporidium was found in the age group 0 – 2 years (57.14%) and no significant difference was found in the detection rates between the two sexes

($p>0.05$) (Table I). The positivity of Cryptosporidium cases increased from 9.41% to 28.57% as the social class decreased from III to V. Thus the lower socioeconomic classes comprised of the maximum number of Cryptosporidium positive cases (Table II). Children who were bottle-fed showed high prevalence of infection (15.4%). Most of the patients who were positive were residing in rural areas and were using insanitary wells for purpose of drinking water. Majority of these patients were using insanitary latrines (16.7%) and 18.2% had animals at their residence (Table III).

DISCUSSION: Cryptosporidium should be regarded as a major public health problem as there are reports of major outbreaks of cryptosporidiosis in the United States, the United Kingdom, and Australia due to contamination of drinking water supplies[4,13].

In this study, Cryptosporidium oocysts were detected in 21 (11.86%) children out of 177 with diarrhoea and none of the controls. Cryptosporidium was found at a higher frequency in the lower socioeconomic classes. Saredi et al.[2] and Nagamani et al.[14] have also shown that majority of the children in their respective studies belonged to lower socioeconomic group. The prevalence of Cryptosporidium was higher in those children being or having been bottle-fed (15.4%) or receiving liquids or foods in addition to breast milk (12.3%) than those children on exclusive breast feeding (5.1%). This finding is in concurrence with the findings of other workers[15,16,17].

The rate of infection in children who lived in rural areas was 12.5% whereas in children from urban areas, it was slightly lower (11.2%). Similar rural to urban variations in infection rates is reported by Mahgoub et al.[18] and Urbina et al.[19] in their respective studies. This is attributed to the insanitary living conditions in slum areas – paucity of clean drinking water supplies, mixed dwelling habits (i.e. domestic/pet animals are kept near or inside the houses), improper sewage or waste disposal facilities, intake of contaminated food, etc.[20]. It was found that the rate of infection among those who drank from wells was 42.9%, compared to those who had access to hand pump (6.4%) and tap water (7.3%). No case was found in children who drank boiled or filtered water. Unsafe drinking water as a source of infection was reported by other workers also[2,21,22].

Close contact with animals has been found to be a predisposing factor for infection; the infection rate was 18.2% among children in close vicinity to animals in comparison with 9.8% among those who lived in compounds with no animals. Spread of Cryptosporidium from an animal source was documented in other studies also [18, 21]. Among the children who went to open fields for disposal of excreta, the infection rate was 16% while it was 16.7% for insanitary latrine users. However, only 7.1% children who used sanitary latrines were infected.

CONCLUSIONS: This study highlights the importance of sociodemographic factors affecting the rate of Cryptosporidium infection. The poor sanitary conditions prevailing in our country may contribute to the disease burden. Since there is no effective, specific therapy against infection with this parasite, preventive measures are of great importance. Such measures include extensive hand washing, avoiding direct contact with stool from animals or humans, avoiding the accidental ingestion of water used in recreational activities, and taking measures to ensure the safety of the drinking water.

REFERENCES:

1. Govt. of India (2006), Health Information of India 2005, Ministry of Health And Family Welfare, New Delhi.
2. Saredi N, Bava J. Cryptosporidiosis in pediatric patients. Rev Inst Med Trop S Paulo 1998; Vol. 40 No. 3.
3. Tzipori S. Cryptosporidiosis in animals and humans. Microbiol Rev 1983;47:84-96.
4. Fayer R, Morgan U, Upton SJ. Epidemiology of Cryptosporidium: transmission, detection and identification. Int J Parasitol 2000; 30 (12-13): 1305-22.
5. Guerrant RL. Cryptosporidiosis: an emerging, highly infectious threat. Emerg Infect Dis 1997;3:51-57.
6. Guardis M del V- Cryptosporidiosis. In: Basualdo JA; Coto CE & de Torres RA. Microbiologia biomedica. Buenos Aires, Editorial Atlante SRL, 1996; 943-947.
7. Hendrickson DA and Krenz HM – Reagents and stains. In: Ballows A; Husler WJ; Hermann KL; Isemberg HD & Shadowy HJ. Manual of clinical microbiology. 5. ed. Washington, American Society for Microbiology, 1991;1289-1314.
8. Basic Laboratory Methods in Medical Parasitology. WHO, Geneva 1991;9-33.
9. Henriksen SA, Pohlenz JFL. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet Scand 1981;22:594-596.
10. Baxby D, Blundell N. Sensitive, rapid, simple methods for detecting Cryptosporidium in faeces. Lancet 1983;ii:1149.
11. Weber R, Bryan RT, Bishop HS, Wahlquist SP, Sullivan JJ, Juranek DD. Threshold of detection of Cryptosporidium oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. J Clin Microbiol 1991;29:1323-1327.
12. Bhaskara Rao T. (2002). Sociology of Medicine. 1st Edition, pp.76.
13. Meinhardt PL, Casemore DP, Miller KB. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. Epidemiol Rev 1996;18:118-136.
14. Nagamani K, Rajkumari A, Gyaneshwari. Cryptosporidiosis in a tertiary care hospital in Andhra Pradesh. Indian J Med Microbiol 2001;19:215-216.
15. Sethi S, Sehgal R, Malla N, Mahajan RC. Cryptosporidiosis in a tertiary care hospital. Natl Med J India 1999;12:207-9.
16. Abdel-Messih IA, Wierzba TF, Abu-Elyazeed R, Ibrahim A, Ahmed SF, Kamal K, et al. Diarrhoea associated with Cryptosporidium parvum among young children of the Nile River Delta in Egypt. J Trop Pediatr 2005;51:154-159.
17. Adamu H, Endeshaw T, Teka T, Kifle A, Petros P. The prevalence of intestinal parasites in paediatric diarrhoeal and non-diarrhoeal patients in Addis Ababa hospitals, with special emphasis on opportunistic parasitic infections and with insight into the demographic and socio-economic factors. Ethiopian J Health Dev 2006;20:39-46.
18. Mahgoub ES, Almahbashi A, Abdulatif B. Cryptosporidiosis in children in a north Jordanian paediatric hospital. East Mediterr Health J 2004;10:494-501.
19. Urbina A, Mata L, Pizarro D. Cryptosporidium en ninos de Costa Rica: cuadro clinico, variacion estacional y tratamiento. Acta Med Costarric. 1984;27:191-198.
20. Palit A, Sur D, MitraDhar K, Saha MR. Asymptomatic Cryptosporidiosis in a Periurban Slum setting in Kolkata, India – a Pilot Study. Jpn J Infect Dis 2005;58:110-111.
21. Gambhir LS, Jaiswal JP, Nath G. Significance of Cryptosporidium as an aetiology of acute infectious diarrhoea in elderly Indians. Trop Med Int Health 2003;8:415-419.

22. Solorzano-Santos F, Penagos-Paniagua M, Meneses-Esquivel R, Miranda-Novales MG, Leanos-Miranda B, Angulo-Gonzalez D, et al. Cryptosporidium parvum infection in malnourished and non malnourished children without diarrhoea in a Mexican rural population. Rev Invest Clin 2000;52:625-31.

TABLE I AGE AND SEX DISTRIBUTION OF CHILDREN EXCRETING CRYPTOSPORIDIUM (n=177)

Age group (in yrs)	Specimens examined	Cryptosporidium detected		
		Male (%) (n=95)	Female (%) (n=82)	Total
0 – 2	96	7 (58.33)	5 (41.66)	12 (57.14)
2 – 4	37	3 (60)	2 (40)	5 (23.81)
4 – 6	17	1 (50)	1 (50)	2 (9.52)
6 – 8	10	1 (50)	1 (50)	2 (9.52)
8 – 10	8	0	0	0
10 – 12	9	0	0	0
Total	**177**	**12 (57.14)**	**9 (42.86)**	**21 (11.86)**

Figures in parentheses indicate percentage

TABLE II DISTRIBUTION OF CRYPTOSPORIDIUM POSITIVE CASES IN RELATION TO SOCIO-ECONOMIC STATUS* (n=177)

Socio-Economic Class	Total Cases	Cryptosporidium Positive Cases (%)
Class I (> Rs. 10,000)	13	2 (15.38)
Class II (Rs. 5000 – 9999)	38	3 (7.89)
Class III (Rs. 3000 – 4999)	85	8 (9.41)
Class IV (Rs. 1500 – 2999)	34	6 (17.65)
Class V (< Rs. 1500)	7	2 (28.57)
Total	177	21

Figures in parentheses indicate percentage

*Modified Prasad's Classification (Bhaskara Rao, 2002)[12]

TABLE III RATE OF INFECTION BY CRYPTOSPORIDIUM IN RELATION TO SOCIODEMOGRAPHIC CHARACTERISTICS

	Characteristics	Total Cases (%)
1.	Diet	
	i.Exclusively breastfed	2/39 (5.1)
	ii.Partially breastfed	9/73 (12.3)
	iii.Bottle fed	10/65 (15.4)
2.	Locality	
	i.Urban	10/89 (11.2)
	ii.Rural	11/88 (12.5)
3.	Source of Water Supply	
	i.Insanitary well	12/28 (42.9)
	ii.Handpump	3/47 (6.4)
	iii.Tap water	6/82 (7.3)
	iv.Boiled/Filtered water	0/20 (0)
4.	Animals at residence	
	i.With	8/44 (18.2)
	ii.Without	13/133 (9.8)
5.	Place of defaecation	
	i.Field defaecation	8/50 (16.0)
	ii.Insanitary latrines	7/42 (16.7)
	iii.Sanitary latrines	6/85 (7.1)

Figures in parentheses indicate percentage

CORRELATION OF INCREASED FIBRINOGEN & OXIDATIVE STRESS IN PROGNOSIS OF DIABETIC FOOT ULCER

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ABSTRACT:BACKGROUND: Diabetic foot ulcer has poor prognosis and is a leading cause of amputation. Oxidative stress is associated with the pathogenesis of chronic wounds. Fibrinogen is a prognostic marker of peripheral vascular disease**. AIMS:** This prospective case-control study was designed to (i) evaluate and compare oxidative stress, protein carbonyl and fibrinogen levels, (ii) assess the correlation of the above parameters with prognosis in diabetic foot ulcer patients and healthy controls and (iii) whether fibrinogen levels can be used as prognostic markers. **MATERIAL AND METHODS:** The study included 100 diabetic patients (40 diabetic without complication, 40 diabetic foot ulcer grade 1 and 20 grade 2 patients) and 60 volunteer healthy controls. Oxidative stress was evaluated by estimating the amount of oxidant load of lipid peroxides by ferrous oxidation products in xylenol orange assay in conjunction with triphenylphosphine version 2 (FOX2 assay) and protein carbonyl. The antioxidant status level was estimated by ferric reducing ability of serum (FRAP assay). Fibrinogen and glycated hemoglobin was measured using commercial kits. SPSS version 17 was used for statistical analysis. **RESULTS:** Oxidative stress was higher in diabetic foot ulcer patients compared to non ulcer ($p<0.05$) and controls ($p<0.01$). Increased oxidative stress and plasma fibrinogen correlated with poor prognosis. **CONCLUSION:** Increased oxidative stress and plasma fibrinogen are associated with poor prognosis irrespective of glycemic control.
KEY WORDS: diabetic foot ulcer, fibrinogen, fox2, frap.

INTRODUCTION: Diabetes mellitus type 2 (T2DM) and its vascular complications are a major worldwide health problem. Access to better health care, use of oral hypoglycemic drugs, insulin devices has resulted in increased longevity of diabetic patients and increased prevalence of vascular complications of T2DM (1). The vascular complications of T2DM are either due to microangiopathy or macroangioathy (2). The macroangiopathy in T2DM is a form of accelerated atherosclerosis affecting carotid, coronary and peripheral arteries. Thus, increasing the incidence and prevalence of diabetic foot ulcer (DFU) (2, 3).

DFU is one of the most common causes of nontraumatic lower extremity amputation and also the the most frequent reason for hospitalisation in T2DM patients (4, 5). In T2DM patients small injuries from shoes and trivial trauma are not perceived owing to neuropathy and these injuries subsequently result in nonhealing DFUs. The etiopathology of DFU includes various causes such as hyperglycemia, neuropathy, endothelial dysfunction and oxidative stress (6). The

deviant metabolic state in T2DM involves chronic hyperglycemia, dyslipedemia and insulin resistance. This aberrant metabolic condition affects the function of endothelial cell, smooth muscle cells and cells associated with inflammation. The endothelial cells of the vascular compartment secrete various bioactive substances, which regulate vascular function and inflammation (6). In T2DM, the secretion of vascular relaxing factor is impaired and the existing hyperglycemia induces the production of reactive oxygen species (ROS). In T2DM insulin resistance causes excessive release of fatty acids from the adipose tissue, which results in concomitant inhibition of phosphatidylinositol-3 kinase (IP-3) pathway and activation of protein kinase C, thus increasing the production of ROS. Besides an increased production of ROS, the diabetic state induces the generation of many vasoactive substances and vasoconstrictors which lead to vascular smooth muscle hypertrophy. The hyperglycemia, dyslipidemia and increased ROS levels in T2DM enhances atheromatous plaque formation (6,7). During normal health ROS and antioxidant levels remain in balance. In T2DM this balance is disrupted resulting in oxidative stress, as there is an increase in oxidant load and a decrease in the antioxidant level of the serum (8). In addition to oxidative stress, T2DM also increases the coagulability of blood (10). T2DM patients have impaired fibrinolysis and an enhanced production of procoagulants. Thus, an increased oxidative stress, enhanced levels of procoagulants and impaired fibrinolysis nepotises initiation and persistence of atherosclerosis and thromboembolic episodes in T2DM patients (10). Many epidemiological studies have correlated the role of oxidative stress and plasma fibrinogen levels in cardiovascular diseases (CVD) (11, 12). Hence this study was designed to (1) assess the correlation between oxidative stress, plasma fibrinogen levels in DFU patients and (2) to compare the above parameters in T2DM patients with and without foot ulcer with controls and (3) to evaluate the role of these parameters in the prognosis of DFU, using the rate of limb amputation as the basis for assessment.

METHODS: The study was approved by the Institute Ethical committee. The study included 100 diabetic patients (40 diabetic without complication, 40 diabetic foot ulcer grade 1 and 20 grade 2 patients) and 60 volunteer healthy controls. The DFU patients were classified as per the University of Texas Diabetic Wound classification for foot ulcer (13). T2DM patients included in the study were selected on the basis of the following criteria: no episodes of diabetic ketoacidosis, no associated CVD, retinopathy, nephropathy; age > 30 years when diagnosed with DM; not on insulin therapy; not undergone lower limb vascular surgery or amputation. In order to avoid confounding factors like preexisting atherosclerosis or nephropathy contributing to an raised plasma fibrinogen level T2DM patients with clinical evidence of CVD and microalbuminuria were excluded. Patients on hormone replacement therapy were also excluded. Group 1 included 40 DFU patients with superficial ulcers not involving tendon, capsule or bone and Group 2 included 20 DFU patients whose ulcer involved the tendon or joint capsule. The biochemical parameters of the DFU patients were compared with 40 T2DM patients without any complications and 60 healthy volunteers. Of the T2DM patients 76 were on oral hypoglycemic drugs (59% on sulphonylurea monotherapy, 12% on metformin and the rest on a combination of sulphonylurea and metformin).

Fasting venous blood sample was collected for estimating biochemical parameters. Plasma fibrinogen was estimated by immunoturbimetric method using kits from Tulip diagnostics adapted to EM360 Erba Transasia Autoanalyser. Glycated hemoglobin was estimated in whole blood by ion exchange chromatography method using kits from Teco

diagnostics, Germany. Lipid profile parameters such as total cholesterol, triglycerides, HDL-Cholesterol were measured using kits from Erba diagnostics, Germany. LDL-Cholesterol was calculated using Freidewalds equation.

The oxidative stress was evaluated by estimating the amount of oxidant load of lipid peroxides was determined by ferrous oxidation products in xylenol orange assay in conjunction with triphenylphosphine version 2 (FOX2 assay) (14). The inter assay and intra assay coefficient of variation for FOX2 were 4.9% and 2.7% respectively. Antioxidant power of serum was measured by ferric reducing ability of serum (FRAP assay) (15). The inter assay and intra assay coefficient of variation for FRAP were 3.0% and 1.0%, respectively. Plasma protein carbonylation (PC) was evaluated by Levine method (its millimolar extinction coefficient is 22.01$mmol^{-1}$ cm^{-1}) (16).

Data are represented as mean ± standard deviation (SD). The data was analyzed by one-way ANOVA followed by post Hoc Tukey's honestly significant differences test. Correlation coefficient was derived by Pearson's correlation analysis. A p value <0.05 was considered significant. Statistical analysis was done using SPSS version 17 software.

RESULTS: Table 1 depicts baseline clinical data of the diabetic patients. The mean age of the diabetic patients was 58.6 ±6.37 years and that of the controls59.8± 6.28 years. The mean duration of disease was 7.7 years. Table 2 shows the biochemical data of the study groups and controls. We observed a significant increase in plasma fibrinogen levels in DFU patients than diabetic patients without complications and controls. The DFU patients had increased levels of oxidative stress as observed by increased FOX2 levels, decreased total antioxidant levels, FRAP and increased protein carbonyl levels. DFU patients also exhibited a higher level of total cholesterol, triglycerides, LDL cholesterol and lower levels of HDL cholesterol. The plasma fibrinogen and levels of oxidative stress markers were significantly higher in diabetic patients without foot ulcer. The above parameters were observed to be increased in Grade 2 DFU patients as compared to Grade 1 DFU cases.

Table 3 shows the correlation between glycated hemoglobin levels, oxidative stress markers and plasma fibrinogen. All the diabetic patients plasma fibrinogen correlated significantly with glycated hemoglobin ($r=0.746$; $p<0.01$). A significant positive correlation was observed between oxidant load, protein carbonyl (PC) and plasma fibrinogen (plasma fibrinogen vs FOX2 $r=0.0778$; $p<0.01$, plasma fibrinogen vs PC $r= 0.792$; $p<0.01$). we observed a significant negative correlation between plasma fibrinogen and total antioxidant status ($r= -0.702$; $p<0.01$). a significant positive correlation existed between glycated hemoglobin, oxidant load and PC (FOX2 $r=0.812$; $p<0.01$, PC $r=0.0836$; $p<0.01$). A negative correlation was seen between glycated hemoglobin and total antioxidant status ($r=-0.860$; $p<0.01$).

The receiver operating curve (ROC) was calculated to assess whether plasma fibrinogen levels can predict for amputation of foot. The ROC curve (figure 1&2) showed an area under curve 0.976 and $p<0.001$. The optimum cutoff value for plasma fibrinogen to predict for foot amputation was 300.4mg%, with 100% sensitivity and 99.2% specificity.

DISCUSSION: This study was designed with the objective to estimate the association of oxidative stress, plasma fibrinogen and prognosis of DFU. Oxidative stress is considered to instigate the development of insulin resistance, β cell dysfunction and impaired tolerance to glucose in T2DM patients (16). Oxidative stress is also associated with the long term complications of DM such as microvascular and macrovascular complications (17). Various in

vitro studies have correlated an increase in oxidative stress in cells when exposed to a hyperglycemic environment (18, 19). Oxidative stress occurs as a consequence to an increased oxidant load and decreased antioxidant status (18. 19). Oxidative stress causes cell damage by forming lipid peroxides and protein carbonyl (19). We observed significantly high levels of oxidant load, FOX2 levels, and protein carbonyl in DFU patients than DM. The above parameters were increased in DM without complications as compared to controls. These findings are in concordance with previous studies (16-19). These findings imply a positive correlation of DFU with oxidative stress.

An increased plasma fibrinogen levels was observed in diabetic patients in our study, which is similar to the findings of previous studies (22-24). The higher level of plasma fibrinogen is attributed to increased synthesis and impaired clearance (23-25). We observed higher levels of plasma fibrinogen in DFU grade 2 case as compared to grade 1; higher levels of fibrinogen in DFU cases as compared to diabetics without complication and controls. The level of fibrinogen was increased in diabetics as compared to controls. This implies a positive association of increased plasma fibrinogen with DFU.

An association between oxidative stress and plasma fibrinogen has been observed in diabetics (26, 27). Fibrinogen synthesis is regulated by a feedback mechanism by thrombin activation (28-30). In diabetics thrombin formation is induced by free radicals (31, 32). Thus, oxidative stress is a link between increased fibrinogen levels in diabetics. We observed a positive correlation between glycated hemoglobin, oxidative stress and plasma fibrinogen levels. This is in agreement with previous studies (27-32).

Subsequent to follow up period of 10 months, we observed 18 grade 2 DFU patients underwent lower limb amputation. In these patients the plasma fibrinogen level was ≥ 300.4 mg%. This indicates that increased oxidative stress and high plasma fibrinogen levels are markers of poor prognosis in DFU patients.

The limitations of this research work are it is a prospective non randomized study and the data regarding the vascular status of the patients were not recorded. Nevertheless, the results are interesting and indicate further research in a controlled study to illustrate whether plasma fibrinogen levels and oxidative stress markers can be used as prognostic markers to prevent limb amputation in DFU. The associations with vascular parameters such as ankle brachial pressure indices, toe pressure and tissue oxygen tension should be assessed to provide a better prospect to both the patients and the clinical fraternity.

REFERENCES:

1. 1.Wild S, Sicree R, Roglic G, King H, Green A. Global prevalence of diabetes. Diabetes Care. 2004;27;1047-1053.
2. Diabetes Drafting Group, WHO. Prevalence of small vessel and large vessel disease in diabetic patients from 14 centers. The World Health Organization multinational study of vascular diseases in diabetics. Diabetologia.1985;28:615-640.
3. Peripheral arterial disease in people with diabetes: American Diabetes Association Consensus Statement. J Vasc Surg. 2004;39:1356-1357.
4. Lavery LA, Ashry HR, van Houtum W, Pugh JA, Harkless LB, Basu S. Variations in the incidence and proportion of diabetes related amputations in minorities. Diabetes Care. 1996;19:48-52.

5. Armstrong DG, Lavery LA, Ouebedeaux TL, Walker SC. Surgical morbidity and the risk of amputation due to infected puncture wounds in diabetics versus nondiabetic adults. South Med J. 1997;90:384-389.
6. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcer in an Indian tertiary care hospital. Diabetes Care. 2006;29:1727-1732.
7. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis epidemiology, pathophysiology and management. JAMA. 2002;287: 2570-2581.
8. Rosen P, Nawroth PP, King G, Möller W, Tritschler HJ,Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications. Diabetes Metab Re Rev. 2001;17:189-212.
9. Inoguchi T, Li P, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes.2000;49:1939-1945.
10. Carr ME. Diabetes mellitus: a hypercoagulable state. J Diabetes Complications. 2001;15:44-54.
11. Zuanetti G, Latini R, Maggioni AP, Santoro L, Franzosi MG. Influence of diabetes on mortality in acute myocardial infarction: data from GISSI-2 study. J Am Coll Cardiol. 1993;22:1788-1794.
12. Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. JAMA. 1999;281:1291-1297.
13. Lavery LA, Armstrong DG, Harkless LB. Classification of diabetic foot wounds. J Foot Ankle Surg. 1996;35: 528-531.
14. Halliwell B, Gutteridge JMC, eds. Free radicals in biology and medicine. 3rd ed. New York: Oxford University Press1999:pp298–299.
15. Benzie FFI, Strain JJ. The ferric reducing ability of plasma as a measure of antioxidant power: the FRAP assay. Analytical biochemistry 1996; 239: pp70-76.
16. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 1994;233:346-357.
17. Wright E, Jr, Seism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycemia. Int J Clin Pract. 2006;60:308-314.
18. Guigliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996; 19:257-267.
19. Ouagliaro L, Piconi L, Assalone R, et al. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes. 2003;52:2795-2804.
20. Schiekofer S, Andrassy M, Chen J, Rudofsky G, Schnieder J, Wendt T. Acute hyperglycemia causes intracellular formation of CML and activation of ras, p42/4 MAPK and nuclear factor kappaB in PBMCs. Diabetes. 2003;52:621-633.
21. Berlett BS, Stadman ER. Protein oxidation in ageing, disease and oxidative stress. J Biol Chem. 1997;272: 20313-20316.
22. Dubry SW, Reaveley DR, Seed M, et al. Risk factors for cardiovascular disease in IDDM. A study of identical twins. Diabetes. 1994;43:831-835.
23. Vague P, Juhan-Vague I. Fibrinogen, fibrinolysis and diabetes mellitus: a comment. Diabetologia. 1997;40:738-740.

24. Jones RL, Jovanovic L, Forman S, Peterson CM. Time course of reversibility of accelerated fibrinogen disappearance in diabetes mellitus: associations with intravascular volume shifts. Blood. 1984;63:22-30.
25. Jones RL, Peterson CM. Reduced fibrinogen survival in diabetes mellitus: a reversible phenomenon. J Clin Invest. 1979;63:485-493.
26. Stucker M, Moll C, Rudolph T, et al. Fibrinogen adsorption a new treatment option for venous ulcers? Vasa. 2003;32:173-177.
27. Collier A, Rumbley AG, Paterson JR, Leach JP, Lowe GDO, Small M. Free radical activity and haemostatic factor in NIDDM patients with and without microalbuminuria. Diabetes. 1992;41:909-913.
28. Knobl P, Schernthaner G, Schack C, et al. Thrombogenic factors are related to urinary albumin excretion in type 1 and type 2 diabetic patients. Diabetologia. 1993;36: 1045 - 1050
29. Ceriello A, Taboga C, Giacomello R, et al. Fibrinogen plasma levels as marker of thrombin activation in diabetes. Diabetes. 1994;43:430-432.
30. Ceriello A, Pirisi M, Giacomello R, et al. Fibrinogen plasma levels as marker of thrombin activation: new insights in the role of fibrinogen as a cardiovascular risk factor. Thromb Haemost. 1994;71:593-595.
31. Giansante C, Fiotti N, Caattin L, Da Col PG, Calabresse S. Fibrinogen, D-dimer and thrombin-antithrombin complexes in a random population sample: a relationship with other cardiovascular risk factors. Thromb Haemost. 1994;71:581-586.
32. Ceriello A, Giacomello R, Stel G, et al. Hyper-glycemia induced thrombin formation in diabetes: a possible role of oxidative stress. Diabetes. 1995;44:924-928.

Table 1 baseline clinical data of diabetic patients

Number	100
Age (years)	58.6 ±6.37
Sex (M/F)	93/7
Duration of Diabetes (years)	7.7 years
Body Mass Index (BMI) (Kg/m^2)	27.4 ±4.3
Systolic blood pressure (mm Hg)	124±14
Diastolic blood pressure (mm Hg)	76±10
Smoking (n)	12
Site of ulcer	
Forefoot	26
Mid-foot	4
Hind foot	30
Grade of ulcer	
Grade 1	40
Grade 2	20

Table 2 comparision of biochemical parameters in controls, diabetic foot ulcer patients without foot ulcer and diabetic foot ulcer patients (DFU)

Biochemical parameters	Controls	Diabetic patients without foot ulcer	DFU Grade 1	DFU Grade 2
Glycated hemoglobin (%)	6.53±0.22	7.46±0.54 b	8.22±0.56 [b]	8.76±0.48 b,d
Total cholesterol (mg/dl)	141.26±5.51	189.36±5.92[c]	190.78±4.98[b]	244.42±21.6[b,d]
Triglycerides (mg/dl)	61.22±5.55	100.40±6.08 [c]	106.4±8.0 [b]	116.92±8.92 [b,d]
HDL cholesterol (mg/dl)	56.76±7.77	48.12±6.22 [c]	50.02±6.66 [b]	43.44±4.54 [b,d]
LDL cholesterol (mg/dl)	100.4±6.06	122.36±5.12[b]	116.58±5.56 [b]	131.68±4.4 [b,d]
Fibrinogen (mg/dl)	230.72±5.14	250.48±6.48 [b]	274.88±14.2[b,e]	313.2±5.68[b,d,e]
FOX2 (µmol/L)	4.33±1.7	8.44±2.2b [b]	10.96±4.4 [b,e]	18.0±4.9 [b,d,e]
FRAP (µmol/L)	423±15.23	388.2±10.22 [b]	112.66±8.8 [b,e]	99.87±7.48 [b,d,e]
Protein carbonylation (nmol/mg of protein)	0.74±0.008	1.86±0.62 [b]	2.36±0.14 [b,e]	2.81±0.20 [b,d,e]

HDL=high density lipoprotein; LDL= low density lipoprotein
All data is represented as mean ± Standard deviation.

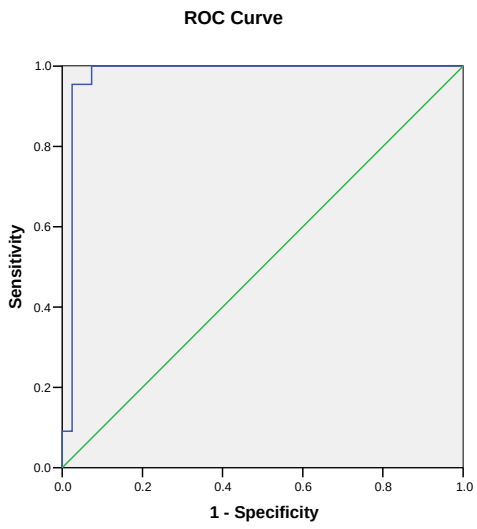
b $p<0.001$ compared to controls
c $p<0.05$ compared to controls
d $p<0.01$ compared to controls
e $p<0.005$ when mean of DFU Grade 1 and DFU Grade 2 comared with diabetics without complication.

Table 3 Correlation of Plasma Fibrinogen with Glycated Hemoglobin and Oxidative Stress Markers in Diabetic foot Ulcer patients

	FOX2 r value	FRAP r value	PC r value	fibrinogen r value
Glycated hemoglobin	0.812*	-0.860*	0.836*	0.746*
Plasma fibrinogen	0.778*	-0.702*	0.792*	

r = pearson's correlation coefficient; * $p<0.01$

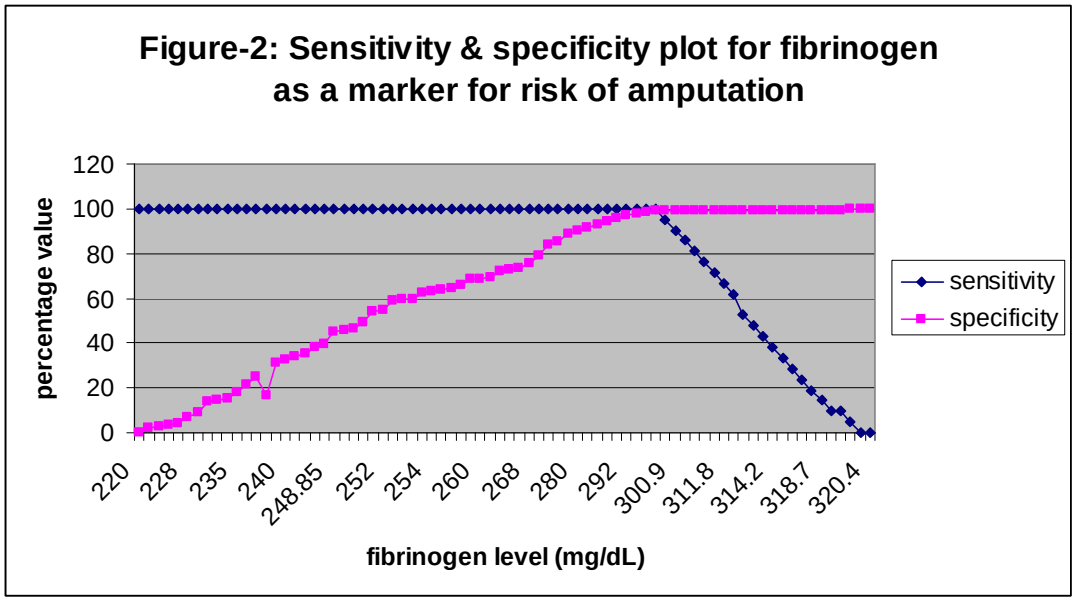
Figure-1: Receiver Operating Curve of plasma fibrinogen



[1] Area under the curve
Test Result Variable(s): Fibrinogen

Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.976	.022	.000	.932	1.019

a: Under the nonparametric assumption
b: Null hypothesis: true area = 0.5



STUDY OF OXIDATIVE STRESS & ROLE OF ANTIOXIDANTS IN SENILE CATARACT

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ABSTRACT: **OBJECTIVE:** Senile cataract is by far commonest cause of visual impairment and blindness globally and also in developing countries like India .It has been hypothesized that oxidative damage may be involved in the pathogenesis of cataract . The aim of our present study is to evaluate the role of oxidative stress in cataract. **METHODS:** We tried to assess the role of oxidative stress in patients by estimating the levels of lipid peroxidation assessing plasma Malondialdehyde(MDA) levels and antioxidant status by reduced glutathione(GSH), Serum Glutathione-S-Transferase(GST) and Vitamin-C in blood. For this, we have taken 80 cases of senile cataract patients compared with 100 age matched controls. **RESULTS:** A significant increase in the levels of Serum MDA, whereas significant decrease in the levels of reduced glutathione, Serum GST and Vit-C were observed. **CONCLUSION:** This suggests that oxidative stress and reduced anti oxidant defense mechanism play an important role in the pathogenesis of Senile cataract which needs further studies.
KEYWORDS: Senile cataract, oxidative stress, antioxidants.

INTRODUCTION: Anatomically, an opacity in the lens or its capsule, whether developmental or acquired is called a cataract. Clinically, it is opacification of the lens which interferes with vision and which obstructs the normal red glow on direct or indirect ophthalmoscope. Cataract can be classified etiologically into congenital and acquired types whereas Senile or age related cataract is the most common type. According to WHO, there are about 42-45 million blind suffering from cataract and about 15 million of these are in developing countries like India and china. Over 50% of all cases of blindness can be attributed to cataract and more than 20 million people worldwide are affected(1,2).

Oxidative stress is implicated in the pathogenesis of a variety of human diseases(3). Oxidative damage occurs to biomolecules like lipids, proteins, carbohydrates and nucleic acids and other extracellular components like collagen and hyaluronic acid which are very deleterious(4)resulting in lipid peroxidation, mutagenesis and carcinogenesis. Free radicals are easily generated in the course of normal metabolic activities and may also be produced by external agents such as electromagnetic and

particulate radiation, air pollutants, tobacco smoke or through the metabolism of drugs(doxorubicin)(5).They can be produced and act inside the cell or they can be generated within the and released to extracellular space. However, the body's defense mechanisms play an important role in the form of antioxidants that help to minimize the damages which are caused by oxidative stress. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. Oxidative stress occurs when there is an imbalance between reactive oxygen species(ROS) and antioxidants reaction capacity which stimulate the development of a disease such as cataract. Oxidative damage can result in a number of molecular changes that contribute to the development of glaucoma, cataract, and other eye diseases(6). If the free radical theory of aging is applied to the eye, an altered oxidant/antioxidant balance should be evident for age related ocular diseases, such as age-related macular degeneration, cataract, and glaucoma.(7).

The present study was planned to evaluate the possible role of oxidative stess and antioxidant status in the pathobiology of senile cataract patients.

MATERIALS AND METHODS: The present study was conducted in the Departments of Ophthalmology and Biochemistry , S. V. Medical college, Tirupati, AP. 80 diagnosed senile cataract cases in the age group of 45-70 years were chosen for study. Out of 80, 60 were senile cortical cataract cases(30 cases of immature & 30 cases of mature) &20 patients are of senile nuclear(10 cases of immature nuclear&10 of mature nuclear) cases. 80% of the cases both female and male included population who are mainly manual labourers working under the sun.100 age matched controls having visual activity of 6\6 without any lens opacities in either eyes are taken . The controls included mainly population with life style where there is less exposure to sunlight. We have excluded the patients having diabetes mellitus, hypertension and patients having any other systemic illness like thyroid disorders. Informed consent was obtained from all individuals included in the study.

10ml of fasting blood samples were collected by venipuncture and for the separation of sera, 5ml of blood was centrifuged at 3000rpm for 5min and the remaining 5ml of blood was taken into a plain vial containing EDTA and was centrifuged at 3000rpm for 10min for the separation of plasma. Aqueous humor is also collected from each cataract case preoperatively with BCG syringe on same day of blood sample collection. The oxidation is mainly brought about by free radicals .Direct estimation of blood oxidant levels is difficult because of the very short half life of free radicals. So, the plasma MDA levels were estimated by using thiobarbituric acid reacting substances(TBARS) by the method of Yagi(8) and Sinnhuber et al(9).Reduced glutathione was determined by the method of Beutler et al(10). Serum GST is measured by following the increase in absorbance at 340nm using 1,chloro2,4 dinitrobenzene as substrate(11).Plasma ascorbic acid level is estimated by using 2,4 dinitrophenylhydrazine & reading the absorbance at 520nm(12). The findings were expressed as mean $\pm$ standard deviation & evaluated for statistical significance.

RESULTS: In this study significant increase in the levels of serum MDA is observed in senile cataract when compared with controls.. There is significant decrease in levels of GSH and serum GST levels in senile cataract and also in its cortical and nuclear types when compared with controls. There is significant decrease in plasma Vit-C levels in

senile cataract and also in its cortical and nuclear types when compared with control groups.

DISCUSSION: The current interest is in oxidative stress, nutrient & enzymatic antioxidant status in pathophysiology of senile cataract which is responsible for the largest number of blindness cases in the country. Oxidative stress has been implicated in cataractogenesis (13). In our study, a statistically significant relationship was found between the presence of cataract and plasma MDA levels. The plasma MDA level, a byproduct of lipid peroxidation, is a reliable and commonly used biomarker of overall lipid peroxidation. ROS causes damage to biopolymers including nucleic acids, proteins, PUFA & carbohydrates which are the basic mechanisms underlying diseases including cataract. The solid mass of the lens is 98% protein. Because these proteins undergo minimal turnover as the lens ages, they are subjected to chronic stresses of exposure to light and oxygen. So, these proteins are extensively damaged in aged lenses. Lens opacities develop as the damaged proteins aggregate and precipitate(14).Lipid damage to the fiber cell membrane is also associated with lens opacities(15). Smoking and ultraviolet light, which appear to induce oxidative stress(16), are also associated with elevated cataract risk(17). Photo oxidative stress has important consequences in the lens because the lens never sheds its cells & there is no turnover of lens proteins throughout the life. The constituents of the young lens differ chemically from the older lens. These differences between young and old lenses are a result of three major processes. First, there are post translational changes in protein in the inner region of the lens where protein synthesis is insignificant and particular protein macromolecules have been present for many years. There is posttranslational modification of proteins like racemization, glycation (18), COOH terminal degradation, deamidation and non-covalent aggregation. Second, the crystallins the major structural proteins are produced by many genes and any gene is not necessarily active all throughout life. Finally, with aging certain key metabolically active components involved in protecting the lens from stress appear to decrease in activity In the normal, young human lens, there is no oxidation of the cytosolic protein and no oxidation in the membrane fraction. All thiols are buried in the interior of the macromolecular structure. In normal lenses, some membrane protein oxidation is apparent by the age of 60-65, but there is still no oxidation of cytosolic protein. At this stage, only about 50% of the protein thiols remain buried. In cataract, the picture is dramatically different. All thiols are exposed, and massive oxidation of thiol to both protein and mixed disulfides (probably with GSH) as well as cysteic acid is observed. . Oxidation of membrane lipids precedes high molecular weight protein aggregation. This often exposes buried functional groups & lead to conformational change. Oxidation of membrane lipids also causes polymerization & cross-links between lens proteins & membranes. If these damaged proteins accumulate, eventual opacification occurs. Cataracts are thought to result from photo oxidation of lens proteins that results in protein damage, accumulation, aggregation and precipitation in the lens(19).

Photo oxidative stress results essentially from light absorption by the constituents of the lens like structural proteins, enzymes, DNA & membranes. Direct protection is offered mainly by dietary antioxidants like Ascorbic acid, Tocopherols & carotenoids and antioxidant enzymes. So, the rise in plasma MDA levels indicate that oxidative stress may be responsible in the pathogenesis of cataract.

We also observed a significant decrease in the levels of reduced glutathione in the cases as compared to the controls. In young lenses, damaged proteins are usually

maintained at harmless amounts by defense systems. Primary defenses that directly protect the lens against the initial oxidative insult include small molecule antioxidants (eg, vitamins C and E and carotenoids) and antioxidant enzyme systems (eg, superoxide dismutase, catalase, and the glutathione redox cycle).The lens also has secondary defense systems, which include proteolytic enzymes that selectively identify and remove damaged or obsolete proteins. Accumulation of photo oxidized (and/or otherwise modified) proteins in older lenses indicates that protective systems are not keeping pace with the insults that damage lens proteins. This occurs in part because similar to bulk proteins, enzymes that compose some of the protective systems are damaged by photooxidation.The intracellular depletion of reduced glutathione can be either due to the formation of a direct complex with a electrophilic agent or due to the inhibition of synthesis or due to the subjection of the cell to oxidative stress(20). When a cell is subjected to oxidative stress, there is increased utilization of glutathione, thus leading to its depletion. Many enzymes are GSH dependent and their activity may be regulated by the thiol disulphide exchange. They are thus dependent on the GSH status. Another possible explanation for reduced GSH levels may be due to defective intracellular synthesis. The liver is the major site for GSH synthesis. The precursors necessary for this synthesis are L-glutamate, L-cysteine and L-glycine. Although both glutamate and glycine are important, it seems that the major determinant of the rate of GSH synthesis is the availability of the amino acid cysteine.(21).Cysteine results from the metabolism of homocysteine and any interruption in the homocysteine-cysteine pathway would result not only in the accumulation of homocysteine but also in less available quantities from the second amino acid which may affect GSH synthesis. So, reduced glutathione levels here indicate there is depletion which is because of increased defense mechanisms.

We also observed a significant decrease in the levels of Serum GST in the cases as compared to the controls. Glutathione –S- transferase (GST) is reduced which are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification(22,23). This reduction is due to the reduced levels of GSH.

There is also a decrease in the levels of non-enzymatic anti-oxidants such as Vit-C, which states that there is an increased defense mechanism against oxidative damage in cataract. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to an increased turnover for preventing oxidative damage in these patients, thus suggesting an increased defense against oxidative damage. Our results support the researchers who reported decreases in the antioxidant level and increases in lipid peroxidation level(24,25).Several other researchers showed over expression of antioxidants to be associated with cataract.(26,27).

In conclusion, the present study revealed an increase in the levels of MDA and decrease in the levels of reduced glutathione ,serum GST and Vit-C in cases compared to controls suggesting the role of oxidative stress as a pathogenic mechanism in the development of cataract and further extensive studies are required in future to establish oxidative stress as a biomarker in the development of cataract.

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REFERENCES:

1. Bunce GE, Kinoshita J, Horwitz J. Nutritional factors in cataract. Annu Rev Nutr 1990;10:233-254.
2. Taylor A, Nowell T.Oxidative stress and antioxidant function in relation to risk for cataract. Adv Pharmacol 1997;35:515-556.
3. Beck MM, Levander OA. Dietary oxidative stress and potentiation of viral infection. Annu Rev Nutr,1998; 18: 93-116.
4. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. Am J Med,1994; 97: S5-S13.
5. Halliwell B, Gutteridge JMC. Free radicals in biology & medicine.3rd edition. Oxford university press,1999.
6. Berman ER.Biochemistry of the eye.Plenum Press:New York,1991.
7. Webb M. Toxicological significance of metallothionein. Exp Suppl 1987;52:109-134.
8. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids, 1978; 45: 337-351
9. Sinnhuber RO, Yu TC. Characterization of red pigment formed in thiobarbituric acid determination of oxidative rancidity. Food Res,1958; 23: 626-630.
10. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med, 1963;61:882-888.
11. Habig WH, Pabst MJ, Jacoby WB. Glutathione-s-transferase, the first enzymatic step in mercapturic acid formation. J Biochem 1974;249:7130-7139.
12. Kuether CA, Roe HJ. Detection of ascorbic acid in whole blood & urine through the 2,4 dinitrophenylhydrazine derivative of dehydroascorbic acid. Journal of Biological chemistry,1943;147:399-407.
13. 13.Halliwell B. Antioxidants: the basics-what they are and how to evaluate them. Adv Pharmacol 1997;38:3-19.
14. Taylor A, Jacques PF, Dorey CK. Oxidation and aging: impact on vision. J Toxicol Ind Health 1993;9:349-71.
15. Jacques PF, Chylack LT Jr. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr 1991;53(suppl):352S-5S.
16. Schectman G, Byrd JC, Gruchow HW. The influence of smoking on vitamin C status in adults. Am J Health 1989;79:158-62
17. Christen WG, Manson JE, Seddon JM, et al. A prospective study of cigarette smoking and risk of cataract in men. JAMA 1992;268:989-93.
18. Patrick J.S, Thorpe S.R, Baynes J.W. Non enzymatic glycosylation of proteins does not increase with age in normal human lenses. J.Gerontol.1990;45:B18-B23.
19. Taylor, A. Cataract: relationship between nutrition and oxidation. J. Am. Coll. Nutr.1993;12:138-146.
20. 20.Deneke SM, Farberg TZ. Regulation of cellular glutathione lung cells. Mol.Physiol,1994 ;1163-1173.

21. Lu SC.Regulation of hepatic glutathione synthesis:current concepts and controversies.Faseb J.1999;13:1169-1183.
22. Listowsky I,Abramovitz M, Homma H et al. Intracellular binding and transport of hormones and Xenobiotics by glutathione-S-transferase. Drug Metab Res,1988;19:305-318.
23. Ketley JN, Habig WH, Jacoby WB. Binding of non substrate ligands to glutathione-s-transferases. J Biol Chem,1976;250:8670-8673.
24. Gonenc A, Erten D, Aslan S, et al. Lipid peroxidation andantioxidant status in blood and tissue of malignant breast tumor and benign breast disease. Cell Biol Int 2006;30:376-80.
25. Yeh CC, Hou MF, Tsai SM, et al. Superoxide anion radical, lipid peroxides and antioxidant status in the blood of patients with breast cancer. Clin Chim Acta 2005;361:104-11.
26. Nourmohammadi I, Ladan G, Mehdi M, Abbas G-J. Evaluation of erythrocyte glutathione peroxidase, superoxide dismutase and total antioxidants in cataract patients. Arch Iranian Med 2001;4:123-126.
27. Delcourt C, Carriere I, Delage M, Descomps B, Cristol JP, Papoz L. Associations of cataract with antioxidant enzymes and other risk factors: the French age related eye diseases(POLA) prospective study. Ophthalmology 2003;110:2318-2326.

Table 1

Comparison of levels of MDA, enzymatic and non-enzymatic antioxidants in cases and controls

Parameters	Senile cortical cataract(n=60)	Senile nuclear cataract(n=20)	Controls(n=100)	P value
MDA (nmol /ml)	8.99±0.13	7.93±0.13	2.18±0.12	<0.001(highly significant)
GSH (mg/g Hb)	5.87±0.11	6.43±0.97	14.66±0.13	<0.001 (highly significant)
Serum GST (IU/L)	39.01±6.92	40.05±5.67	72.73±9.33	<0.001 (highly significant)
Vitamin-C (mg/dl)	2.14±0.19	2.32±0.84	7.58±0.16	<0.05 (significant)

A STUDY OF ARTERIAL SUPPLY OF VERMIFORM APPENDIX IN HUMANS

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ABSTRACT: The surgical procedures like appendicectomy, demands a precise knowledge of vascular anatomy of ileocolic region. The aim of this study is to study the arterial supply of the appendix, findings of which may reveal more anatomical facts about the arteries of appendix and their variations. Total 52 specimens of caecum and appendix with their arteries intact were collected, cleaned and dissected. The ileocolic artery and its branches to the appendix were traced carefully and observations were recorded. The ileocolic artery arises independently from superior mesenteric artery in 96.88% of cases and ends by dividing into superior and inferior division in 93.76% of cases. The appendicular artery arises from inferior division in 46.88%, ileal branch 28.13%, ileocolic artery 18.75% and from arterial arcade in 6.25% of cases. 21.87% of cases showed additional appendicular artery.
KEYWORDS: Caecum, appendix, ileocolic artery, appendicular artery.

INTRODUCTION: Vascular anomalies always pose a great challenge to the anatomists and surgeons. The surgical trauma to the sustaining blood vessels is irreparable and lead to fatal necrosis of the part involved.
Surgical procedures like appendicectomy, which is one of the common surgical procedures in case of appendicitis, appendicular carcinoid tumors etc. require good knowledge of arteries supplying it and the possible variations to avoid intra and post-operative complications like hemorrhage.

MATERIALS AND METHODS: The arterial supply of the appendix was studied in 52 human specimens. The specimens (caecum with appendix and part of ascending colon and ilem) were collected with their arteries intact from the postmortem centre and dissection hall (Department of anatomy) of J.J.M medical college. Davangere and S.I.M.S & R.C .Mangalore .
Thus collected specimens were preserved in 5% formalin. After the preservation the specimens were dissected cleaned and numbered. The ileocolic artery and its branches to the appendix were traced carefully and observations were recorded.

RESULTS: The arterial supply to the vermiform appendix was studied by dissection method in 52 specimens. The arteries to the appendix were carefully traced from their origin to termination. The findings are noted down.

In the present study of 52 specimens the appendicular had variable origin as follows,

In the present study of 52 specimens, 12 specimens (23.07%) showed an additional appendicular artery.

Out of these 12 specimens, the additional appendicular artery was originating from posterior caecal artery in 11 specimens (21.15%) and in one specimen (1.92%) it was originating from common caecal artery.

The specimen no.50 showed an anastomosis between appendicular and posterior caecal arteries. The specimen no.40 showed anastomosis between appendicular and ileal branches. Specimen no 2 showed anastomosis of appendicular artery with the common caecal artery.

DISCUSSION: 52 specimens were studied for the arteries supplying the vermiform appendix. The findings of the study have been compared with those of previous workers on the subject.

24 (46.15%) out of 52 specimens studied, showed the origin of appendicular artery from the inferior division of ileocolic artery.Cunningham[1]and Michel R B [2] illustrate the origin of appendicular artery from the descending branch of the ileocolic artery. Susan Standring[3] in Gray's Anatomy mentions the origin of appendicular artery from the inferior division.

In 16 (30.76%) specimens of the present study, the appendicular artery originated from the ileal branch. Barry J Anson[4] and others[5,6] have mentioned the origin of appendicular artery from ilealbranch.Bergmann[7] mentioned the origin of appendicular artery from the ileal branch in 35% of cases. (Un-published report of Beaton, Anson, Swigart and Jamieson).Schumpelick Volker et al[8] mentioned the origin of appendicular artery from the ileac ramus of the ileocolic artery in 35% of cases.

In 10 (19.23%) specimens the appendicular artery originated directly from the ileocolic artery. This type of origin has also been mentioned by Haller[9]and others(4,6, 10, 11, 12, 13).Luzsa[14] state that the appendicular artery arises from the ileocolic artery in 1/3 of cases.Bergmann[7] mentions the origin of appendicular artery from the ileocolic artery in 48.5% of cases. Schumpelick Volker et al[8] state that the appendix gets its blood supply from the appendicular artery, which originate from the ileocolic artery in 28% of cases.

In the present study one specimen (1.92%) showed the origin of appendicular artery from the arterial arcade between posterior caecal and ascending colic branch. One more specimen (1.92%) from the arcade between ileal and common caecal branch. Anson and Mcvey[6] have mentioned the origin of appendicular artery from the arcade between colic and ileal branches. Kozmith et al [15] mentioned the origin from the ileal side of the ileocolic loop. Michel Simon et al[13] mentioned the origin of appendicular artery from the ileocolic arcade.

In the present study of 52 specimens, 12 specimens (23.07%) showed an additional appendicular artery. The variation is even mentioned by Barry J Anson [4], and others (6, 14, 16). Katzarski M et al [17],have demonstrated more than one appendicular artery in 39.8% of cases. Ajmani M L Ajmani[18] demonstrated more than one appendicular artery in 39% of cadavers.

In the present study, out of 12 specimens the additional appendicular artery originated from the posterior caecal artery in 11 specimens(21.15%) and in one specimen(1.92%) from

the common caecal artery.Piersol[19],Shah and Shah [20], and other workers (4, 6, 13, 14, 16)have mentioned the origin of appendicular artery from the posterior caecal artery. Bergmann [7] mentioned the origin of appendicular artery from posterior caecal artery in 5% of cases. Schumpelick Volker[8] states the origin of appendicular artery from posterior caecal artery in 12% of cases.

Specimen no 50 showed an anastomosis between appendicular and posterior caecal arteries. This observation is even mentioned by Susan Standring[3] in Gray's anatomy, Mc Minn R M H [16] in Last's anatomy.

Specimen no 40 showed anastomosis between appendicular and ileal branches. Specimen no 2 showed anastomosis of appendicular artery with the common caecal artery. Michel Simon[13] mentioned the anastomosis between the appendicular artery and the ileal branch of the superior mesenteric artery.

CONCLUSION: The appendicular artery originated from inferior division in 46.15%. Other sites of origin are ileal branch of inferior division (30.76%) and directly from the ileocolic artery in 19.23% and an arterial arcade in 3.84% of cases.23.07% of specimens showed additional appendicular artery, which originated from posterior caecal artery (21.15%) or common caecal artery (1.92%)5.76% of specimens showed anastomosis of the appendicular artery with the posterior caecal, ileal and common caecal branches.

From the inferior division of ileocolic artery	24 specimens	46.15%
From ileal branch	16 specimens	30.76%
Directly from ileocolic artery	10 specimens	19.23%
From an arterial arcade between posterior caecal and ascending colic branch	1 specimen	1.92%
From an arcade between ileal and common caecal branch	1 specimen	1.92%

REFERENCES:

1. Romanes GJ. Cunninghams's Text book of Anatomy 12th ed. Wallan street Oxford. Oxford University press. 1981; p.926-27.
2. Michael RBK, Norman SW. Surgery of Anus, Rectum and Colon. Vol. 1. Philadelphia. W.B. Saunders Company. 1993; p.11.
3. Jeremiah CH, Neil RB. Abdomen and Pelvis. Section 7 in: Susan S, Harold E, Jeremiah CH, David J, Andrew W, Patricia C, et al. Gray's Anatomy. 39th ed. Edinburgh. Elsevier Livingstone. 2005; p. 1187-90.
4. Barry JA. Morri's Anatomy 12th ed. Newyork. McGraw-Hill Book Company. 1966;p.744.
5. Patrick WM, David HL, Martin AL, Anthony JS, Surgery of the colon, rectum and anus. 1st ed. Philadelphia. W. B. Saunders Company. 199; p.18.
6. Anson BJ, McVey CB. Surgical Anatomy. 5th ed. Philadelphia. W.B. Saunders Company 1971.

7. Bergmann RA, Thompson SA, Afifi AK, Saadeh FA. Compendium of Human Anatomic Variation. 1st ed. Munich. Urban Schwarzenberg. 1988; p.406.
8. Volker S, Bernhard D, Kerstin O, Andreas P. Appendix and Cecum Embryology, Anatomy and surgical applications. The surgical clinics of North America. Feb 2000; 80(1): p.301-302.
9. Haller V. Icones of Haller 1756.
10. Hollinshed WH. Anatomy for Surgeons. Vol.2, 1956; p.492-95.
11. Solanke TF. The blood supply of Vermiform Appendix in Nigerians. J Ant 1968;102: p.353-362.
12. Pitynski K, Skawina A, Gorezyca J, Kitlinski M, Kitlinski Z. Arterial vascularization of the Vermiform appendix in human fetus. Folia Morphol(Warsz) 1992; 51(2): p.159-64.
13. Simon AM, Birnbaum BA, Jacobs JE. Isolated infarction of the cecum: CT findings in two patients. Radiology 2000 Feb; 214(2): p.513-16.
14. Luzsa G. X-ray Anatomy of the vascular system London. Butterworth and Co. Ltd. 1974; p.237.
15. Kornith PK, Scot, Boley, Blain SW. Anatomy of the sphlanchnic Circulation. The surgical clinics of North America. Feb 1992; 72(1): p.12-15.
16. Mcminn RMH. Last's Anatomy. 9th ed. Edinburgh. Churchill Livingstone. 1994; p.338-39.
17. Katzarski M, Gopal Rao VK, Brady K. Blood supply and position of the Vermiform appendix in Zambians. Med J Zambia 1979 Apr-May;13(2); p. 32-34.
18. Ajmani ML, Ajmani K. The position, length and arterial supply of Vermiform appendix. AnatAnz 1983; 153(4): p.369-74.
19. Piersol. Human Anatomy. Philadelphia. JB Lippincott Company. 1907.
20. Shah MA, Shah M. The arterial supply of Appendix. Anatomical Record. 1946 May-Aug; 95:p.1457-60.

A STUDY OF ARTERIAL SUPPLY OF CAECUM IN HUMANS.

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ABSTRACT: The surgical procedures on the caecum demand a precise knowledge of vascular anatomy of ileocolic region. The aim of this study is to study the arterial supply of the caecum, findings of which may reveal more anatomical facts about the arteries of caecum and their variations. Total 52 specimens of caecum and appendix with their arteries intact were collected, cleaned and dissected. The ileocolic artery and its branches to the caecum, and ileum were traced carefully and observations were recorded. The ileocolic artery arises independently from superior mesenteric artery in 96.88% of cases and ends by dividing into superior and inferior division in 93.76% of cases. The anterior and posterior caecal arteries arise by a common trunk in 56.25%. The ileocolic artery arises from the superior mesenteric artery independently in 96.88% and terminates into superior and inferior division in 93.76% of cases. Common caecal artery seen in 56.25% of cases, arises from inferior division (43.75%), superior division (9.38%) and ileocolic artery (3.12%).Anterior caecal artery arises from superior division (12.5%), inferior division (15.63%), ileocolic artery (3.12%), ileal branch (6.25%) and arterial arcade (6.25%).The posterior caecal artery arises from superior division (18.76%), inferior division (9.38%), ileal branch (3.12%), ileocolic artery (3.12%), arterial arcade (6.25%) and from ascending colic branch of inferior division (3.12%). 21.87% of cases showed additional anterior and posterior caecal arteries.

KEYWORDS: Caecum, ileocolic artery, anterior caecal artery, posterior caecal artery.

INTRODUCTION: Holstead, a pioneer American surgeon has said that the best way to avoid injury to the blood vessel is to know how, when and where to ligate them. The responsibility of studying the arterial variations lies with the anatomists, the knowledge of which helps the surgeons.

Caecum has got great importance because it is prone for many pathological conditions. So surgical procedures on caecum demand a precise knowledge of vascular anatomy of ileocolic region. Literature pertaining to the vascular anatomy of caecum is vast in western population. There is need for the study on Indian population. In the present study an attempt is made to study the variations of arteries supplying caecum.

MATERIALS AND METHODS: The arterial supply of the caecum was studied in 52 specimens. The specimens (caecum with appendix and part of ascending colon and ileum) were collected with their arteries intact from the postmortem centre and dissection hall (Department of anatomy), of J.J.M medical college Davangere and S.I.M.S & R.C Mangalore.

Thus collected specimens were preserved in 5% formalin. After the preservation the specimens were dissected cleaned and numbered. The ileocolic artery and its branches to the caecum were traced carefully and observations were recorded.

RESULTS: The arterial supply of human caecum was studied by dissection method in 52 specimens. All the branches of the ileocolic artery have been dissected and traced till their termination with special attention to the caecal branches and variations have been noted down.

ORIGIN OF ILEOCOLIC ARTERY: In the present study the ileocolic artery originated from the superior mesenteric artery in 50 specimens (96.15%) and in 2 specimens (3.84%) it was originating in common with the right colic artery.

TERMINATION OF ILEOCOLIC ARTERY: The ileocolic artery ends by dividing into superior and inferior divisions in 50 specimens (96.15%); into ascending colic, common caecal and ileal branches in 1 specimen (1.92%); and into anterior caecal, posterior caecal, appendicular and ileal branches in 1 specimen (1.92%).

COMMON CAECAL ARTERY: The common caecal artery arises from inferior division in majority of cases 48 specimens (92.30%); from superior division in 3 specimens (5.76%) and from ileocolic artery in one specimen (1.92 %).

ANTERIOR CAECAL ARTERY: The anterior caecal artery arises from the common caecal artery in 30 specimens (57.69%); inferior division in 10 specimens (19.23%); superior division in 7 specimens (13.46%); ileal branch and arterial arcade in 2 specimens each (3.84%) and in one specimen directly from ileocolic artery (1.92%).

One specimen showed an additional anterior caecal artery (1.92%).

POSTERIOR CAECAL ARTERY: The posterior caecal artery arises from the common caecal artery in 30 specimens(57.69%);superior division in 12 specimens (23.07%);inferior division in 5 specimens (9.61%); ileocolic artery, ascending colic branch of inferior division and ileal branch in one specimen each (1.92% each) and from an arterial arcade in 2 specimens (3.84%).

3 specimens showed additional posterior caecal arteries (5.765).
2 specimens showed two anterior and two posterior caecal arteries (3.84%).

DISCUSSION: In the present study, 52 specimens were studied for the arteries supplying the human caecum. The findings of the study have been compared with those of previous workers on the subject.

ORIGIN OF ILEOCOLIC ARTERY: In the present study the ileocolic artery originated from the superior mesenteric artery in 50 specimens (96.15%) and in 2 specimens (3.84%) it was

originating in common with the right colic artery. Barry j.Anson[1]mentions the origin of ileocolic artery independently from the superior mesenteric artery in 65% of cases and in 35% of cases it arises in common with the right colic artery. The origin of ileocolic artery either independently or in common with the right colic artery has also been mentioned byPiersol[2],and others (3, 4)

TERMINATION OF ILEOCOLIC ARTERY: In our study, the ileocolic artery ends by dividing into superior and inferior divisions in 50 specimens (96.15%); into ascending colic, common caecal and ileal branches in 1 specimen (1.92%); and into anterior caecal, posteriorcaecal, appendicular and ileal branches in 1 specimen (1.92%).

Solanke. T. F[5] mentions that the division of ileocolic artery into medial and lateral branches of unequal caliber is found in only 15% of cases; remaining 85% of the cases it remains single. Cunnigham[3] illustrates the termination of ileocolic artery into ascending and descending branches.

Michel R B[4] also describes the termination of the ileocolic artery into ascending colic and the descending branch which divides into anterior caecal, posterior caecal, appendicular and ileal branches. Patrick W [6] states the termination of the ileocolic artery into colic and ileal branches, which is similar to findings of the present study.

In the present study in one specimen (1.92%) the ileocolic terminates by dividing into ascending colic, common caecal, and ileal branches. The findings are similar to the same finding by,Schaffer[7],Vandamme J P [8]and others (2, 9)Grant's [10] states the similar pattern of division of ileocolic artery into four branches anterior caecal, posterior caecal, appendicular and ileal as in our present study.

COMMON CAECAL ARTERY: In the present study the anterior and posterior caecal arteries take their origin by a common trunk in 30 specimens(57.69%).Piersol[2], Anson and Mcvey[11] mentioned the same in their study that the anterior and posterior caecal arteries arise by a common trunk (common caecal artery).Michel and co-workers [12] in their study found the anterior and posterior caecal arteries arising from a common trunk in 36% of cases. Ures et al. [13] mentioned the same in 76.2% of cases and Bergmann [14] in 13.5% of cases (unpublished report of Beaton, Anson, Swigart, and Jamieson).

ORIGIN OF COMMON CAECAL ARTERY (IN 57.69%): In the present study, the common caecal artery arises from inferior division in majority of cases 26 specimens (50%); from superior division in 3 specimens (5.76%) and from ileocolic artery in one specimen (1.92 %).

Michel and co-workers [12] mentioned the origin of the common caecal artery from an arcade between colic and ileal branches in 76%, less frequently from either ascending colic or ileocolic trunk. Anson and Mcvey[11] have mentioned the origin of common caecal artery from the arcade between colic and ileal branches or separately from colic and ileal branches. Ures et al. [13] mentioned the origin of common caecal artery from the right colic artery in 15% and from the ileal branch in 61.2% of cases. Bergmann [14]mentioned the origin of common caecal artery from the ileocolic artery.

ANTERIOR AND POSTERIOR CAECAL ARTERIES

In the present study,

The anterior and posterior caecal arteries arise from a common trunk(common caecal artery) in 30 specimens(57.69%) The anterior and posterior caecal arteries had separate origin without a common trunk in 22 specimens (42.30%).
The various sources of anterior and posterior caecal arteries are as follows:

ORIGIN OF ANTERIOR CAECAL ARTERY: The anterior caecal artery arises from the common caecal artery in 30 specimens (57.69%); inferior division in 10 specimens (19.23%); superior division in 7 specimens (13.46%); ileal branch and arterial arcade in 2 specimens each (3.84%) and in one specimen directly from ileocolic artery (1.92%).

ORIGIN OF POSTERIOR CAECAL ARTERY: The posterior caecal artery arises from the common caecal artery in 30 specimens(57.69%);superior division in 12 specimens (23.07%);inferior division in 5 specimens (9.61%); ileocolic artery, ascending colic branch of inferior division and ileal branch in one specimen each (1.92% each) and from an arterial arcade in 2 specimens (3.84%).

Schaffer[7]and several other workers (1, 4, 15, 16, 17) have mentioned that the anterior and posterior caecal arteries originate directly from the ileocolic artery.

Michel and co-workers [12] stated that the anterior and posterior caecal arteries arise separately in 64% of cases. The most common origin for both arteries is from an arcade in between colic and ileal branches in 76%, less frequently from colic, ileal or ileocolic artery.

Hamilton [18] has mentioned the origin of anterior and posterior caecal arteries from the inferior division of the ileocolic artery. Ures et al [13] have mentioned the origin of anterior and posterior caecal arteries separately in 23.7%. In 8.7% of cases the anterior caecal artery had its origin from the right colic artery and posterior caecal artery directly from the ileal branch. In 13.8% of cases both caecal arteries originated directly from the ileal branch and in 1.2% of cases the anterior caecal artery originated from the right colic artery and posterior caecal artery from the ileocolic artery. Bergmann [14] mentioned the origin of both caecal arteries directly from the ileocolic artery in 28.5% cases and in 4% of cases from the arcade between right colic and ileal branches. Kozmithet al.[19]mention the origin of both caecal arteries from the ileocolic loop formed between ileal and colic branches. Patrick W [6] states the origin of both the caecal arteries from the ileal branch.
In the present study some of the specimens showed additional anterior or posterior caecal artery.

Specimen no.18 showed an additional anterior caecal artery from ileal branch. Specimen no.1 showed additional posterior caecal artery from an arcade between posterior caecal and ascending colic branch. Specimen no.42 and 47 showed additional posterior caecal arteries from superior and inferior divisions respectively.

Anson and Mcvey[11] described one anterior caecal and two posterior caecal arteries originating from an arcade by a common trunk. Bergmann [14] mentioned the origin of three posterior caecal and one anterior caecal arteries from the ileocolic artery in 6.5% of cases (unpublished report of Beaton, Anson, Swigart and Jamieson).

CONCLUSION: In the present study on the arterial supply of human caecum shows that the origin of ileocolic artery is from the right side of the superior mesenteric artery independently and it terminates by dividing into superior and inferior divisions in majority of cases.

There is common caecal artery in more than half of the cases (57.69%), which originated from inferior division in 50%; superior division 5.76% ; and ileocolic artery in 1.92% of cases.

In 57.69% of the specimens the anterior and posterior caecal arteries originate from the common caecal artery. Other sites of origin for anterior caecal artery are superior division 13.46%; inferior division 19.23%; ileocolic artery 1.92%; ileal branch and arterial arcade in 3.84 specimens each.

Other sites of origin of posterior caecal artery are superior division 23.07%; inferior division 9.6%; from ileocolic artery, ascending colic branch of inferior division and ileal branch in 1.92% each; from an arterial arcade in 3.84% of cases.

7.69% of specimens showed additional anterior and posterior caecal arteries.

Separately from superior mesenteric artery	50 specimens	96.15%
In common with right colic artery	2 specimens	3.84%

Superior and inferior division	50 specimens	96.15%
Ascending colic, common caecal, &ileal branches	1 specimen	1.92%
Anterior caecal, posterior caecal, appendicular &ileal branches	1 specimen	1.92%

Superior division	3 specimens	5.76%
Inferior division	26 specimens	50%
Ileocolic artery	1 specimen	1.92%

Common caecal artery	30 specimens	57.69%
Ileocolic artery	1 specimen	1.92%
Superior division	7 specimens	13.46%
Inferior division	10 specimens	19.23%
Ileal branch	2 specimens	3.84%
Arterial arcade	2 specimens	3.84%

Common caecal artery	30 specimens	57.69%
Ileocolic artery	1 specimen	1.92%
Superior division	12 specimens	23.07%
Inferior division	5 specimens	9.61%
Ileal branch	1 specimen	1.92%
Arterial arcade	2 specimens	3.84%
Ascending colic branch of inferior division	1 specimen	1.92%

REFERENCES:

1. Barry JA. Morri's Anatomy 12th ed. Newyork. McGraw-Hill Book Company. 1966; p.744.
2. Piersol. Human Anatomy. Philadelphia. JB Lippincott Company. 1907.
3. Romanes GJ. Cunninghams's Text book of Anatomy 12th ed. Wallan street Oxford. Oxford University press. 1981; p.926-27.

4. Michael RBK, Norman SW. Surgery of Anus, Rectum and Colon. Vol. 1. Philadelphia. W.B. Saunders Company. 1993; p.11.
5. Solanke TF. The blood supply of Vermiform Appendix in Nigerians. J Ant 1968;102: p.353-362.
6. Patrick WM, David HL, Martin AL, Anthony JS, Surgery of the colon, rectum and anus. 1st ed. Philadelphia. W. B. Saunders Company. 199; p.18.
7. Schaffer WJ. Morri's Human Anatomy. 11th ed. Toranto. 1953; p.709.
8. Vandamme JP, Bonte J. Anew look at the blood supply of the ileocolic angle. Acta Anat(Basel). 1982; 113(1): p.1-14.
9. Mcminn RMH. Last's Anatomy. 9th ed. Edinburgh. Churchill Livingstone. 1994; p.338-39.
10. Basmajian JV. Grant's method of Anatomy. 8th ed. Calcutta scientific Book Agency 1972.
11. Anson BJ, McVey CB. Surgical Anatomy. 5th ed. Philadelphia. W.B. Saunders Company 1971.
12. Michels NA, Siddharth P, Kornblith PL, Parke WW. The variant blood supply to the small and large intestines, its importance in regional resections. A new anatomic study based on 400 dissections with complete review of literature. J Int Coll Surgeons 1963; 39:p.127.
13. Ures J, Stacchini A, Prates JC, Gatto IM, Ures S. Angiographic anatomy of the cecal artery. ArqGastroenterol. 1979 Jan-Mar; 16(1):p.8-11.
14. Bergmann RA, Thompson SA, Afifi AK, Saadeh FA. Compendium of Human Anatomic Variation. 1st ed. Munich. Urban Schwarzenberg. 1988; p.406.
15. Hollinshed WH. Anatomy for Surgeons. Vol.2, 1956; p.492-95.
16. Luzsa G. X-ray Anatomy of the vascular system London. Butterworth and Co. Ltd. 1974; p.237.
17. Simon AM, Birnbaum BA, Jacobs JE. Isolated infarction of the cecum: CT findings in two patients. Radiology 2000 Feb; 214(2): p.513-16.
18. Hamilton WJ. Text book of human anatomy. 1st ed. London. Mac Millan and Company Ltd. 1976.
19. Kornith PK, Scot, Boley, Blain SW. Anatomy of the sphlanchnic Circulation. The surgical clinics of North America. Feb 1992; 72(1): p.12-15.

A STUDY OF SUPRACONDYLAR PROCESS OF HUMERUS

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ABSTRACT: BACKGROUND: Supracondylar process, in human, is a rare, anomalous, beak-like bony process on the anteromedial surface of the humerus. It represents the embryologic vestigial remnant of climbing animals and seen in many reptiles, most marsupials, cats, lemurs and American monkeys. Aim is to study the supracondylar process of humerus. **MATERIALS AND METHODS:** 80 adult dry humeri were collected from Anatomy Department, Gauhati Medical College and were examined. **RESULTS:** Out of 80 humeri, we found one humerus of left side with a bony projection from antero-medial surface of its distal shaft. The bone was then examined, studied, photographed and its dimensions were recorded. **CONCLUSION:** Knowledge of this variation may be of great importance to anatomists and anthropologists, because of possible link to the origins and relations of the human races.

KEY WORDS: supracondylar process, humerus, Struther's ligament.

INTRODUCTION: Race estimation from skeletal data has always been a central focus in anthropology[1]. Also, knowledge of variations in anatomy which is important to anatomists, radiologists, anesthesiologists and surgeons, has gained more importance due to wide use and reliance on computer imaging in diagnostic medicine[2]. Morphological differences are the tools being used to find the missing links between the different stages of evolution. One of such variations is the "supracondylar" processes.

The spur of the humerus or supracondylar process was first reported by Struthers in 1849. It has been referred to as the "supraepitrochlear", "supracondyloid" "epicondyloid" or "a supratrochlear spur" by various authors[3]. It is a normal anatomical structure in climbing animals[4]. In human, it is a rare, anomalous, beak-like bony process on the anteromedial surface of the humerus. It represents the embryologic vestigial remnant of climbing animals and seen in many reptiles, most marsupials, cats, lemurs and American monkeys[5]. It is usually found 5 - 7 cm above the medial epicondyle. The process projects anteroinferomedially from the distal third of the humerus and presents in 0.7 to 2.7% of the population[4]. A ligament called Struthers' ligament extends from the apex of the process to the medial epicondyle[3].

MATERIALS AND METHODS: The study was conducted on 80 humeri which were collected from the 1st M.B.B.S students and from the osteology laboratory, Department of Anatomy, Gauhati Medical College. The bones were examined for any osseous projection from distal part

under day light. Only one humerus of left side was found with an osseous spine on its distal anteromedial surface. Dimensions of the projection were recorded by a vernier caliper.

RESULTS: The bony projection was extending obliquely, medially and downward from the anteromedial surface of the distal humeral shaft approximately 4.4 cm above the medial epicondyle. This spine was reported & referred to as supracondylar process.
Out of 80 humeri, 48 were of left sided and rests were of right sided bone.

DISCUSSION: Gupta RK[23] et al. and Oluyemi KA[3] et al. reported presence of supracondylar process in one humerus among 380 and 40 humeri in their study respectively. Measurements calculated in their study are tabulated below-

The incidence of the supracondylar process of the humerus is very low and the percentage of incidence, as given by different authors, varies. Gruber[17] found the incidence of supracondylar process as 2.7 %, while Danforth[18] found it as 0.5%, Adachi[19] as 0.8%, Hrdlicka[20] as 1%, Dellon[21] as1.15% and Natsis[22] as 1.3% in different races.

There is a high incidence of unilateral supracondylar process of the humerus in 'Cornelia de Lange syndrome', an autosomal recessive trait, occurring in approximately one in every 10,000 live births[6].

It is usually clinically silent, but may become symptomatic by presenting as a mass or can be associated with symptoms of median nerve compression and claudication of the brachial artery[7]. The process ends in a roughened point at which a dense fibrous band (ligament of Struthers) continues to the medial epicondyle[5]. From embryological point of view, the Struthers ligament lies between the tendon of the latissimus dorsi and the coracobrachialis and corresponds to the lower part of the tendon of the vestigial latissimo- condyloideus, a muscle found in climbing mammals which extends from the tendon of insertion of the latissimus dorsi muscle to the medial epicondyle[8]. Rarely, this fibrous band may ossify forming a supracondylar foramen, a tunnel which transmits the median nerve and the brachial artery and sometimes a variant ulnar artery[9] or the ulnar nerve[10]. In lower mammals, the osseo-fibrous tunnel formed by the humerus, supracondylar process and the Struthers' ligament serves to protect the nerves and vessels going to the forearm[10]. In human, the presence of supracondylar process and the Struthers' ligament is usually asymptomatic, but also it is an important entrapment site for the median nerve and brachial artery. Entrapment of brachial artery and median nerve by this ligament at the level of supracondylar process is known as the supracondylar process syndrome which can be treated by surgical removal of the process and ligament[11]. The compression symptoms include severe paresthesia and hypersthesia of the hand and fingers, ischemic pain of the forearm, embolization of the distal arm arteries and disappearance of the radial or ulnar pulse on full extension and supination of the forearm[8,10,16]. More rarely, ulnar nerve compression can also occur if the fibromuscular band from the process, instead of being attached to the medial epicondyle, extends downward as a band which blends with the fibrous arch between the two heads of the flexor carpi ulnaris[13,14,15]. The anterior surfaces of the humerus are also covered by the brachialis muscle. The spine is thus likely to be within the substance of the brachialis muscle. This could probably impair the function of the muscle[3]. Terry (1925) states that supracondylar process gives rise to the pronator teres, and occasionally affords insertion to a persistent lower part of the coracobrachialis[12].

A supracondylar process should be differentiated from osteochondroma. The spur is oriented distally, towards the elbow joint and there is no discontinuity in the cortex of the

humerus. An osteochondroma points away from the joint. X-ray films of the supracondylar process show an intact underlying humeral cortex, whereas in an osteochondroma, the cortex of the tumor is continuous with the humeral cortex. Heterotopic bone such as myositis ossificans may also mimic a supracondylar process[14].

The anteroposterior radiographic view is most important since the lateral view may fail to show the spur on the anteromedial surface of the humerus[14].

Treatment consists of excision of the supracondylar spur and the associated ligament of Struthers. The spur has been reported to recur, and it is therefore recommended that the spur be removed together with the overlying periosteum[24,25].

CONCLUSION: The supracondylar process is frequently misjudged as a pathological condition of the bone rather than as a normal anatomical variation. Though the supracondylar process is a very rare vestigial structure in humans, yet it is known to have racial variations. Along with the anatomists and anthropologists, the supracondylar process is equally important for clinicians as it may be overlooked and there may be misdiagnosis.

REFERENCE:

1. William PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson MWJ, (Ed).Gray's Anatomy. 38th Ed. Churchill Livingstone. New York,1999:626.
2. Harry WG, Bennett JD, Guha SC. Scalene muscles and the brachial plexus: anatomical variations and their clinical significance. Clin. Anat. 1997; 10: 252.
3. Oluyemi Kayode A, Okwuonu Uche C, Adesanya Olamide A, Akinola Oluwole B, Ofusori David A, Ukwenya Victor O and Odion Blessing I. Supracondylar and infratubercular processes observed in the humeri of Nigerians. African Journal of Biotechnology. 2007 Novemver 5; Vol. 6 (21), pp. 2439-2441.
4. Aydinlioglu A, Cirak B, Akpinar F, Tosun N, Dogan A. Bilateral Median Nerve Compresion at the Level of Struthers' Ligament. J. Neurosurg. 2000; 92: 693-696.
5. Parkinson C. The supracondylar process. Radiology. 1954; 62: 556-558.
6. Peters FLM. Radiologic manifestations of the Cornelia de Lange syndrome. Pediatr Radiol. 1975; 3:41-46.
7. Subasi M, Kesemenli C, Necmioqlu S, Kapukaya A, Demirtas M. Supracondylar process of the Humerus. Acta Orthop. Belg. 2002; 68 (1):72-75.
8. Kessel L, Ring M. The supracondylar spur of the humerus. J Bone Joint Surg. 1976; 48:765-766.
9. Barnard LB, Mccoy SM. The supracondyloid process of the humerus, J Bone Joint Surg, 1946; 28(4):845–850.
10. Mittal RL, Gupta BR. Median and ulnar-nerve palsy: an unusual presentation of the supracondylar process. Report of a case, J Bone Joint Surg Am, 1978; 60(4):557–558.
11. Pecina M, Boric I, Anticevic D. Intraoperatively Proven Anomalous Struthers' Ligament Diagnosed by MRI. Skeletal Radiol. Sep 2002; 31(9): 532-535.
12. Terry RJ. On the Racial Distribution of the Supracondyloid Variation. Am. J. Phys. Anthropol., 1930; 14: 459-462.
13. Al-Qattan MM, Husband J.B. Median nerve compression by the supracondylar process:A case report. J. Hand Surg. 1991; 16B: 101-103.

14. Fragiadakis EG, Lamb DW. An unusual case of ulnar nerve compression. Hand, 1970; 2: 14-16.
15. Thomsen PB. Processus supracondyloidea humeri with concomitant compression of the median nerve and the ulnar nerve. Acta Orthop. Scand., 1977; 48: 391-393.
16. Talha H, Enon B, Chevalier JM, L'hoste P, Pillet J. Brachial artery entrapment: compression by the supracondylar process, Ann Vasc Surg, 1987; 1(4):479–482.
17. Gruber, W.; Ein Nachtrag zur Kenntnis des Processus supracondyloideus (internus) humeri des Menschen. Arch. Anat. Physiol. Wissen. Med. 1865; 267:367-376.
18. Danforth CH. The Heredity Of Unilateral Variations In Man; Genetics; 1924; 9: 199.
19. Adachi B. Das Arteriensystem der Japaner. Verlag der Kaiserliche- Japanische University zu Kyoto, Kenyusha Press, Tokyo.1928.
20. Hrdlicka, A. Incidence of the supracondyloid process in whites and other races. Am. J. Phys. Anthropol.1923; 6:405-412.
21. Dellon, Lee. Musculotendinous variations about medial humeral epicondyle. Journal of Hand Surgery (Br), 1986; 11B: 175- 181.
22. Natsis K. Supracondylar process of the humerus: study on 375 Caucasian subjects in Cologne, Germany. Clin Anat. 2008 Mar; 21(2):138-41.
23. Gupta RK, Mehta CD. A Study of the Incidence of Supracondylar Process of the Humerus. J. Anat. Soc. India, 2008. 57 (2):111-115.
24. Ivins G K, Fulton MO. Supracondylar process syndrome : A case report. J. Hand Surg., 1996, 21-A, 279-281.
25. Spinner RJ, Lins RE, Jacobson SR, Nunley J A, Durham NC. Fractures of the supracondylar process of the humerus. J. Hand Surg. 1994, 19-A, 1038-1041.

Table1. Showing various measurements of the supracondylar process (bony spine)

Measurement Of Spine (supracondylar process)	**Value(in cm)**
Length of spine	1.1
Distance of spine from medial epicondyle	4.4
Distance of spine from nutrient foramen	6.5
Breadth at the base of spine	1.5

Table2. Showing measurements of supracondylar process as reported by Gupta RK and Oluyemi KA.

Measurement Of Spine (supracondylar process)	In Gupta RK study	In Oluyemi KA study
Length of spine	0.3 cm	1.6 cm
Distance of spine from medial epicondyle	6.5 cm	5.5 cm
Breadth at the base of spine	1.1 cm	_
Distance of spine from nutrient foramen	_	5.3 cm

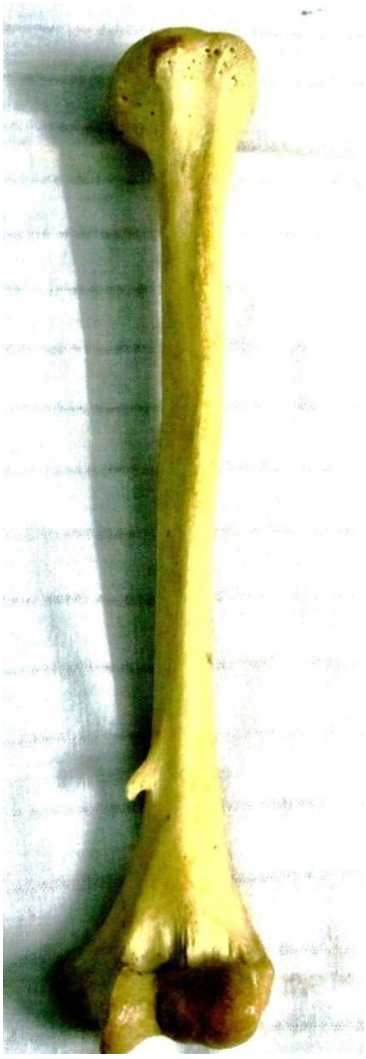

Fig 1: Showing left sided humerus with supracondylar process.

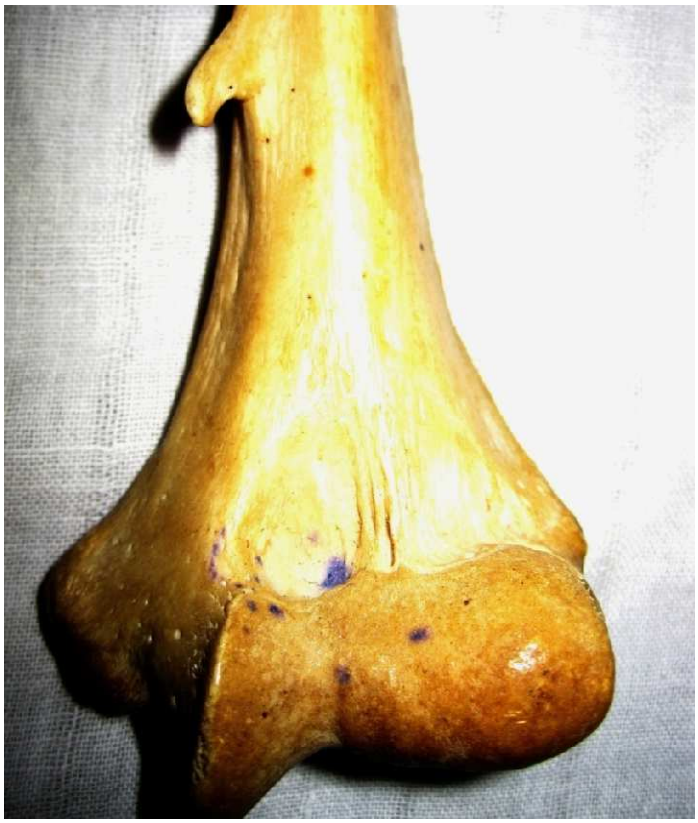

Fig 2: showing only the distal part of the humerus with supracondylar process

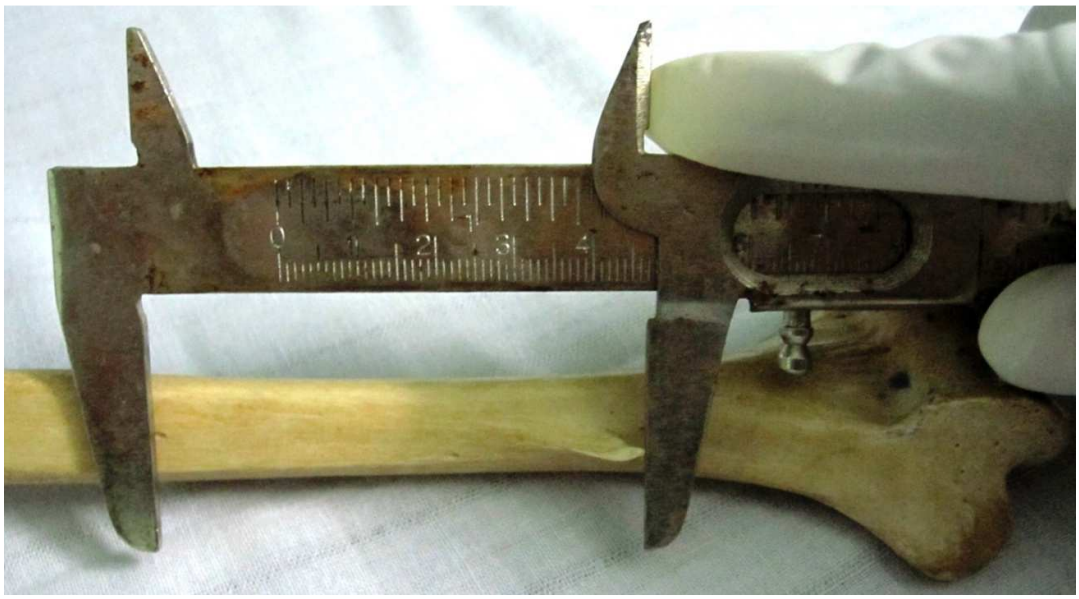

Fig 3: Showing the measurement of distance of supracondylar process from nutrient foramen with vernier calliper

STUDY OF ONYCHOMYCOSIS AT A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT:
BACKGROUND: Onychomycosis is common infection in adults and account for 20% of all nail diseases. The clinical presentation is often confused with other conditions, making laboratory diagnosis and confirmation necessary. Accurate identification of etiological agent is important, as the treatment is different for dermatophytes and non dermatophytes. **AIMS AND OBJECTIVES:** 1. To isolate and identify the etiological agents of onychomycosis. 2. To study the occupational status of the study group. 3. Correlation between nail involvement and sex. **METHODOLOGY:** To identify the aetiological agents of onychomycosis, the present study was carried out in the department of Microbiology, S. S. Institute of medical sciences and research centre. Nail clippings were collected from a total of 120 clinically suspected patients of onychomycosis attending the outpatient department of Dermatology and Venereology and were processed and identified by standard laboratory techniques. **RESULTS:** Fungi was demonstrated in 66 (55%) either by KOH preparation and/or culture. Sixty (50%) were positive by direct microscopy and 54 (45%) were culture positive. Out of the 54 culture isolates,32 (59.26%)were dermatophytes followed by yeasts 13 (24.07%) and non dermatophytemoulds 9 (16.67%). Among the dermatophytes,T.rubrum18 (33.33%) was the most common followed by T.mentagrophyte 8 (14.81%) and T.tonsurans 4 (7.41%).Among the yeasts, C.albicans11 (20.37%) was predominate followed by C.tropicalis 2 (3.7%).Among the non dermatophytemoulds,Aspergillusflavus3 (5.55%) was the commonest followed by Fusarium spp 2 (3.7%).The finger nails were more commonly involved 66 (55%) than toe nails 54 (45%). Most of the patients were farmers 38 (31.67%) followed by housewives 32(26.67%).
CONCLUSION: Dermatophytes remain the predominant cause of onychomycosis, with T.rubrum as the most common aetiological agent, but less commonly yeasts like C.albicans and non dermatophytic moulds like Aspergillus spp can also cause nail infection and hence accurate diagnosis is needed ,since the treatment is different for each group.
KEYWORDS: Onychomycosis, Dermatophytes, Trichophyton rubrum, Candida albicans

INTRODUCTION: Onychomycosis is common infection in adults and account for 20% of all nail diseases[1].The factors that increase the prevalence of onychomycosis include increasing age, male sex, underlying conditions such as diabetes and immunodeficiency[2].Although not life

threatening, this may have significant clinical consequences such as secondary bacterial infections, chronicity, therapeutic difficulties and disfigurement in addition to acting as a reservoir of infection.[3]The symptomatic disease can be a source of embarrassment and potential cause of morbidity[4]. Onychomycosis can be classified Into several clinical types: Distal and lateral subungual onychomycosis, proximal subungual onychomycosis, white superficial onychomycosis and Total dystrophic onychomycosis[1]. Most of the cases are due to dermatophytes, but yeasts and non dermatophytic fungi can also cause nail infection, particularly after trauma or diseases causing nail dystrophy.[1] The clinical presentation may often be confused with other conditions like psoriasis, lichenplanus, onychodystrophy and nail trauma[1], making laboratory diagnosis and confirmation necessary. Fungal cultures are essential for accurate identification of the causative organism. This is of paramount importance because the clinical outcome of antifungal agents varies as to whether the aetiological agent is a dermatophyte, yeast or a non-dermatophytic mould.[5]

The present study was undertaken to isolate and identify the aetiological agents of onychomycosis.

MATERIAL AND METHODS: The present study of onychomycosis was carried out in the department of Microbiology, S. S. Institute of medical sciences and research centre. A total of one hundred and twenty clinically diagnosed randomly selected cases of nail infection, of all age groups and of both sexes, attending Dermatology and Venereology outpatient department of S. S. hospital, Davangere, were studied. A detailed history of selected cases was taken in relation to name, age, sex, address, occupation and involvement of more than one site. Patients under antifungal treatment were excluded from the study group.

The affected area was cleansed with 70% ethyl alcohol and the nail specimen was collected by taking clippings of the infected part and scrapings beneath thenail[1,6.] One portion of the specimen was subjected to 20%potassium hydroxide(KOH) wet preparation for 1 to 2 hours at room temperature in a moist chamber[7-9],and the remaining material was inoculated onto two sets of test tubes, one containing Sabouraud's dextrose agar with 0.05% chloramphenicol and the other containing Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide and incubated at 28^0C for up to 4 weeks.[6,7,9] Cultures were read initially at 24 to48 hours for non dermatophytes and then on periodically for up to 4 weeks for dermatophytes. If no growth was found after 4 weeks, it was taken as negative for the growth of fungi and discarded. Repeat cultures were performed in cases where culture was negative for dermatophytes but positive for non dermatophytic moulds or yeasts to rule out the possibility of contamination.[7,10,11] The criteria used to report non dermatophytic moulds or yeasts as pathogens was direct microscopy positive and isolation of same fungus in three consecutive samples at intervals of 7 days.[11]

Fungal isolate was identified based on colony morphology, reverse pigmentation, growth rate, microscopy (LPCB), slide culture and special tests like hair perforation test, urease test, germ tube test, sugar fermentation and assimilation tests [1,6,8,12].

RESULTS: A total of 120 patients with clinical suspicion of onychomycosis were included in the study. Most of the patients were farmers 38 (31.67%) followed by house wives 32 (26.67%).

The finger nails were more frequently involved 66 (55%) than toe nails 54 (45%).

Out of 120 clinically suspected cases of onychomycosis, fungi were demonstrated in 66 (55%) either by direct microscopy and / or culture. Forty Eight (40%) were positive by both

microscopy and culture. Twelve (10%) were positive by microscopy and negative by culture. Six (5%) were negative by microscopy but culture positive. Fifty four (45%) were negative both by microscopy and culture.

Overall out of the 54 culture isolates, 32 (59.26%) were infected with dermatophytes, followed by Candida species 13 (24.07%) and non dermatophytemoulds 9 (16.67%).

Various fungi isolated is shown in table 3.

DISCUSSION: In the present study,120 clinically suspected cases of onychomycosis attending Dermatology and Venereology out patient department of S. S. hospital, Davangere were studied.

In the present study, Males(60%) were more commonly affected than females(40%), which is comparable with the studies of Adhikari L et al[14],NeupaneS et al[2], where as Madhuri JT et al[9] (51.96%) and Bokhari et al[8](72%) have reported higher prevalence in females. Male predominance may be due to increased outdoor physical activity and increased opportunity for exposure. Toe nail infection(52.78%) was commoner in males, while finger nail infection(66.67%) was common in females. This may be due to increased exposure to wet work in females, as most of them were house wives.

Onychomycosis was most commonly seen in farmers(31.67%), followed by house wives(26.67%), where as students were least affected (9.17%), which is comparable with the study of Veer P et al[10], where as NeupaneS et al[2] reported that students were more commonly affected(31.3%) followed by house wives(28.5%).High prevalence in farmers and house wives may be due to increased outdoor physical activity and increased exposure to wet work respectively. It is less frequently seen in students due to rapid growth of nails which causes elimination of dermatophytes.

In the present study, out of 120 clinically suspected cases of onychomycosis, fungus was demonstrated in 66 (55%) either by direct microscopy and/or culture. Direct microscopy was positive in 60 (55%) and culture in 54 (45%),which is comparable with the studies of Madhuri JT et al[9] and Das NK et al[12], where as Malik NA et al[7]has reported only 16% culture positivity. This variation in direct microscopy and culture may be due to non viability of fungal elements in some cases.

Out of the 54 fungal isolates, dermatophytes 32 (59.26%) were the commonest, followed by yeasts 13 (24.07%) and non dermatophytic moulds 9 (16.67%).Among the dermatophytes, T.rubrum was the predominate isolate 18 (33.33%) followed by T.mentagrophyte 8 (14.81%) ,T.tonsurans 4 (7.41%) and E.floccosum 2 (3.7%).

Among the yeasts, C.albicans11 (20.37%) was the most common followed by C.tropicalis 2 (3.7%) and most of them were females due to immersion of hands frequently in water.

Among the non dermatophyticmouldsisolated,A.flavus3 (5.55%) was the most common followed by Fusariumspp2 (3.7%).A.niger and curvularia were isolated one each(1.85%) and two isolates(3.7%) could not be identified. This is comparable with the studies of Das NK et al[12], Veer P et al[10] and Malik NA et al[7], where as Vijaya D et al [11] and Adhikari L et al [14]reported yeasts and T.tonsurans as predominant fungi respectively in their study.

CONCLUSION: Onychomycosis is very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of fungi and also majority of the people are agriculturists. It can no longer be considered as simple cosmetic problem, but can considerably impair patients quality of life.

Dermatophytes remain the predominant cause of onychomycosis, with T.rubrum as the most common aetiological agent, but less commonly yeasts like C.albicans and non dermatophytic moulds like Aspergillus spp can also cause nail infection and hence accurate diagnosis is needed ,since the treatment is different for each group.

REFERENCES:

1. Chander J(2009).Text book of Medical mycology.3rded.Newdelhi:Mehta publisher;pg:132-4, 56-63.
2. Neupane S, Pokhrel DB and PokhrelBM.onychomycosis:Aclinicoepidemiologicalstudy.Nepal Med Coll J 2009;11(2):92-95.
3. ErbaqciZ, TuncelA,Zer Y et al. A prospective epidemiological survey on the prevalence of onychomycosis and dermatophytosis in male boarding school residents.Mycopathologica 2005;159:347-52.
4. GuptaAK,TabordaP,etal.Epidemiology and prevalence of onychomycosis in HIV positive individuals.Int J Dermatol 2000;39:746-53.
5. RamaniR,Srinivas CR, et al.Moulds in onychomycosis.Int J Dermatol 1993;32:877-8.
6. ColleeJG,Fraser AG, et al(1996).Mackie and McCartney practical medical microbiology.14thed.Edinburgh:Churchhill livingstone:pg:695-714.
7. MalikNA,Raza N, et al.Nondermatophytemoulds and yeasts as causative agents in onychomycosis.Journal of Pakistan Association of Dermatologists 2009;19:74-78.
8. Bokhari MA et al. onychomycosis in Lahore.PakistanInt J Dermatol 1999;38:5915.
9. MadhuriJT,Rama RGR, et al.Onychomycosis:A significant medical problem.Indian J DermatolVenereol Leprol2002;68(6):326-9.
10. Veer P et al.A study of onychomycosis:Prevailing fungi and pattern of infection. Indian J Med Microbiol2007;25:53-6.
11. Vijaya D ,Anandkumar BH, et al. Study of onychomycosis. Indian J DermatolVenereolLeprol 2004;70(3):185-6.
12. DasNK,Ghosh P, et al.A study on the aetiological agent and clinico-mycological correlation of finger nail onychomycosis in eastern india. Indian J Dermatol 2008;53:75-9.
13. VelezA,Linares MJ et al.study of onychomycosis in Corboda,Spain:Prevailing fungi and pattern of infection.Mycopathologica 1997;137:1-8.
14. Adhikari L, Gupta AD et al.Clinico-aetiologic correlates of onychomycosis in Sikkim.Indian J PatholMicrobiol 2009;52:194-7.

Table 1 shows occupational status of the study group.

Occupation	Frequency	Percentage
Farmers	38	31.67%
Housewives	32	26.67%
Office workers	13	10.83%
Students	11	9.17%
Miscellaneous	26	21.67%

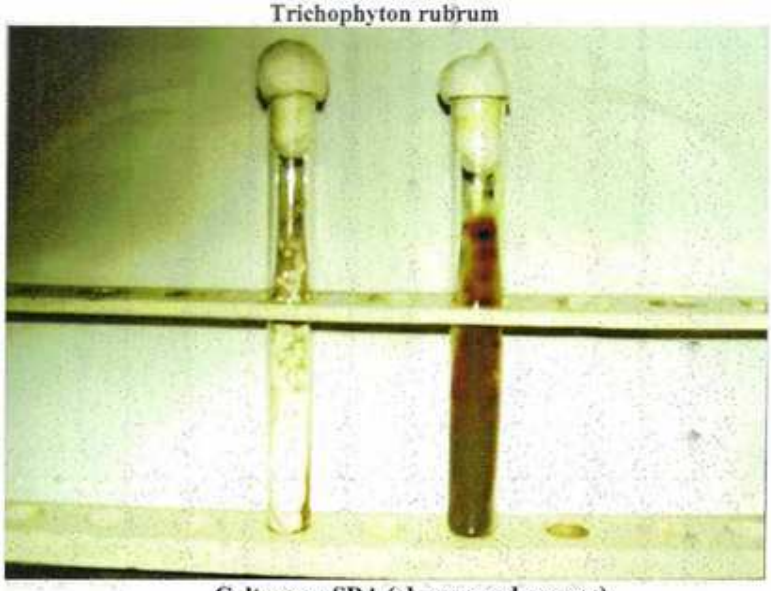
Table 2 shows nail involvement according to gender.

Gender	site		Total
	fingernail	Toe nail	
Male	34 (47.22%)	38 (52.68%)	72
Female	32 (66.67%)	16 (33.38%)	48
Total	66	54	120

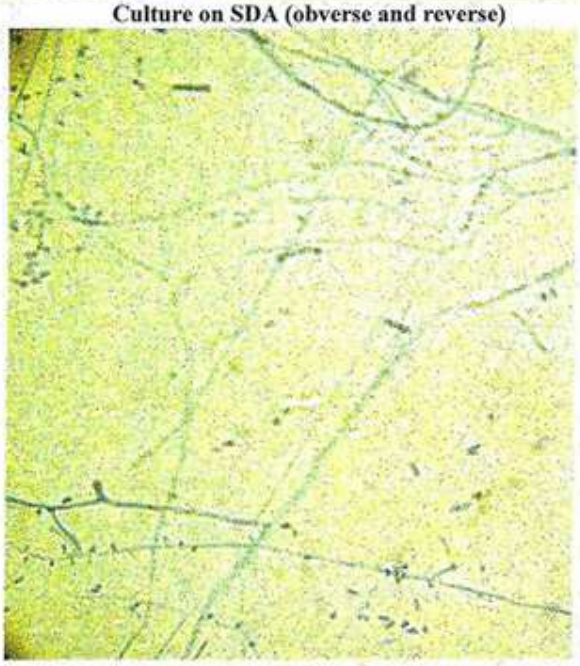
Various fungi isolated is shown in table 3.

Causative agent	Finger nail	Toe nail	Total	percentage
Dermatophytes				
T. rubrum	10	8	18	33.33%
T. mentagrophyte	5	3	8	14.81%
T. tonsurans	2	2	4	7.41%
E. floccosum	0	2	2	3.70%
Yeasts				
C. albicans	8	3	11	20.37%
C. tropicalis	1	1	2	3.70%
Non-dermatophytes				
A. flavus	1	2	3	5.55%
Fusariumspp	1	1	2	3.70%
Curvulariaspp	1	0	1	1.85%
A. niger	1	0	1	1.85%
unidentified	0	2	2	3.70%
Total	**30**	**24**	**54**	**100%**

Trichophyton rubrum

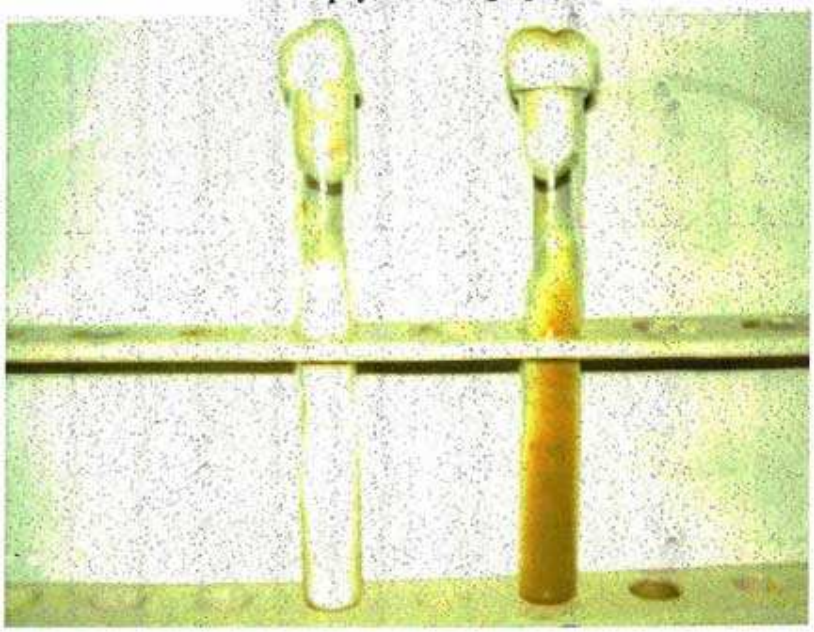


Culture on SDA (obverse and reverse)

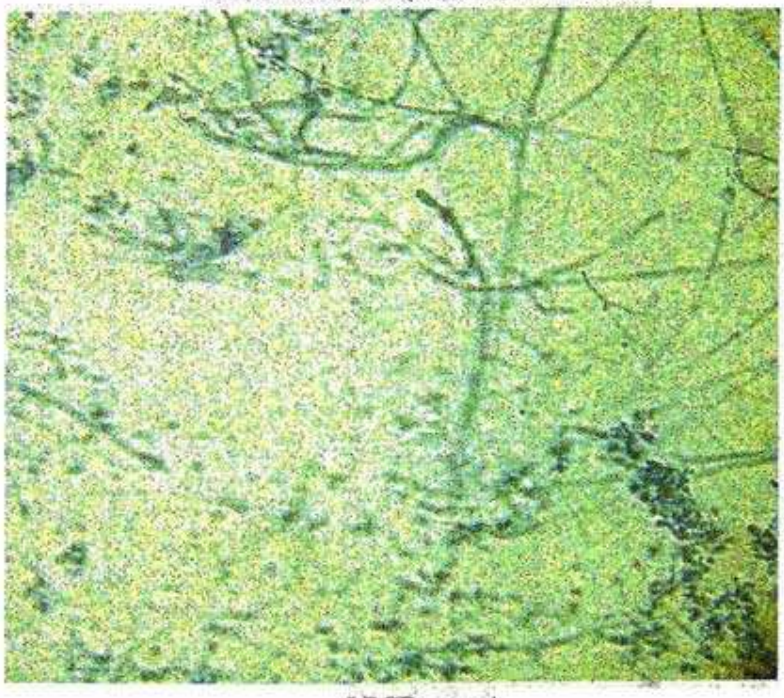


LPCB mount

Trichophyton mentagrophytes



Culture on SDA (obverse and reverse)



LPCB mount

SOCIO-DEMOGRAPHIC PROFILE OF HIV PATIENTS ATTENDING ART CENTRE, VIMS: A HOSPITAL BASED DECRIPTIVE STUDY

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ABSTRACT: BACKGROUND: The human immunodeficiency virus (HIV) infection is a global pandemic.HIV continues to be a burden globally and presents serious public health problems in the developing countries, especially in India. According to the UNAIDS and World Health Organization (WHO) reports of November 2010, there are approximately 33.3 million people living with HIV/Acquired immunodeficiency syndrome (AIDS) worldwide, with a global prevalence of 0 .8%. It is now the leading cause of adult deaths in the world due to infectious diseases. HIV has become the first truly international epidemic easily crossing the oceans & borders. **METHODOLOGY:** A case-series study was conducted among HIV positive patients coming to the ART centre for seeking treatment at ART Centre, Vijayanagara Institute of Medical Sciences Bellary, Karnataka from March 2011 to September 2011. The sample size of the study was 500 and consecutive sampling technique is used. Permission from respective authorities and written consent from study participants was obtained. Data was collected using a pre tested semi structured questionnaire. Data was analyzed using SPSS 15.0. **RESULTS:** The study subjects included both males (54%) and females (46%). The educational status is less than primary schooling in 88% of study subjects and 90% are married. Hetero sexual route of transmission was most common and only 16.1% of study subjects used contraceptives. **CONCLUSION:** Both males and females were equally infected with HIV and productive age group is more commonly affected. Use of contraception is very low.
KEY WORDS: HIV, ART, Mode of transmission

INTRODUCTION: Since the recognition of AIDS pandemic, many efforts have been concentrated on the prevention of HIV transmission[1]. In INDIA as stated by NACO people living with AIDS for the year 2009 estimates 23.1 lakhs which equates to a prevalence of 0.3%. Well this may seem low compared to large population of our country, but a mere 0.1% increase in HIV prevalence would increase the estimated no of people living with HIV by over half a million!!!![2]. Since India

has emerged as a major player in the global HIV epidemic, and, the lack of information on knowledge, perceptions, and behaviors regarding HIV risk and preventive behaviors among Indian adolescents is alarming!![3].

Nowadays, interventions to stem the spread of the Human immunodeficiency virus (HIV) throughout the world are as varied as the contexts in which we find them. Not only is the HIV epidemic dynamic in terms of treatment options, Prevention strategies and disease progression, but sexual Behavior which remains the primary target of AIDS Prevention efforts worldwide, is widely diverse and deeply embedded in individual desires, social and cultural Relationships, and environmental and economic Processes. This makes prevention of HIV, which could be an essentially simple task, enormously complex involving a Multiplicity of dimensions .To respond effectively to heterosexual acquired HIV infection, it is important to understand sexual behavior of persons infected with HIV that facilitate or protect against transmission[4].Behavioral change has been responsible for the prevention successes to date. Strategies to modify risk behaviors need to remain a main priority for HIV prevention[5]. By behavioral strategies we mean an attempt to delay onset of first intercourse, decrease the number of sexual partners, increase the number of sexual acts that are protected, provide counseling and testing for HIV, encourage adherence to biomedical strategies preventing HIV transmission, decrease sharing of needles and syringes, and decrease substance use. Behavioral strategies to accomplish these goals can focus on individuals, couples, families, peer groups or networks, institutions, and entire communities [5].

OBJECTIVE: To study the socio demographic profile of HIV patients

METHODOLOGY: A case-series (descriptive) study was conducted among HIV positive patients coming to the ART centre for seeking treatment at ART Centre, Vijayanagara Institute of Medical Sciences Bellary, Karnataka from March 2011 to September 2011. Totally 10,000 patients have registered and among them 5000 patients were on treatment at ART centre. Hence this study considered patients only who were on treatment. Totally 500 patients were included for the study. These 500 patients were selected based on non probability purposive sampling technique.

Data was collected using a pre designed semi structured questionnaire which contained information regarding socio demographic details and behavior. After obtaining written informed consent, data was collected by interview technique and after the data collection health education was given to patients on Prevention & treatment of HIV. Patients who were seriously ill and those who did not give consent were excluded from the study

Data was entered in Microsoft excel and analyzed in SPSS 15.

RESULTS: Among 500 study subjects, 54% were males and 46% were females. More numbers of study subjects were in 30 – 39 years of age group (39%) followed by 20 – 29 years(32%), 40 – 49 years (25%) and more than 50 years(4%). It was observed that majority of study subjects were between 20 to 39 years (71%). Among males, more number of study subjects were in 30 – 39 years of age group(40.8%) and whereas among females, more number of study subjects were in 20 – 29 years of age group(43.6%) (Table 1)

Among 500 study subjects, 16.4% of them were not known how they might have acquired HIV infection but it was found that heterosexual route of transmission of HIV was more common among study subjects i.e. 83%. Blood transfusion and inject able drug abuse contributed 0.60% collectively (Table.no.2).

Out the 500 subjects taken for our study 45% of them were illiterates, 43% of subjects had just primary education, 9% of subjects had high school education and 2% of them had completed their education till pre university level.

In our study about half of the study subjects were unskilled workers primarily daily wage workers followed by semi skilled and skilled workers who formed 22% and 19% respectively; 49% were unskilled; 7% were professionals.

Among our study subjects, 62% of them earned an income of about 3000-6000 rupees per month; 17% of them earned an income less than 3000 per month ; 21% of them earned an income of more than 6000 per month. In our study it was found that only 10% of the HIV positive subjects are unmarried (Table.no.3).

Among our study subjects, 99% of the subjects confessed they had indulged in a sexual intercourse at least once.

Out of the 495 study subjects who had intercourse at least once, 84.8% of them had an intercourse out of their own interest. Whereas sizeable number of 15.2% of the study subjects were forced.

Out of the 495 study subjects who had indulged in sexual intercourse, 82.8% of the subjects had first act of sexual intercourse been 16 to 25 years age group; 14.2% fell below age 15 years & 3% were above 25 years.

In our study, out of 495 study subjects, 62.6% of the study subjects had their first sexual encounter with their spouse. Sizeable numbers of the study subjects have had their first sexual encounter with a Commercial Sex Worker (19.1%) and the rest with either friend or relative.

Use of contraceptives was found only among 16.1% of study subjects who had indulged in sexual intercourse (Table.no.4).

DISCUSSION: In our study out of the 500 subjects, 54% were male subjects and 46% were female subjects. On a total, maximum subjects fell between age group 30-39 years. Compared to a similar study in INDIA, 41% were female subjects and 59% were male subjects; majority of them fell between age groups 30-34 years[6]. Compared to a similar study done in Goa with only males as the sample, maximum subjects fell between age group 35- 49 years, similar to our study[7]. In another study as well maximum subjects fell between age group 35-49 years[4].

Illiteracy is one of the major reasons for the rampant increase in the number of HIV positive cases. Out of the 500 subjects taken for our study, 45% of them were illiterates. Compared to the similar study done, 30% were illiterate; 24% had a primary education; 36% had a high school education & 10% had a higher education; whereas in our study there were significantly more number of illiterates and less number of subjects with pre-university education[6]. Compared to the similar study done in Chennai**,** 30% were illiterates; 34% had primary education; 25% had secondary education & 11% were professionals whereas in our study there was significantly more number of illiterates [8]. Compared to another similar study done in south India, 25% were illiterate; 50% were educated till high school; 25% were educated till Pre University, whereas in our study there were significantly more number of illiterates and less number of subjects with high school and pre-university education[9].

Ever since the first case of HIV was diagnosed in Chennai in the year 1986, HIV-AIDS was thought to be, primarily, a disease of Commercial Sex Workers and Truck drivers. But in the past decade the infection has crept into the richer sections of the society. In our study, about half of the study subjects were unskilled workers primarily daily wage workers followed by semi skilled and skilled workers. Compared to a study in INDIA, 19% were unemployed; 81%

were employed[6].Whereas in our study we have a large population who are unemployed. Compared to a study done in Kasturba Medical College Hospital, Mangalore, Karnataka; laborers and hotel workers constituted 19.9%., housewives constituted 26.97%; beedi rollers, business and semi-skilled professionals constituted a sizeable number. Drivers constituted only 3.08%[10]. Compared to another study done in Goa, 66.3% were employed, 15.7% were unemployed and 18% are students[7]. Whereas in our study, we have a larger unemployed population.

Poverty is root cause of all the evil in India including the high rates of HIV. Among our study subjects, a maximum number of study subjects earned an income of about 3000-6000 rupees per month (62%). Compared to a similar study, 15% are poorest; 15% were poorer; 3% were middle class; 32% were rich; 15% were richest[6]; whereas in our study 17% have a very low income & there is no question about rich or richest in our study. Compared to other study, 16% had a low standard of living index; 32% had a medium standard of living index& 52% had a high standard of living index[11].

Marital status of the patient was known to generally access the sexual behavior among the subjects. Those who are unmarried are assumed to have Pre marital sex whereas those who are married are assumed to either have sex with either their wives or indulge in extra marital sex. In our study, it was found that only 10%of the HIV positive subjects are unmarried therefore it can be assumed that the pre marital sex leading to HIV is quite low. Compared to a study done in Chennai 55% are married; 35% were single; 7% were separated; 2% were divorced[8]. Whereas in our study a significant large number of them are married. Compared to a study done in south India, 57% are married; 43% were unmarried (43%)[9], whereas in our study a significant large no of them are married and less no of them are unmarried. Compared to a study done in Goa, 45.3% were single and 54.7% were married[7]. Whereas in our study maximum subjects are married.

Among our study subjects, 99% of the study subjects confessed they had indulged in a sexual intercourse at least once. . Late adolescent age group is at threat age group; teenage pregnancies are slowly rising as well. Out of 495 subjects, 14% of the subjects had first act of sexual intercourse before 15 years of age. Compared to a similar study[5], about 12% of study subjects had sexual intercourse before 15 years of age, the findings match with our study .

The scenario of Sexual intercourse before marriage which is very common in western countries has shown a rampant shift into our country over the years. Shear inquisitiveness pushes many into this, in our study out of the 495 study subjects who had intercourse at least once, 420 of them had an intercourse out of their own interest(84%). Whereas sizeable number of the study subjects was forced, 16% study subjects, it could have been due to peer pressure or rape. Compared to a study done in Government Medical College & SSG Hospital, Vadodara, which goes by name “Adolescent HIV/AIDS: Issues and challenges”[12], 26% Of the interviewed female sex workers (FSWs), “Devadasi” FSWs (socially accepted FSWs) had initiated sex work at a much younger age (mean 15.7 vs. 21.8 years) due to childhood sexual abuse; the finding is almost similar to our study where 16% were forced. Compared to a similar study “sexual behavior in India with risk of HIV/AIDS transmission[13]”, 25% reported experience of premarital sex out of interest & 25 per cent of male students in a Delhi school and 28 per cent of male college students in Hyderabad reporting premarital sexual experience .

Heterosexual is the most common mode of transmission worldwide. As such in this study 83% of seropositive cases accounted for this commonest mode of transmission which is supported by various studies in India as well as other parts of world[14,15,16].

CONCLUSION: Illiteracy and low socio economic status are still remaining as major determinants of HIV and productive age group of life is more commonly affected so strategies for tackling HIV infection in our country should be based on profile of the patients.

REFERENCES:

1. B Cribier, M P Schmitt, C Le Coz, and E Grosshans. Changes in sexual behaviour of patients attending an HIV testing centre: a prospective study 1988-1994. Genitourin Med. 1996 February; 72(1): 37–42.
2. National AIDS Control Organization. Annual Report. 2008-2009, http://nacoonline.org/upload/Publication/Annual_Report_NACO_2008-09.pdf accesesed on 07/09/2011
3. Quinn TC, Overbaugh J. HIV/AIDS in women: an expanding epidemic. Science. 2005;308(5728):1582–1583.
4. Journal of Urban Health: Bulletin of New York Academy of Medicine, Vol-83, No.4. 2006
5. Coates TJ, Richter L, Caceres C. Behavioural strategies to reduce HIV transmission: how to make them work better. Lancet. 2008 Aug 23;372(9639):669-84.
6. Perkins JM, Khan KT, Subramanian SV (2009) Patterns and Distribution of HIV among Adult Men and Women in India. PLoS ONE 4(5): e5648. doi:10.1371/journal.pone.0005648
7. Vaz FS, Ferreira AM, Kulkarni MS, Motghare DD. Sexual risk behaviors and HIV/AIDS awareness among males in a rural community in Goa. J Commun Dis. 2006 Mar;38(1):74-8.
8. Solomon SS, Celentano DD, Srikrishnan AK, Vasudevan CK, Anand S, Kumar MS, Solomon S, Lucas GM, Mehta SH. Mortality among injection drug users in Chennai, India (2005-2008). AIDS. 2009 May 15;23(8):997-1004.
9. Chandra PS, Satyanarayana VA, Satishchandra P, Satish KS, Kumar M. Do men and women with HIV differ in their quality of life? A study from South India. AIDS Behav. 2009 Feb;13(1):110-7. Epub 2008 Jul 25.
10. S Jayarama, Shaliny Shenoy, B Unnikrishnan, John Ramapuram, and Manjula Rao. Profiles of Attendees in Voluntary Counseling and Testing Centers of a Medical College Hospital in Coastal Karnataka. Indian J Community Med. 2008 January; 33(1): 43–46.
11. An Lwin, Thein Z., "An Examination of the Association between HIV Related Knowledge, Attitudes, and Behaviors and HIV Infection Status in Five High HIV Prevalence States in India." (2011).Public Health Theses. Paper 168. http://digitalarchive.gsu.edu/iph_theses/168
12. Smriti Naswa and Y. S. Marfatia. Adolescent HIV/AIDS: Issues and challenges. Indian J Sex Transm Dis. 2010 Jan-Jun; 31(1): 1–10.
13. Moni Nag. Sexual behaviour in India with risk of HIV/AIDS transmission. Health Transition Review, Supplement to Volume 5, 1995, 293-305.
14. Verma S, Mahajan A, Singh JB, Sharma M. Clinical Profile of HIV/AIDS patients in Jammu. JK Practitioner. 2007; 14(2):79-83.
15. Destura RV, Berba RP, Mendoza MT et al. Profile of HIV/AIDS Patients at the Philippine General Hospital: Revisting 9 years of Clinical Experience. Philippine journal of Microbiology and Infectious Diseases. 2003; 32:111-21.
16. Khopkar U, Raj S, Sukthankar A, Kulka. Clinical profile of HIV Infection. Indian J Dermatol Ven. 1992; 58(3):155-158.

Table 1: Age sex wise distribution of study subjects

Age group	Male	Female	Total
20 – 29 years	60 (22.3%)	100(43.6%)	160 (32%)
30 – 39 years	110 (40.8%)	85 (36.9%)	195 (39%)
40 – 49 years	80 (29.6%)	45 (19.5%)	125 (25%)
50 – 59 years	15 (05.5%)	00	15 (03%)
>59 years	05 (01.8%)	00	05 (01%)
Total	270 (100%)	230 (100%)	500 (100%)

Table 2: Mode of Transmission of HIV among study subjects

Mode of Transmission	Frequency	Percentage
Heterosexual	415	83.00%
Blood transfusion	002	00.40%
Inject able drug abuse	001	00.20%
Don't know	082	16.40%
Total	500	100%

Table 3: Socio demographic characters of study subjects

Socio demographic characters	Frequency	Percentage
Education		
Illiterate	225	45.0%
Primary	215	43.0%
High school	045	09.0%
Pre university	010	02.0%
Degree & above	005	01.0%
Occupation		
Unskilled	245	49.0%
Semiskilled	110	22.0%
Skilled	095	19.0%
Professional	015	03.0%
Business & others	035	07.0%
Income (monthly)		
<3000 Rs	085	17.0%
3000 – 6000 Rs	310	62.0%
>6000 Rs	105	21.0%
Marital status		
Married	450	90.0%
Unmarried	050	10.0%

Table 4: Distribution of study subjects based on sexual behavior

Sexual behaviors	Frequency	Percentage
Ever indulged in sex?		
Yes	495	99.0%
No	005	01.0%
Age at first act of sex		
<15 years	070	14.2%
15 – 25 years	410	82.8%
>25 years	020	03.0%
First time sex with		
Spouse	310	62.6%
Relative	020	04.0%
Friend	075	15.3%
Commercial sex worker	095	19.1%
Sex before marriage		
Out of interest	420	84.8%
Out of force	075	15.2%
Use of contraception		
Yes	080	16.1%
No	415	83.9%

USES OF HYPERBARIC OXYGEN THERAPY: A REVIEW

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ABSTRACT: In the last three decades great strides in Hyperbaric Oxygen research has raised the value of this unique therapy. Studies have expanded the list of conditions usefully treated with compressed oxygen. Despite the promising experimental and clinical data, the major criticism to most HBO studies has been the lack of controlled prospective analysis for its use. This article discusses the use of HBO, including staffing and equipment considerations, side effects and reviews the published experience in this subject.
KEYWORDS: hyperbaric oxygen therapy, HBO, uses of HBO.

INTRODUCTION: HBO therapy was initially used to treat patients involved in diving accidents or with decompression sickness. However, its indications have increased over the past few decades. Currently, there are twelve indications for HBO therapy approved by the Undersea and Hyperbaric Medical Society (UHMS) in the United States.[1] There are, however, over a hundred indications internationally, although most of them have not been proven by controlled studies. The committee on hyperbaric medicine defines hyperbaric oxygen therapy as "A mode of medical treatment in which the patient is entirely enclosed in a pressure chamber and breathes 100% oxygen at a pressure greater than 1 atmosphere absolute (ATA)". ATA is the units of pressure and 1 ATA is equal to 760 mm of mercury or pressure at sea level.[1]As with most areas of medicine, in hyperbaric medicine there is a constant struggle to balance enthusiasm for progress in the field with the need to apply it on the basis of established evidence.The lack of sound scientific evidence of the efficacy of HBO has bred uncertainty in the wider medical community regarding its legitimacy.

PHYSIOLOGICAL BASIS: The arterial partial pressure of O2 is 100 mm Hg, Hb is 95% saturated and 100 ml of blood carries 19 ml of O2in combination with Hb and 0.32 ml dissolved in plasma. If the inspired O2 concentration is increased to 100%, O2combined with Hb can increase to a maximum of 20 ml when the Hb is 100% saturated and the amount of O2 dissolved in plasma may increase to 2.09 ml. During HBO in addition to the Hb which is 100% saturated the amount of O2 carried in solution will increase to 4.4 ml% at a pressure of 2 ATA to 6.8 ml % at 3 ATA which is almost sufficient to supply the resting total oxygen requirement of many tissues

without a contribution from oxygen bound to hemoglobin. Itis this increased oxygen in plasma which is responsible for most of the beneficial effects of hyperbaric oxygen.[2]

HISTORY OF HYPERBARIC OXYGEN THERAPY: The first pressurized room used to treat health problems was built by an Englishman named Henshaw in 1662.[3]in1788; hyperbaric air was put to large scale use in a diving bell for underwater industrial repairs of an English bridge. The first deep sea diving suit, invented in 1819 by August Siebe, used compressed air supplied to the helmet for generous underwater movement.[4]Dr. John S. developed the first diving tables for the Royal Navy. His legacy gives him the title "Father of Oxygen Therapy" and physicians continue in his line of work to this day.[4] In 1918 Dr. Orval Cunningham built the world's largest functional hyperbaric chamber, a 64' steel sphere "hyperbaric medical hotel" with five floors of living space after he found that denser air helped people fight infection suffering from flu. The Great Depression in the 1930's ended his project and the giant chamber was scrapped for the war effort in the 1940's.[5] The Hyperbaric Oxygen Committee was developed by the UHMS in 1976 to oversee the ethical practice of hyperbaric medicine.[5]

THERAPEUTIC EFFECTS OF HBO THERAPY:

Therapeutic effects of HBO can be attributed to its mechanical or hyperoxygenation effects as shown in the table

Therapeutic effects	Mechanism
Reduces bubble size	Direct mechanical effect.
Immune stimulation	Restores WBC function, enhances phagocytic capabilities and neutrophil mediated killing of bacteria.
Neovascularization	Augmentation of fibroblastic activity which promotes capillary growth.
Reduces edema and tissue swelling	Hyper oxygenation.
Bactericidal	For anaerobic organisms such as Clostridiwelchii, and also inhibits the growth of aerobic bacteria at pressures greater than 1.3 ATA.

ADMINISTRATION: HBO therapy can be given in a monoplace chamber in which a single patient is placed in a chamber pressurized with 100% oxygen or it can be given in a multiplace chamber where many patients can be treated at the same time. To be effective, hyperbaric oxygen must be inhaled in the atmosphere or through an endotracheal tube in a monoplace chamber and in multiplace chamber masks, tight-fitting hoods, or endotracheal tubes can be used. Monoplace chambers are the most common type of chamber used due to their portability, minimal personnel requirements and low cost.[6]

Time: The duration of single treatments varies from 45 minutes for carbon monoxide poisoning to almost 5 hours for some severe decompression disorders. For treatment of wounds - most protocols average 90 minutes for each of 20 to 30 treatments.

GENERAL EQUIPMENT CONSIDERATIONS: All equipment used inside hyperbaric chambers must adhere to the guidelines of the National Fire Protection Association (NFPA) .[7] Chamber fires result in catastrophic consequences.[8]The primary cause of mishaps is the introduction of prohibited items into the chambers, specifically when chamber personnel do not adhere to NFPA fire safety rules. Equipment inside chambers must be intrinsically electrically safe, follow NFPA guidelines, and be tested for the pressures to which they will be exposed.[7]

HYPERBARIC CHAMBER SELECTION, LOCATION AND STAFFING: Hyperbaric oxygen can be offered to critically ill patients inmonoplace and multiplace chamber. Monoplace chambers can be located inside the intensive care unit, where they can be staffed by ICU personnel and are then an extension of the ICU.[9, 10]However; hands-on care cannot be provided to a patient inside a monoplace chamber. Although multiplace chambers do allow hands-on care, experienced staff must be available inside the chamber. Because most hyperbaric chambers are not located within or adjacent to the ICU, the potential benefits of HBO2 to a critically ill patient must be balanced by the risks from transporting the patient as well as the risks from HBO.[11, 12] Personnel working as inside attendants of multiplace chambers must be medically suitable for hyperbaric exposure (e.g., able to equalize ears, no claustrophobia, no pulmonary or cardiac disease, etc). Staff supporting critically ill patients during HBO2 could include Certified Hyperbaric Registered Nurses, physicians; critical care respiratory therapists, and paramedics.

INDICATIONS: The following indications are approved uses of hyperbaric oxygen therapy as defined by the Hyperbaric Oxygen Therapy Committee.

1. Air or Gas Embolism
2. Carbon Monoxide Poisoning
Carbon Monoxide Poisoning Complicated By Cyanide Poisoning
3. Clostridial Myositis and Myonecrosis (Gas Gangrene)
4. Crush Injury, Compartment Syndrome and Other Acute Traumatic Ischemias
5. Decompression Sickness
6. Arterial Insufficiencies:
    Central Retinal Artery Occlusion
    Enhancement of Healing In Selected Problem Wounds
7. Severe Anemia
8. Intracranial Abscess
9. Necrotizing Soft Tissue Infections
10. Osteomyelitis (Refractory)
11. Delayed Radiation Injury (Soft Tissue and Bony Necrosis)
12. Compromised Grafts and Flaps
13. Acute Thermal Burn Injury
14. Idiopathic Sudden Sensorineural Hearing Loss.

USES OF HBO

ARTERIAL GAS EMBOLISM: Arterial gas embolism, occurs when air bubbles in the circulation. There are many causes, including mechanical ventilation; central line placement, haemodialysis, severe diving injury and pulmonary barotrauma.[13]The bubbles cause tissue deformation and vessel occlusion, impairing tissue perfusion and oxygenation. Biochemical effects at the blood-

gas interface cause endothelial damage, changes in haemostasis and activation of leukocytes.[14] Clinical symptoms include muscle and joint pain, arrhythmias, ischemia, confusion, focal neurological deficits and loss of consciousness.

RATIONALE OF TREATMENT WITH HBO:

•HBO reduces bubble size in accordance with Boyle's law—at 3ATA, bubble volume is reduced by about two-thirds.[15]

•Hyperoxia increases the diffusion gradient with the embolized gas, moving gas into solution where it can be metabolized.[16]

HBO is widely accepted as the only life-saving treatment, UHMS suggests maximal benefit with 100% oxygen at 2.8 ATA, and repeated treatments until no further improvement is seen, typically after no more than 5–10 treatments.[17]19 patients in the USA with iatrogenic cerebral arterial gas embolism, showed significant improvement in symptoms with HBO treatment, but the loophole in the study was the control group and end-points were not clearly defined.[18] HBO is most effective when initiated early, but can be successful after hours or even days.[17]

CARBON MONOXIDE POISONING: Loss of consciousness (syncope, seizures, and coma), neurologic deficits, pulmonary edema, myocardial ischemia, and severe metabolic acidosis are the most common symptoms of carbon monoxide poisoning caused primarily from smoke inhalation and suicide attempts. Less severely poisoned patients may have headache, nausea, and other constitutional symptoms. All victims of carbon monoxide poisoning are at risk for delayed neuropsychological sequelae.CO combines preferentially with hemoglobin to produce COHb, displacing oxygen and reducing systemic arterial oxygen (O2) content. CO binds reversibly to hemoglobin with an affinity 200- 230 times that of oxygen.[19]consequently, relatively minute concentrations of the gas in the environment can result in toxic concentrations in human blood. Toxicity includes decrease in the oxygen carrying capacity of blood and alteration of the dissociation characteristics of oxyhemoglobin. It also causes decrease in cellular respiration by binding with cytochrome a3 and binding to myoglobin, which leads to myocardial and skeletal muscle dysfunction.[19]

The rationale for the use of HBO:

- HBO induces cerebral vasoconstriction, which may reduce intracranial pressure and cerebral edema.[20]
- HBO results in more rapid dissociation of CO from respiratory cytochromes.[20]
- HBO may antagonize the oxidative injury that occurs after CO poisoning.[20]

Thom has demonstrated that oxygen at 3 ATA, but not at 1 ATA prevents brain lipid peroxidation when administered to rats beginning 45 minutes subsequent to CO poisoning.[21] undersea and Hyperbaric Medical Society recommends HBO for those patients with signs of serious intoxication regardless of their COHB levels. This includes patients with a history of unconsciousness, presence of neurological signs, cardiovascular dysfunction or severe acidosis. Pregnant women should be evaluated with liberal criteria for HBO due to the increased toxicity risk to the fetus.[1]

GAS GANGRENE: Infection with Clostridium Perfringens in devitalized tissue is the most common cause of gas gangrene. Clostridial microorganisms are anaerobes that produce local and systemic toxins. Wide surgical debridement and appropriate antibiotic therapy remain the standard treatment modality. Adjunctive HBO is known to have antibacterial and anti-toxin effects.[22]case reports support combined therapy with HBO, antibiotics and surgery in these conditions, reducing need for drastic surgery and amputation.[23]

Rationale for treatment:

• HBO therapy induces high oxygen partial pressure in all tissues; achievable tissue oxygen levels are lethal to some obligate anaerobic bacteria such as Clostridium perfringens.[22]

•Anti-edema effect, causes activation of fibroblasts and macrophages, and stimulates angiogenesis.[22]

The UHMS recommends that three 90-min treatments should be given at 3.0ATA in the first 24h, followed by twice-daily treatments for 4–5 days, until clinical improvement is seen.[1]

CRUSH INJURIES, COMPARTMENT SYNDROMES AND OTHER ACUTE TRAUMATIC PERIPHERAL ISCHEMIA'S: Acute traumatic peripheral ischemia's (ATPIs) results in progressive, self-perpetuating ischemia, edema and inadequate healing due to extravasation of intravascular fluid. There is severe damage centrally, with progressive improvement in adjacent tissues. Ischemia and edema may continue even when the primary injury is controlled.[24]

COMPARTMENT SYNDROME: Severe pain on passive stretching of the muscles involved decreased sensation in branches of the involved peripheral nerves and Elevated intra compartmental pressures on direct manometry are the symptoms associated with compartment syndrome.

RATIONALE FOR TREATMENT:

• HBO improves tissue oxygen tensions by increasing plasma-based oxygenation and increasing erythrocyte deformability.[25]

• Intermittent hyperoxia stimulates fibroblast and collagen synthesis, enabling angiogenesis, tissue repair and optimal healing. Hyperoxic vasoconstriction resolves oedema without impairing oxygen delivery, and reverses the ischaemia-oedema cycle.[26]

• HBO also antagonizes free-radical-associated lipid peroxidation, reducing reperfusion injury.[21]

Published research is limited, but a randomized controlled trial in 1996 demonstrated significant improvement in healing with HBO.[27]The UHMS recommends treatment within 4–6h of injury, given at 2.0–2.5ATA at least once daily for several days, although guidelines vary depending on the type of injury.

DECOMPRESSION SICKNESS: Decompression sickness (DCS) occurs mainly when inert gas (mainly nitrogen) comes out of solution during ascent and decompression, forming bubbles in the capillaries and tissues in scuba divers.[18] physical distortion, vessel occlusion, clotting and immune changes lead to symptoms such as fatigue, joint pains, rash, neurological and cardio-respiratory symptoms, coma and death . Predisposing factors include dehydration, injury, exertion at depth and cold exposure.[28] since 1930, HBO is the only established lifesaving treatment for DCS.[29]

Rationale for treatment:

• HBO recompresses bubbles and forces gas back into solution for a more controlled ascent.
• Inert nitrogen is replaced by rapidly-metabolized oxygen, and bubbles move either to the lungs where they are excreted, or to smaller vessels where obstruction is less important, and surface tension forces eventually collapse the bubbles.
• HBO also counteracts platelet and leukocyte activation and endothelial interactions.[30]
UHMS recommend rapid treatment at 2.8 ATA, repeated up to ten times if symptoms persist.[1]

HYPERBARIC OXYGEN THERAPY IN NON-HEALING WOUNDS: Diabetic foot ulcers, non-healing traumatic wounds, and peripheral vascular insufficiency ulcers develop in compromised hosts with local and systemic factors contributing to impairment of tissue repair. Wound healing is slowed down due to decreased collagen production, poor capillary angiogenesis, and impaired oxygen-dependent intracellular leukocyte killing.

Rationale for treatment
• HBO therapy promotes neovascularization and increases endothelial cells, fibroblast proliferation and collagen deposition.[31]
• Modifies the cellular functions of the activated neutrophil, resulting in increased oxidative microbial killing and decreased neutrophil-endothelial adhesion.[32]
• HBO up-regulates platelet-derived growth factor receptor messenger RNA activity.[33]
• Has synergistic effect with growth factor.[34]
Kalani found that the wound-healing rate was 76% in the group with HBO therapy and 48% in the group without HBO therapy in 38 patients treated for diabetic foot ulcers over a period of three years. [35]Patients are generally treated at 2.0 to 2.5 ATA for 90-120 minutes per day and receive 20-30 treatments.[35]

EXCEPTIONAL BLOOD LOSS ANEMIA: Severe hemorrhagic shock and blood loss anemia may lead to tissue hypoxia and ischemia. Where whole blood transfusion is not possible, for religious or practical reasons, HBO may compensate for such a hemoglobin deficiency. HBO is used as a short-term measure, but is inconvenient and expensive, and the risk of oxygen toxicity limits its treatment.
Rationale for treatment:

HBO increases levels of plasma-dissolved oxygen to enable oxygenation while erythrocyte regeneration occurs.[36]
Hart described 70% survival in 26 patients who received HBO after losing >50% of their circulating volume.[37]
UHMS recommend treatments at up to 3ATA for 2–4h periods, three or four times a day, until hypoxic symptoms have resolved and red blood cells have been regenerated.[1]

INTRACRANIAL ABSCESS: HBO can be used as an adjuvant therapy in patients with severe infection or immune compromise, who may be unresponsive to standard aspiration and antibiotic treatment.[1]

Rationale for treatment
- HBO inhibits the predominantly anaerobic micro-organisms.[20]
- reduces cerebral oedema.[20]

- modifies the immune response.[20]

Kurschel reported HBO therapy to be safe and effective in treating children with brain abscesses.[37]

UHMS recommends HBO for multiple, deep or dominantly-located abscesses, or in patients with immune compromise, poor surgical risk, or resistance to conventional treatment.[1] Treatments are once or twice daily, at 2.0–2.5ATA for 60–90min. The average number of treatments is thirteen, and a utilization review is recommended after 20treatments.[1]

NECROTISING SOFT TISSUE INFECTIONS: Necrotising fasciitis is commonly seen in patients with diabetes mellitus, cirrhosis, and intravenous drug abuse. Reports of mortality range from 30% to 75%.[1] It is a rapidly-progressive traumatic bacterial infection of the deep fascia with secondary subcutaneous and cutaneous involvement. Hemolytic streptococci are typical pathogens, but polymicrobial infection, host diabetes and vascular disease are all common. An obliterative endarteritis occurs, causing tissues to become hypoxic, hypovascular and hypocellular. Leukocytes become sequestered in vessels, impairing local immunity, and incomplete substrate oxidation results in hydrogen and methane accumulation in the tissues. Tissue necrosis occurs, with purulent discharge and gas production.[1]

Rationale for treatment

- In animal studies, HBO has a direct bactericidal effect.
- Improves tissue oxygen tension, leukocyte function and bacterial clearance.[38]
- Integrin inhibition decreases leukocyte adherence, reducing systemic toxicity.[21]

HBO has been reported to reduce mortality by up to two-thirds.[39] HBO is particularly indicated in bacterial gangrene and non-clostridial myonecrosis (which have high mortality and morbidity), and in compromised or unresponsive hosts.[40]

The UHMS recommends twice-daily treatments for 90–120min at 2.0–2.5ATA, reduced to once daily when the patient's condition is stabilized. Further treatments may be given to reduce relapse, and a utilization review is recommended after 30 treatments.[1]

HYPERBARIC OXYGEN AS ADJUNCTIVE THERAPY FOR OSTEOMYELITIS: Bone infections that fail to respond to surgical and antibiotic therapy due to systemic host and local immune compromised factors lead to refractory osteomyelitis. Failure of treatment or recurrence of osteomyelitis often leads to amputation. Hyperbaric oxygen can be used as an adjunctive treatment in chronic refractory osteomyelitis along with culture-directed antibiotics, surgical debridement, and nutritional support.

Rationale for treatment:

• HBO enhances oxygen-dependent leukocyte killing through the production of hydrogen peroxide and superoxide by providing increased oxygen tension in the hypoxic tissue.
• Transient reversal of hypoxia might increase clearance of bacteria.[31]
• Optimal tissue oxygen tension enhances osteogenesis and neovascularization.[41]
• HBO has also been shown to enhance osteoaclastic activity on necrotic dead bone to remove bony debris.[42]
• Synergistic effects of HBO on bone healing with bone morphogenic protein were also demonstrated .[43]

• potentiate the antimicrobial effects of aminoglycosides, and possibly sulpha drugs and vancomycin, in the killing of susceptible bacteria.[38]

The results of multiple clinical studies have suggested a beneficial effect with the addition of adjunctive hyperbaric oxygen therapy to conventional treatment regimens for osteomyelitis in terms of reduced hospital stay and amputation rates. [44]whereas other clinical studies have failed to demonstrate such a benefit.[45]Patients with osteomyelitis are usually treated at 2.0 to 2.5 ATA for 90-120 minutes per day and typically receive 20-40 treatments.[1]

SKIN FLAPS AND GRAFTS (COMPROMISED): Skin grafts survive as oxygen and nutrients diffuse into them from the underlying wound bed. Long-term survival depends on a new blood supply forming from the wound to the graft. When the wound bed does not have enough oxygen supplied to it, the skin graft will at least partially fail. Common causes for this are previous radiation to the wound area, diabetes mellitus, and certain infections. Significant improvements with HBO in skin grafts and flaps have been reported since 1967.[46]
Rationale for treatment:

• Increased angiogenesis, healing and increased microvasculature.[47]
• Reduce endothelial leukocyte adherence.[25]
• Prevents progressive vasoconstriction of reperfusion injury.[25]
• Fibroblast stimulation and collagen synthesis.

Nemiroff reported significantly increased microvasculature in animals treated with HBO.[47] The UHMS recommends twice-daily treatment at 2.0–2.5 ATA for 90–120min, reducing to once-daily when the graft or flap has stabilized. A utilization review is recommended after 20 treatments, whether preparing a site for grafting, or maximizing survival of a new graft.[1]

ACUTE THERMAL BURN INJURY: Insufficient oxygen and nutrient supply from the surrounding tissues lead to a central zone of coagulation in cases of severe burn. Burn therapy comprises respiratory care, antibiotics, debridement, and parenteral nutrition, with the aims of reducing oedema, preserving borderline tissue and enhancing host defenses.

Rationale for treatment:
• Reduces haemoconcentration, coagulability and vascular damage in thermal burns.[48]
• Hyperoxic vasoconstriction decreases edema, and increases collagen formation and angiogenesis.[25]
• Phagocytic bacterial killing is also improved, and white cell endothelial adherence is inhibited, preventing capillary damage.[25]
• HBO maintains ATP levels and microvascular integrity, and reduces infection.[25]
HBO has been proved effective in such cases and decreases healing time and reduces need for grafting.[1] However, some studies have found no benefit from HBO in thermal burns and stated that HBO could worsen pulmonary damage in thermal burns.[49] however clinical data is needed to further confirm the benefits. The UHMS recommends three sessions within 24h of injury, and 90-min treatments twice-daily thereafter, at 2.0–2.4 ATA.[1]

IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS: Idiopathic sudden sensorineural hearing loss leads to Tinnitus and a feeling of increased pressure; vertigo is less commonly

associated with the syndrome.[50]Etiology could be a viral infection, such as mumps, trauma, Ménière's disease, acoustic neurinoma, ototoxic medication, multiple sclerosis vascular occlusion, viral infections, labyrinthine membrane breaks, immune associated disease, abnormal cochlear stress response, trauma, abnormal tissue growth, toxins, ototoxic drugs and cochlear membrane damage.

Rationale for treatment:

• Increase of oxygen partial pressure in the inner ear.[51]
•HBO restores the arterial-perilymphatic oxygen concentration difference.

A meta-analysis by Lamm showed a positive effect of HBO in approximately 50% of cases, after failure of classical drug therapy. [52]The UHMS recommends 100% O2 at 2.0 to 2.5 atmospheres absolute for 90 minutes daily for10 to 20 treatments.[1]

OTHER IMPLICATIONS FOR HBO

NEUROLOGICAL ILLNESSES

CEREBROVASCULAR ACCIDENTS (STROKE): Hyperbaric oxygen therapy shows a striking reduction in spasticity possibly due to improved function of neurons in affected areas of the brain and secondly to rise of PO2 in the spastic inactive and hypoxic muscle. Additionally there is an improvement in the cognitive and mental performance.[53]

Potential benefits:

• Increased oxygen delivery.
• decreased cerebral edema.
• decreased lipid peroxidation.
• Inhibition of leukocyte activation.
• Maintenance of blood-brain barrier integrity.[21, 54.]

ACUTE TRAUMATIC BRAIN INJURIES: Acute traumatic brain injury causes a cycle of ischemia, hypoxia, edema and enzymatic derangements. HBO tends to break this vicious cycle. However there has to be a responsive cerebral circulation.

Rationale for treatment

• Improved aerobic metabolism,
• Reduction in lactate levels,
• Increase in creatinine phosphate and ATP levels.
• Elevation of partial pressure of Oxygen increases the diffusion distance, and O2 delivery in abnormal areas is enhanced.[21]

Wang demonstrated that multiple HBOT (3 ATA hourly for 3 or 5 days), delivered 2 days post-injury resulted in significantly reduced overall neurological deficit scores and neuronal apoptosis within brain tissue. [55]

CEREBRAL PALSY: Studies show that HBO therapy can improve some cerebral Palsy symptoms like spasticity, vision, hearing, and speech.[56] however there is a lack of clinical evidence for its use and hence HBO can be used as an adjuvant with other treatment modalities, but it is not a cure.

The rationale for treatment:

- Increased oxygenation of the cerebral ischaemic penumbra.[57]

A study conducted by Collett showed improvement in gross motor function, improved performance in activities of daily living, attention, working memory, and speech in 111 children's with cerebral palsy aged 3-12 years treated with 100% oxygen at 1.75 atmospheres absolute (ATA).[58]

BELL'S PALSY: Steroids and surgical decompression are the only treatment used currently but results are inconclusive as to their benefit. HBO added to other treatment increases the efficacy of the treatment and reduces the period needed for restoration of complete function of the damaged nerve.[59]

Rationale for treatment:

The antioedematous effect and additional oxygenation of hypoxic cranial nerve VII with dissolved oxygen.[60]

In the double blind study, Raeiae in 95.2% cases had full recovery in the average period of 22 days at experimental group of 42 patients treated only with HBO2 under 2.8 ATA and placebo tablets.

ONCOLOGY: A dominant feature of post-radiation change is the obliteration of small blood vessels leading to hypoxia. The oxygen tension inside a tumor drops lower as the tumor enlarges and may drop to zero in the necrotic center of the tumor. Hypoxia increases the resistance of cancer to radiotherapy. With oxygen tension at zero, the amount of radiation required to be effective is three times that required with normal oxygen tension. When irradiation is done immediately after HBO therapy, the well-oxygenated cells will be damaged lethally.

Rationale:

- Stimulates angiogenesis, increases neovascularization, fibroblast and osteoblast proliferation.
- It stimulates collagen formation at wound edges and thus helps in re-epithelialization of ulcers and provides a better nutritive bed to support grafts and pedicle flaps.[61]

AUTISM: Studies have shown that there is cerebral hypo perfusion in approximately 86% of autistic patients.[62] Hypoperfusion could be the result of inflammation around the blood vessels in the brain. HBO is used in successful treatment of vasculitis.

Rationale for treatment:

HBO attenuates the production of proinflammatory cytokines including TNF α, [62] IL-1β and IL-6 and increase the production of anti-inflammatory IL-10.[63]In one case report Heuser treated a 4 year patient with autism using HBO at 1.3 atm and 24% oxygen and reported striking improvement in behavior including memory and cognitive functions.[64]

SIDE EFFECTS OF HBO2: While HBO2 has an admirable safety record, those recommending HBO2 in wound care should be aware of potential side effects and complications.

EAR AND SINUS BAROTRAUMA: Middle ear barotrauma is the most common side effect of HBO2. Patients with a cold, upper respiratory tract infection or allergic rhinitis are not suitable candidates for HBO2. [65]

MYOPIA: Some patients receiving HBO2 will develop reversible myopia. [66] HBO2 may lead to oxidative change of the lens proteins. After cessation of therapy, the refraction usually returns to the pretreatment state within a few weeks.[67]

AGGRAVATION OF CONGESTIVE HEART FAILURE: HBO2 causes increased peripheral vascular resistance from its vasoconstrictive effects. Blood flow to the left ventricle has been noted to decrease during HBO. Patients with a cardiac ejection fraction of less than 35% are generally not treated by HBO.[68]

PULMONARY BAROTRAUMA: injury is related to pressure changes and occurs only on ascent. For the lungs to be injured there must be an obstruction such as a closed glottis or bronchial obstruction. An untreated pneumothorax is an absolute contradiction to HBO2 therapy and patients with a pneumothorax must have a chest tube inserted prior to the treatment dive. If a pneumothorax occurs during the treatment, a chest tube must be inserted prior to ascent to prevent a marked deterioration in the condition of the patient.[69]

COST: analysis has shown that the addition of hyperbaric oxygen to conventional treatment results in significant cost savings due to lesser stay in hospital and shorter course of illness.[53]

CONCLUSION: The Hyperbaric Committee of the Undersea and Hyperbaric Medical Society in the US (UHMS) reviews and publishes once in 2-3 years the indications for HBO, which are supported by adequate medical literature. The Committee usually looks for three kinds of evidence: physiological, animal studies and human studies preferably double blinded, and publishes this list of "approved" indications. In addition to the use of HBO for the "Approved" indications, growth of Hyperbaric Medicine over the past two decades has also led to its popularity and use for some "unapproved" or the so called “Off Label” indications. In diseases for which the use of hyperbaric oxygen is not well supported, the potential benefits must be carefully weighed against the risks of treating. Patient selection for HBOT should be executed carefully and according to accepted guidelines. If safety guidelines are strictly followed, HBO therapy is a modality with an acceptable rate of complications. Doctors in all fields must familiarize themselves with recent evidence on this mode of therapy, so that their patients are not denied the gains of this modern treatment.

REFERENCE:

1. Hampson NB, ed. Hyperbaric Oxygen Therapy: 1999 Committee report. Kensington MD, Undersea and Hyperbaric Medical Society, 1999.
2. Leach RM, Rees PJ, Wilmshurst P. Hyperbaric oxygen therapy.BMJ 1998; 317:1140-3.
3. Simpson A. Compressed air as a therapeutic agent in the treatment of consumption, asthma, chronic bronchitis and other diseases. Edinburgh: Sutherland and Knox, 1857.

4. Acott, C. (1999). "JS Haldane, JBS Haldane, L Hill, and A Siebe: A brief resume of their lives."South Pacific Underwater Medicine Society Journal 29 (3). ISSN 0813-1988. OCLC 16986801.Retrieved 2008-07-12.
5. Kindwall EP, Gottlieb FJ, Larson DL. Hyperbaric oxygen therapy in plastic surgery, plastic and reconstructive therapy.1991; 888:898-908.
6. Hart GB. The monoplace chamber. In: Kindwall EP, ed. hyperbaric medicine practice. Flagstaff, Ariz.: Best,1994:57-82.
7. National Fire Protection Association: NFPA 99: Standard for Health Care Facilities. 2005 Edition.Quincy, MA, NFPA, 2005.
8. Sheffield PJ, Desautels DA: Hyperbaric and hypobaric chamber fires: A 73-year analysis. Undersea Hyperb Med 1997; 24:153–164.
9. Gossett WA, Rockswold GL, Rockswold SB: The safe treatment, monitoring and management of severe traumatic brain injury patients in a monoplace chamber. Undersea Hyperb Med 2010; 37:35–48
10. Weaver LK, Churchill SC, Bell JE: Taking the chamber to the critically ill patient. Abstr. Undersea Hyperb Med 2010; 37:347
11. Waydhas C, Schneck G, Duswald KH: Deterioration of respiratory function after intrahospital transport of critically ill surgical patients. Intensive Care Med 1995; 21:784–789.
12. Shirley PJ, Bion JF: Intra-hospital transport of critically ill patients: Minimising risk. Intensive Care Med 2004; 30:1508–1510.
13. Murphy BP, Harford FJ, Cramer FS. Cerebral air embolism resulting from invasive medical procedures: treatment with hyperbaric oxygen. Ann Surg 1985; 201:242–5.
14. Muth CM, Shank ES. Gas embolism. N Engl J Med 2000; 342:476–82.
15. Branger AB, Lambertsen CJ, Eckmann DM. Cerebral gas embolism absorption during hyperbaric therapy: theory. J ApplPhysiol 2001; 90:593–600.
16. Dexter F, Hindman BJ. Recommendations for hyperbaric oxygen therapy of cerebral air embolism based on a mathematical model of bubble absorption.AnesthAnalg 1997; 84:1203–7.
17. Moon RE, de Lisle Dear G, Stolp BW. Treatment of decompression illness and iatrogenic gas embolism.Respir Care Clin N Am 1999; 5:93–135.
18. Benson J, Adkinson C, Collier R. Hyperbaric oxygen therapy of iatrogenic cerebral arterial gas embolism. Undersea Hyperb Med 2003; 30:117–26.
19. Gosselin RE, Smith RP, Hodge HC, eds. Clinical Toxicology of Commercial Products, 4th edn, Baltimore, MD: Williams and Wilkins, 1984
20. Tibbles PM, Edelsberg JS. Hyperbaric oxygen therapy (Reviewarticle). NEJM 1996:1642-8.
21. Thom SR. Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning. J ApplPhysiol 1992;73:1584-9.
22. Kaye D. Effect of hyperbaric oxygen on Clostridia in vitro and in vivo. ProcSocExpBiol Med 1967; 124:360–6.
23. Hirn M. Hyperbaric oxygen in the treatment of gas gangrene and perineal necrotizing fasciitis.Eur J Surg 1993; 570:1–36.
24. Wattel F, Mathieu D. Proceedings of the 1st European Consensus Conference on Hyperbaric Medicine. Lille (France), 1994:377–82.
25. Zamboni WA, Roth AC, Russell RC, Graham B, Suchy H, Kucan JO et al. Morphological analysis of the microcirculation during reperfusion of ischaemic

skeletal muscle and the effect of hyperbaric oxygen. Plastic ReconstrSurg 1993; 91:1110–23.
26. Nylander G, Lewis D, Lewis D. Reduction of post-ischaemicoedema with hyperbaric oxygen. PlastReconstrSurg 1985; 76:596–601.
27. Bouachour G, Cronier P, Gouello JP. Hyperbaric oxygen therapy in the management of crush injuries: a randomised double-blind placebo-controlled trial. J Trauma 1996; 41:333–9.
28. Hagberg M, Ornhagen H. Incidence and risk factors for symptoms of decompression sickness among male and female dive masters and instructors: a retrospective cohort study. Undersea Hyperb Med 2003; 30:93–102.
29. Yarbrough OD, Behnke AR. Treatment of compressed air illness utilizing oxygen. J IndustHygToxicol 1939; 21:213–18.
30. Ishii Y, Miyanaga Y, Shimojo H, Ushida T, Tateishi T. Effects of hyperbaric oxygen on procollagen messenger RNA levels and collagen synthesis in the healing of rat tendon laceration. Tissue Eng 1999;5: 279-86.
31. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic. The effect of inspired oxygen on infection. Arch Surg 1984;119:199-204
32. Bonomo SR, Davidson JD, Tyrone JW, Lin X, Mustoe TA.Enhancement of wound healing by hyperbaric oxygen and transforming growth factor beta3 in a new chronic wound model in aged rabbits. Arch Surg 2000; 135:1148-53.
33. Zhao LL, Davidson JD, Wee SC, Roth SI, Mustoe TA.Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. Arch Surg 1994;129:1043-9.
34. Kalani M, Jorneskog G, Naderi N, Lind F, Brismar K. Hyperbaric oxygen therapy in treatment of diabetic foot ulcers. Long-term follow-up. J Diabetes 2002; 16:153-8.
35. Gill, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM. 2004 Jul;97(7):385-95.
36. Hart GB, Lamb RC, Strauss MB. Gas gangrene. J Trauma 1983; 23: 991-100.
37. Kurschel S, Mohia A, Weigl V, Eder HG. Hyperbaric oxygen therapy for the treatment of brain abscess in children. Childs Nerv Syst. 2006 Jan;22(1):38-42.
38. Mader JT, Guckian JC, Glass DL, Reinarz JA.Therapy with hyperbaric oxygen for experimental osteomyelitis due to Staphylococcus aureus in rabbits. J Infect Dis 1978; 138:312–18.
39. Riseman JA, Zamboni WA, Curtis A, Graham DR, Konrad HR, Ross DS et al. Hyperbaric oxygen therapy for necrotizing fasciitis reduces mortality and the need for debridements. Surgery 1990; 108:847–50.
40. Hirn M, Niinikoski J, Lehtonen OP. Effect of hyperbaric oxygen and surgery on experimental multimicrobial gas gangrene. EurSurg Res 1993; 25:265–9.
41. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK et al. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000; 135:1293-7.
42. Barth E, Sullivan T, Berg E. Animal model for evaluating bone repair with and without adjunctive hyperbaric oxygen therapy (HBO): comparing dose schedules. J Invest Surg 1990;3:387-92.
43. Okubo Y, Bessho K, Fujimura K, Kusumoto K, Ogawa Y, Iizuka T et al. Effect of hyperbaric oxygenation on bone induced by recombinant human bone morphogenetic protein-2. Br J Oral MaxillofacSurg 2001;39:91-5

44. Davis JC, Heckman JD, DeLee JC, Buckwold FJ. Chronic non-haematogenous osteomyelitis treated with adjuvant hyperbaric oxygen. J Bone Joint Surg 1986;68A:1210-7.
45. Esterhai JL Jr, Pisarello J, Brighton CT, Heppenstall RB, Gellman H, Goldstein G et al. Adjunctive hyperbaric oxygen therapy in the treatment of chronic refractory osteomyelitis. J Trauma 1987;27:763-8.
46. Perrins DJD. Influence of hyperbaric oxygen on the survival of split skin grafts. Lancet 1967; 7495:868–71.
47. Nemiroff PM, Lungu AL. The influence of hyperbaric oxygen and irradiation on vascularity in skin flaps: a controlled study. Surg Forum 1987; 38:565–7.
48. Grossman AR, Grossman AJ. Update on hyperbaric oxygen and treatment of burns. Burns 1982; 8:176–9.
49. Brannen AL, Still J, Haynes M, Orlet H, Rosenblum F, Law E, et al. A randomised prospective trial of hyperbaric oxygen in a referral burn centre population. Ann Surg 1997; 63:205–8.
50. Lehnhardt, R.E. Die akuteInnenohrschwerhörigkeit. HNO 1991; 39: 378-385
51. Murata, K. and U. Fisch. Effect of CO2 on the perilymphatic oxygen tension in cats. Ann OtolRhinolLaryngol 1977; 86: 164-171
52. Lamm, K., Lamm, H. and W. Arnold. Effect of hyperbaric oxygen therapy in comparison to conventional or placebo therapy or no treatment in idiopathic sudden hearing loss, acoustic trauma, noise-induced hearing loss and tinnitus.A literature survey.AdvOtorhinolaryngol. 1998;54:86-99.
53. Sahni T, Jain M. Status of Status of Hyperbaric Oxygen Therapy in Neurological Illnesses – Review of International and Indian experience. Neurosciences Today 2003; 7:126-134.
54. Mink RB, Dutka AJ. Hyperbaric oxygen after global cerebral ischemia in rabbits reduces brain vascular permeability and blood flow. Stroke. 1995; 26: 2307–2312
55. Wang GH, Zhang XG, Jiang ZL, Li X, Peng LL, Li YC et al.: Neuroprotective Effects of Hyperbaric Oxygen Treatment on Traumatic Brain Injury in the Rat. J Neurotrauma 2010, 27:1733-1743
56. Montgomery D, Goldberg J, Amar M. Effects of hyperbaric oxygen therapy on children with spastic diplegic cerebral palsy: A pilot project. Undersea Hyperb Med 1999;26:235-42.
57. Neubauer RA. Hyperbaric oxygen for treatment of closed head injury. South Med J 1994;87:933-6.
58. Collet JP, Vanasse M. Hyperbaric oxygen for children with cerebral palsy; a randomized multicentre trial. HBO-CP Research Group. Lancet 2001;357:582-86.
59. Heiden CH, Biesinger E, Hoing R. Idiopathic facial palsy (Bell's Palsy): an indication for hyperbaric oxygen therapy? Eur J Underwater Hyper baric Med 2003; 4(1): 20-23.
60. Raèiæ G, Denoble P, Sprem N, Bojiæ L, Bota B. Hyperbaric Oxygen as therapy of Bell,s palsy. Undersea Hyperb Med 1997; 24(1):35-8.
61. TarunSahni, Madhur Jain, S. Hukku, G.K. Jadhav. Role of hyperbaric oxygen therapy in oncology and radiation induced tissue damage: Apollo Medicine, Vol.1, December 2004
62. Weisz G, Lavy A, Adir Y, Melamed Y, Rubin D, Eidelman S, Pollack S et al. Modification of in vivo and in vitro TNF-alpha, IL-1, and IL-6 secretion by

circulating monocytes during hyperbaric oxygen treatment in patients with perianal Crohn's disease. J ClinImmunol. 1997 Mar;17(2):154-9.
63. Benson RM, Minter LM, Osborne BA, Granowitz EV. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. ClinExpImmunol. 2003 Oct;134(1):57-62.
64. G Heuser, SA Heuser, D Rodelander Treatment of neurologically impaired adults and children with mild hyperbaric oxygen (1.38 ATA and 24% oxygen) edited by Joiner JT Flagstaff Arizona:best publications 2002:109-15.
65. Plafki C, Peters P, Almeling M, Welslau W, Busch R. Complications and side effects of hyperbaric oxygen therapy. Aviat Space Environ Med 2000; 71(2): 119-24.
66. Palmquist BM, Philipson B, Barr PO. Nuclear cataract and myopia during hyperbaric oxygen therapy. Br J Ophthalmol 1984; 68(2): 113-7.
67. Lyne AJ. Ocular effects of hyperbaric oxygen. Trans OphthalmolSoc UK 1978; 98(1): 66-8.
68. Bergo GW, Risberg J, Tyssebotn I. Effect of 5 bar oxygen on cardiac output and organ blood flow in conscious rats. Undersea Biomed Res 1988; 15(6): 457-70.
69. Davis JC, Dunn JM, Heimbach RD. Hyperbaric medicine: Patient selection, treatment procedures and side effects. In: Davis JC, Hunt TK, editors. Problem Wounds - the role of oxygen. New York: Elsevier, 1988.

PAP SMEAR FOR SCREENING T. VAGINALIS

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ABSTRACT: BACKGROUND: Trichomonas vaginalis is sometimes seen in Papanicolaou stained smears, but because emphasis is placed on malignant cells in Papanicolaou stained smears, not much is done to search for this parasite in smears. In this study, cervical and vaginal specimens were examined by conventional Papanicolaou method for the presence of Trichomonas vaginalis microscopically. **MATERIALS AND METHODS:** Five hundred high vaginal swabs collected from gynaecology OPD were stained with Papanicolaou stain. **RESULTS:** One hundred and fifty (30%) out of 500 Papanicolaou stained smears screened, were positive for Trichomonas vaginalis. Out of them 76.67% of positive smears were from asymptomatic females. Presumptive diagnosis based on perinuclear halo and complete T. vaginalis had the highest sensitivity of 69.33%, while diagnosis based on perinuclear halo alone was 50.66% and 41.33% for diagnosis based on identification of complete organisms in Pap smear.
KEY-WORDS: Papanicolaou smears, Trichomonas vaginalis, perinuclear halo,

INTRODUCTION: Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract. The prevalence of Trichomonas vaginalis has been reported to be as high as 26% among female STD clinic patients and 22% among HIV-positive women.[1] In infected female patients, symptoms include: vaginal discharge, vulvar pruritis, dysuria, and dyspareunia. Classical green, frothy, foul-smelling discharge occurs in 10% of the women. However, up to 50% of the infected female patients are asymptomatic.[2] In women, trichomoniasis may play a role in development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility.[3] The presence of T. Vaginalis in the vagina increases predisposition to HIV seroconversion. Having trichomoniasis may increase the chance that an HIV infected woman passes HIV to her sex partner(s).[4,5]

Diagnosis of T. vaginalis is an important public health issue, as asymptomatic patients may act as a reservoir for its transmission to their sexual partners. Wet mount preparations had a sensitivity of 40 to 75% while T. vaginalis culture had a sensitivity of 86 to 97%.[6,7] However, expert personnel and laboratory support are required and they are not readily available in a primary care setting.[2] For direct microscopy only fresh specimens are of value.[8] Diagnosis by

wet-mount requires visualisation of viable, motile protozoa; therefore, specimens must be examined immediately. The sensitivity of wet-mount microscopy can be further reduced as a result of delays between specimen collection and examination,[7] and with the large numbers of patients attending gynaecological clinics, an 'on the spot' examination of a vaginal swab is virtually impossible.[9]

Stained smears have the advantage that there can be considerable delay between preparation and staining and examination of a smear without loss of reliability in diagnosis. In primary care setting, the Papanicolaou (PAP) smear is a commonly performed screening test for cervical cancer. Thus, detection of T. vaginalis in PAP smear would mean an additional advantage provided the result is accurate.[2] Trichomonas vaginalis is sometimes seen in PAP smears where it is reported, but because emphasis is placed on malignant cells in PAP smears, not much is done to search for this parasite in smears.[3] Moreover, studies have shown that PAP smear may detect Trichomonas vaginalis in a considerable number of culture-negative women.[10] The aim of this study was to determine the suitability of PAP smear in the detection of Trichomonas vaginalis in cervical and vaginal specimens. [2]

MATERIALS AND METHODS: A total of 500 high vaginal swabs were collected from females in reproductive age group attending at gynaecology OPD. These smears were stained by conventional Papanicolaou method, [11] and screened microscopically for Trichomonas vaginalis. The study was carried in department of microbiology, during the period of August 2010 - August 2011.

PAPANICOLAOU METHOD[11] : Each specimen was smeared on a clean grease free slide and fixed in ether-alcohol for 30 minutes. The specimens were then stained by the Papanicolaou method as follows: Harris's haematoxylin without acetic acid for 5 minutes, rinsed in tap water and differentiated in 1% acid alcohol for 30 seconds and blued in Scott's water for 2 minutes. Smears were taken to 95% alcohol and stained in OG6 for 2 minutes, rinsed in 95% alcohol and stained in EA 35 for 2 minutes. Smears were then taken to two changes of absolute alcohol, xylene and mounted in DPX. The stained smears were examined under the light microscope at low and high power objectives for the presence of Trichomonas vaginalis and perinuclear halo.

Identification of one or more of the following morphological characters was considered to be conclusive of T. vaginalis: a pear-shaped, oval to round, cyanophilic organism that ranges in size from 15-30 microns; pale nucleus, vesicular and centrally located; cytoplasmic eosinophilic granules or flagella. The presence of perinuclear halo in the epithelial cells was also used as a presumptive diagnosis for T. vaginalis. These criteria were also used to distinguish trichomonads from cytoplasmic fragments.

RESULTS: One hundred and fifty (30%) out of 500 PAP smears screened were positive for Trichomonas vaginalis. On retrospective analysis 76.67% of positive smears were from asymptomatic females. Only 23.33% had history of vaginal discharge and pruritis. 25(5%) of 150 positive PAP smears showed Candida infection and all were symptomatic women. See table1

Presumptive diagnosis based on perinuclear halo alone was 50.66% while diagnosis based on identification of organisms alone in PAP smear was 41.33%. When both the morphological characters of Trichomonas vaginalis were given equal consideration for

identification, i.e slide was considered positive when any one or both the characters are present; diagnosis of Trichomonas vaginalis rose to the sensitivity of 100%. See table 2.

DISCUSSION: Papanicolaou is the best staining method in cytology, because it helps to effectively differentiate malignant cells from non-malignant cells. It also stains the cytoplasm and its contents. Its ability to differentiate acidophilic materials from basophilic materials as well as its ability to stain non-cellular substances such as fibrin, crystals and pigments, makes it an essential stain in. T. vaginalis, the causative organism for trichomoniasis is the most common curable sexually transmitted organism worldwide. It parasitizes both males and females where it is sometimes asymptomatic in the early stages of the infection. T. vaginalis infection is said to play a role in the development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility. In our study 76.67% of females in reproductive age group were asymptomatic carriers of T. Vaginalis, and are at risk of developing above said complications until treated in time. There is also epidemiological and experimental evidence that PAP smears are beneficial in detecting infections that are risk factors associated with cervical cancer. It can detect certain viral, bacterial, and fungal infections of the cervix and vagina. Culture is a very sensitive method of detecting T. vaginalis but it is expensive and time consuming.

In the present study very broad criteria were used for the identification of Trichomonas vaginalis in PAP smears, to increase the sensitivity. Twenty three percent of the women with Trichomonas - positive PAP smears had genital symptoms, while 77% of women were asymptomatic carriers of Trichomonas vaginalis. Thus our data suggest that PAP smear may detect Trichomonas vaginalis in a considerable number of asymptomatic women. Steven KF Loo. et al[2] reported that Pap smear could diagnose T. vaginalis infection in 42% of asymptomatic carriers.

The comparison of positive results showed that the highest sensitivity was found when diagnosis was based on perinuclear halo and/or T. vaginalis in PAP smear followed by sensitivity of 50.66% when diagnosis was based on perinuclear halo alone. Presumptive diagnosis based on identification of complete organisms alone in Pap smear was 41.33%. Avwioro O G[3] also reported a similar sensitivity pattern of 65.77%, 52.63% and 42.11% for diagnosis based on both perinuclear halo and T. vaginalis, perinuclear halo alone and complete organisms in PAP smear respectively. In our study, sensitivity for the PAP smear was 100 % which is in comparison with previous studies in the diagnosis of T. vaginalis. The reported sensitivities ranged from 83 to 99%.[12,13]

CONCLUSION: Papanicolaou smears used for routine screening of cervical cancers can also be used for screening T. vaginalis infection in females. Papanicolaou smears can detect trichomoniasis in asymptomatic patients and is suggested to be one of the best screening tools for asymptomatic carriers of T.vaginalis in females. This helps in early detection and treatment of infection.

REFERENCES:

1. Frank Sorvillo, Lisa Smith, Peter Kerndt, Lawrence Ash. Trichomonas vaginalis, HIV and African-Americans. Emerging Infectious Diseases 2001;1(6):927-32

2. Avwioro O G. Diagnosis of trichomoniasis in pap smears; how effective is it. Eur J Exp Bio 2011;1(1):10-13
3. Steven KF Loo, William YM, Tang KK Lo. Clinical significance of Trichomonas vaginalis detected in Papanicolaou smear: a survey in female Social Hygiene Clinic. Hong Kong Med J 2009;15:90-3
4. Cotch MF, Pastorek JG, Nugent RP, Hillier SL, Gibbs RS, Martin DH et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 1997;24:353-60.
5. Moodley P, Connolly C, Sturm AW. Interrelationships among human immunodeficiency virus type 1 infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts. J Infect Dis 2002;185:69-73.
6. Joseph G. Lossick, DO. The diagnosis of vaginal trichomoniasis. J of American Med Assoc 1988;259(8):1230.
7. Thomason JL, Gelbart SM, Sobun JF, Schulien MB, Hamilton PR. Comparison of four methods to detect Trichomonas vaginalis. J Clin Microbiol 1988;26:1869-70.
8. J Sydney and McCann. Comparison of direct microscopy and culture in the diagnosis of trichomoniasis. Brit J vener. Dis 1974;50:450-2.
9. P. R. Mason, Heather Super, and P. J. Fripp. Comparison of four techniques for the routine diagnosis of Trichomonas vaginalis infection J clin Path 1976;29:154-7.
10. C S Petersen, L Carl, D Alnor, U Thomsen, H K Thomsen. Ignored trichomonal infestation diagnosed by Papanicolaou smear. Genitourin Med 1995;71:257-8.
11. Anne M. Specimen preparation. In: John Bancroft, editor. Theory and practice of histological techniques, 5th ed. London: Churchill Livingstone; 2005. p. 87- 105
12. Weinberger MW, Harger JH. Accuracy of the Papanicolaou smears in the diagnosis of asymptomatic infection with Trichomonas vaginalis. Obstet Gynecol 1993;82:425-9.
13. Lara-Torre E, Pinkerton JS. Accuracy of detection of Trichomonas vaginalis organisms on a liquid-based papanicolaou smear. Am J Obstet Gynecol 2003;188:354

Table 1: Prevalence of T.vaginalis in PAP smears

Total number of PAP positive specimens	**150**	**100 %**
Presence of genital symptoms	35	23.33%
Absence of genital symptoms	115	76.67%
Concomitant sexually transmitted disease	25	16.66%

Table 2: Comparison of Morphological features for identification of T vaginalis in PAP smears

Investigation	**No.**	**Sensitivity%**
Total positive for perinuclear halo alone (suggestive of T. vaginalis)	76	59.66
Total positive for complete parasite alone seen in PAP smears	62	41.33
Total positive for both perinuclear halo and complete T. vaginalis	12	8
Total positive for perinuclear halo and complete T. vaginalis: alone or both	150	100

RANDOMIZED CLINICAL TRIAL IN CHIKUNGUNYA ARTHRITIS CASES

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ABSTRACT: Chikungunya virus is no stranger to the Indian sub-continent. Since its first isolation in Calcutta[1] in 1963, there have been several reports of chikungunya virus infection in different parts of India[2], [3], [4]. The last outbreak of chikungunya virus infection occurred in India in 1971. Subsequently there has been no active or passive surveillance carried out in the country and therefore, it 'seemed' that the virus had 'disappeared' from the subcontinent[5] However, recent reports of large scale outbreaks of fever caused by chikungunya virus infection in several parts of Southern India have confirmed the re-emergence of this virus. It has been estimated that over 1,80,000 cases have occurred in India since December 2005 [6] Andhra Pradesh (AP) was the first state to report this disease in December 2005, and one of the worst affected (over 80,000 suspected cases) . Over 12% of patients who contract chikungunya virus infection develop chronic joint symptoms [7]. **OBJECTIVE:** To test the efficacy of chloroquine in reducing the pain of chikungunya induced arthritis as compared to paracetamol. **METHODOLOGY:** A Randomized Clinical Trial was carried out in a community attached to urban health centre of PESIMSR, Kuppam during August 2006. Among the 132 cases of arthritis, 86 persons were selected based on their availability and consent to participate. They were divided into two randomly assigned groups namely Category–1(Chloroquine group) and Category–2 (Paracetamol group). Chloroquine tablet -155 mg and Paracetamol tablet - 500 mg were administered as a single dose to the two groups respectively. The groups were followed up for 8 days and the results were analyzed. **STATISTICAL ANALYSIS:** Analysis was carried out by using S.P.S.S. package. Asymptoic test statistic and X^2 MH (Chi square test) were used to evaluate the effect of the drugs. **RESULTS OF THE STUDY:** The decrease of pain in chikungunya arthritis cases was significant in the mild and moderate pain categories with 'p' values of 0.0117 and 0.0129 respectively.

Asymptoic test statistic was 1.70 for chloroquine group and χ^2MH was 2.76 ('P' value between 0.05 to 0.1).OR= 48.59.Incidence Risk Ratio for chloroquine was 1.52 with CI ; 1.14 – 1.90.The efficacy of chloroquine in reducing pain in arthritis was 51.83% (effect size).The logistic equivalents of odds for chloroquine and paracetamol group were 0.41 and 0.02 respectively in logistic regression analysis. **CONCLUSION:** Choloroquine is more efficient in reducing the pain of chikungunya arthritis as compared to paracetamol in both sexes and in all age groups.

KEY WORDS: Chikungunya, Arthritis, Clinical Trial, Chloroquine.

INTRODUCTION: Chikungunya virus is no stranger to the Indian sub-continent. Since its first isolation in Calcutta[1] in 1963, there have been several reports of chikungunya virus infection in

different parts of India[2], [3], [4]. The last outbreak of chikungunya virus infection occurred in India in 1971. Subsequently there has been no active or passive surveillance carried out in the country and therefore, it 'seemed' that the virus had 'disappeared' from the subcontinent[5] However, recent reports of large scale outbreaks of fever caused by chikungunya virus infection in several parts of Southern India have confirmed the re-emergence of this virus. It has been estimated that over 1,80,000 cases have occurred in India since December 2005 [6] Andhra Pradesh (AP) was the first state to report this disease in December 2005, and one of the worst affected (over 80,000 suspected cases) . Over 12% of patients who contract chikungunya virus infection develop chronic joint symptoms [7].

Chikungunya arthritis is a crippling and chronic sequel of chikungunya fever causing a lot of pain and distress to the patients.

Chloroquine is a disease modifying, non steroidal drug known also to have anti-rheumatic effect[8] . The therapeutic dose of chloroquine is 150 mg. It has a half-life of 4 days and the therapeutic effect is said to last upto 16 days after cessation of treatment. It is a safe drug in this dose as it has been extensively used in malaria treatment.

Only one previous study of using chloroquine in patients with chikungunya arthritis has been documented earlier [9]

The present study was carried out to test the efficacy of chloroquine in reducing the pain of chikungunya arthritis patients as compared to paracetamol.

MATERIALS AND METHODS: A randomized double blind clinical trial was conducted in a community attached to the urban health centre of PESIMSR, Kuppam . The study was conducted in the post epidemic period of chikungunya during August 2006.

STUDY POPULATION: The study population constituted the patients having post chikungunya arthritis. A door to door survey was done by health workers to check for these cases in the entire community. The investigators screened all patients and selected 86 arthritis patients with the following criteria.

INCLUSION CRITERIA: Only those subjects with post chikungunya arthritis.

EXCLUSION CRITERIA:

1. Those subjects not giving their consent to participate in the study.
2. Known cases of rheumatoid arthritis /osteo arthritis /other degenerative diseases.

RANDOMIZATION AND ALLOCATION CONCEALMENT: The drugs chloroquine and paracetamol were packed in brown envelopes as a single dosage form separately in 86 sealed covers. These covers were having 43 dosage forms of chloroquine and paracetamol each. The 86 packs were given numbers from a random number table. Thus 86 randomized packs were prepared. The contents of the covers were not noted on the cover. These covers were taken to the field and were administered to the patients recruited on a sequential basis. Thus allocation concealment was done. Thus two groups of patients, one receiving the white capsule and the other receiving the red capsule were formed.

All patients were followed up by investigators for a period of 8 days after initial clinical assessment.

MATERIALS:

1) A pretested proforma and follow up record.
2) Visual analogue scale to assess the pain reduction.
3) Drugs : Chloroquine 150 mg base filled in a white gelatin capsule and paracetamol 500 mg filled in a red gelatin capsule of the same type.

DOSAGE ADMINISTRATION: After formation of the groups the white capsule group was given chloroquine and the red capsule group was given paracetamol as a daily single dose. Directly observed, supervised treatment was administered.

QUALITY CONTROL: The investigators had several meetings along with the patients and had a common clinical assessment agreement. Thus inter and intra observer variation was minimized.

The random number allocation for packets was done by the statistician who was not participating in analysis. The key for the packets were with the same statistician.

EVALUATION OF PATIENTS: Only one end point, viz., the reduction of pain was assessed by the investigators using visual analogue scale. The monitoring of patients was done by the investigators who initially assessed them.

Out of 132 cases of arthritis, 86 persons were selected based on their availability and consent to participate. This study group was divided into 2 randomly assigned groups namely Category – 1(Chloroquine group) and Category – 2 (Paracetamol group). Informed consent was taken from all the respondents.

The trial was double blind and the first author had the key. The two groups were similar in all aspects. Chloroquine tab – 155 mg single dose was given to the chloroquine group and Paracetamol tab – 500 mg single dose was given to the paracetamol group.

The pain was classified into severe, moderate and mild as per the expression of patients. The relief of pain was graded into reduction by 25%, 50%, 75% and above 75% as expressed by patients. The patients were followed up for 8 days and the results were recorded.

STATISTICAL ANALYSIS: Statistical analysis was done by using the SPSS package. Analysis was done on risk approach basis. Asymptoic test and χ^2 (chi square test) with MH treatment were used to evaluate the effect of the drugs.

RESULTS: Response to treatment was favourable for chloroquine irrespective of the degree of pain. The decrease of pain in chikungunya arthritis cases was significant in mild and moderate categories with 'p' values of 0.0117 and 0.0129 respectively.

Increasing age had no effect on the relief of the pain ('r' was not significant). There was no difference in the relief of pain in both sexes with increasing age.

It was observed that the Incidence Risk Ratio for chloroquine was 1.52 with CI ; 1.14 – 1.90.

The efficacy of chloroquine in reducing pain in arthritis was 51.83% (effect size).

The decrease of pain in the chloroquine group was 95.34% and in the paracetamol group it was 62.79% giving a risk difference of 32.55.

In the choloroquine group, the median age was 40.2 and the inter-quartile range was 31.7 to 45.5

In the paracetamol group, the median age was 45.4 and the inter-quartile range was 35.4 to 56.5. No significant difference was seen between the two groups.

The proportion of mild, moderate, and severe pain categories was almost similar in both the groups. The sex ratio of both the groups was also similar.

The logistic equivalents of odds for chloroquine and paracetamol groups were 0.41 and 0.02 respectively.

Results of standardized analysis : OR= 48.59

χ^2_{MH} = 2.76; Asymptoic test statistic = 1.70.

'P' value lies between 0.05 to 0.1.

DISCUSSION: In this study it has been observed that the drug chloroquine is quite efficient in reducing the pain of chikungunya arthritis. Though paracetamol also helps in reducing the pain it is less effective than chloroquine in this regard. These findings are in conformity with an earlier study [8] where it was observed that the symptoms of chikungunya arthritis improved significantly following chloroquine treatment.Very few studies have been reported on the use of chloroquine in chikungunya arthritis cases and these results justify further controlled blind trials of chloroquine in their treatment .

CONCLUSION: Choloroquine is more efficient in reducing the pain of chikungunya arthritis as compared to paracetamol in both sexes and all age groups.

LIMITATIONS: The number of severe cases included in the trial was less. Therefore, the adequacy of chloroquine in reducing the pain in these circumstances cannot be assumed. A larger number of severe cases need to be included in future trials. The dose of chloroquine to be given in severe cases also requires further study.

REFERENCES:

1. Shah KV, Gibbs CJ Jr, Banerjee G. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: isolation of three strains of Chikungunya virus. Indian J Med Res 1964; 52 :676-83.
2. Jadhav M, Namboodripad M, Carman RH, Carey DE, Myers RM. Chikungunya disease in infants and children in Vellore: a report of clinical and haematological features of virologically proved cases. Indian J Med Res 1965; 53:764-76.
3. Dandawate CN, Thiruvengadam KV, Kalyanasundaram V, Rajagopal J, Rao TR. Serological survey in Madras city with special reference to chikungunya. Indian J Med Res 1965; 53 :707-14.
4. Thiruvengadam KV, Kalyanasundaram V, Rajgopal J. Clinical and pathological studies on chikungunya fever in Madras city. Indian J Med Res 1965; 53 :729-44.
5. Pavri K. Disappearance of Chikungunya virus from India and South East Asia. Trans R Soc Trop Med Hyg 1986;80:491.
6. Enserink M. "Massive outbreak draws fresh attention to little-known virus". Science 2006; 311 :1085.
7. CDC. Chikungunya Fever in India. Travelers' Health Outbreak Notice April 21,

2006.http://www.cdc.gov/travel

8. Brighton SW, Simson IW. A destructive arthropathy following chikungunya virus arthritis: a possible association. Clin Rheumatol 1984; 3(2) : 253 – 8

9 Brighton SW. Chloroquine phosphate treatment of chronic chikungunya arthritis: an open pilot study. S Afr Med J 1984; 66(6):217 – 8.

Table – 1: Response to Chloroquine vs Paracetamol :

Arthritis	Chloroquine group		Paracetamol group	
	Cured	Not cured	Cured	Not cured
Mild	27	02	20	10
Moderate	12	00	05	06
Severe	02	00	02	00
TOTAL	**41**	**02**	**27**	**16**

Table – 2: The response to Chloroquine in reduction of pain vs Paracetamol with age and sex.

Age	Male	Female
	Cured	Cured
0 – 20	2	0
20 – 30	4	1
30 – 40	14	4
40 – 50	12	12
50 – 60	06	03
60 – 80	03	07
TOTAL	**41**	**27**

Table – 3: Risk Analysis

	Chloroquine Vs Paracetamol
Risk difference	32.55
IRR	1.52
C.I. with 95% Confidence limits	(1.14 – 1.90) 0.2 to 0.05
Efficacy of chloroquine	51.83

Fig – 1: Comparison of age among Chloroquine and Paracetamol groups:

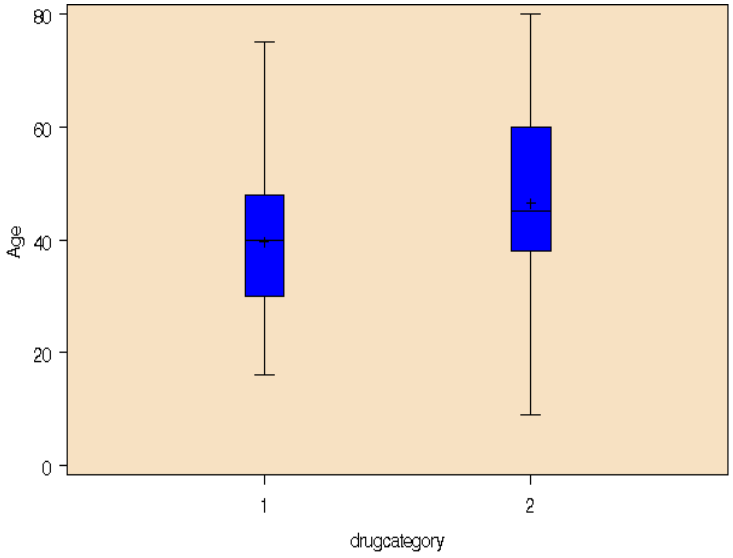


Fig – 2 : Category-wise distribution of cases

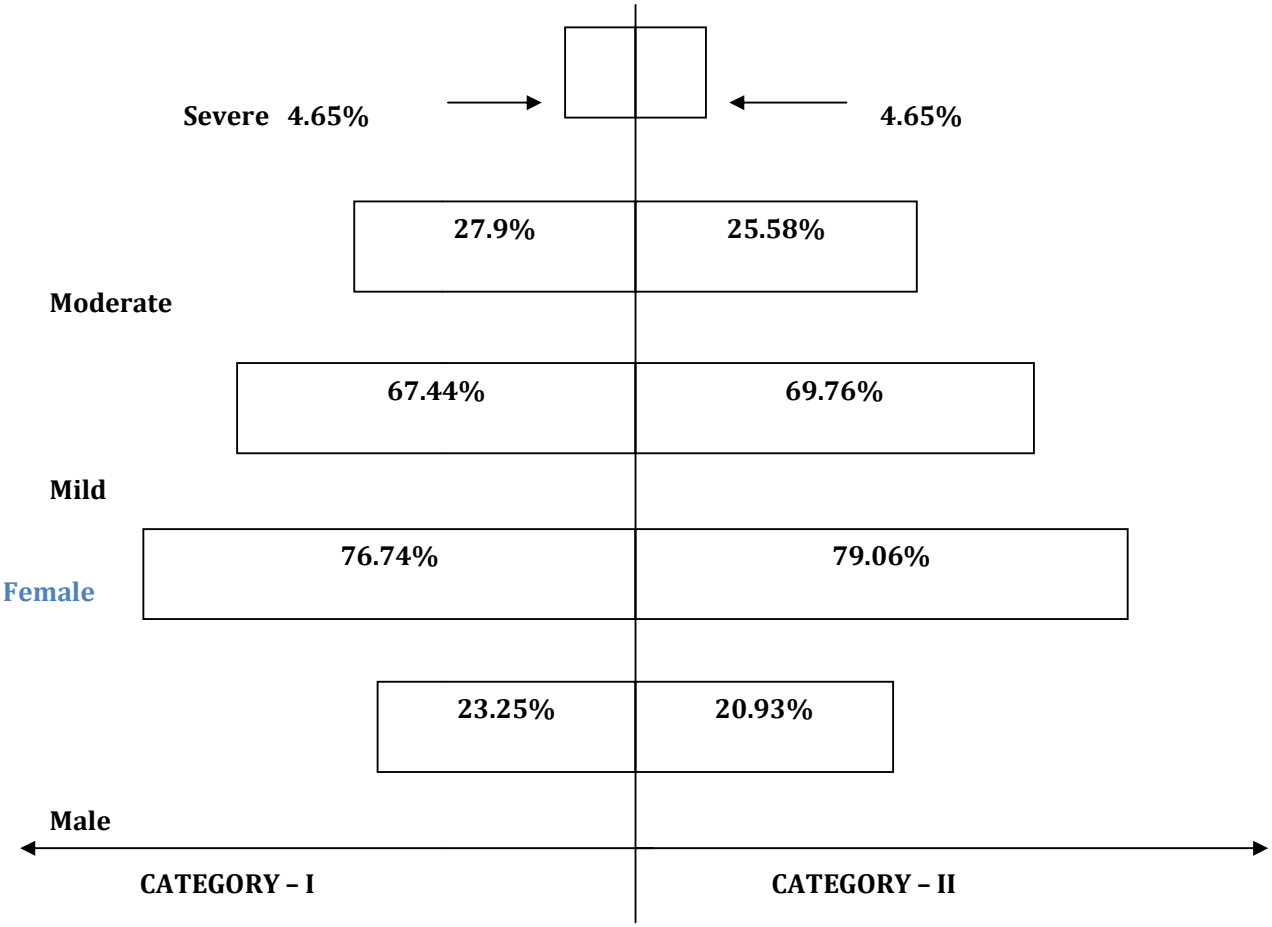
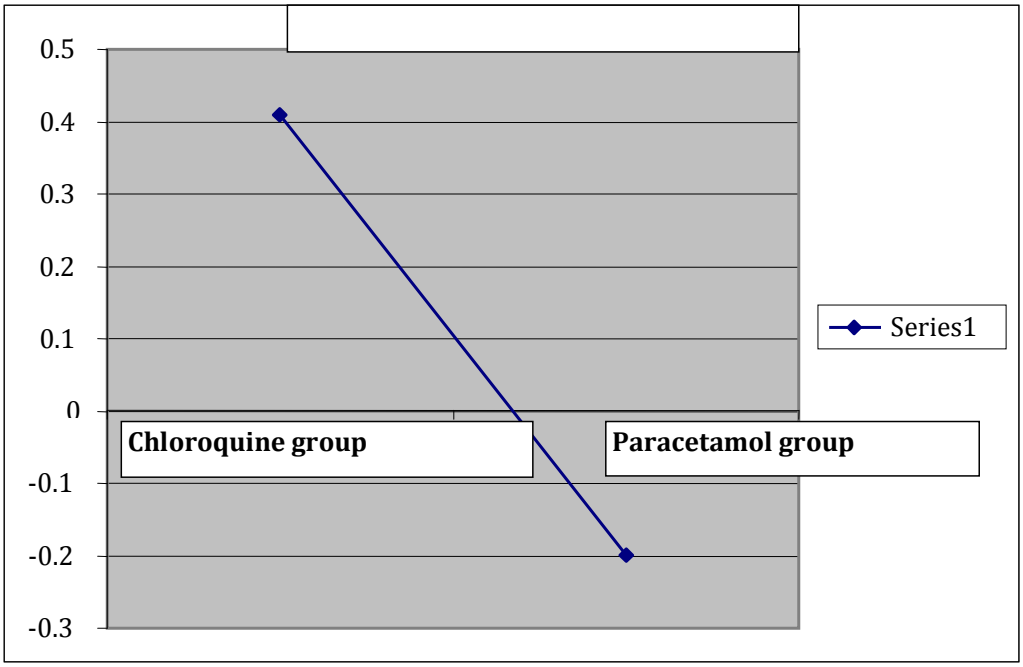


Fig 3 : **Logistic Regression Analysis for Chloroquine Vs Paracetamol**



PRIMARY LARYNGEAL CANDIDIASIS WITH TUBERCULOSIS MIMICKING LARYNGEAL NEOPLASM

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ABSTRACT: A 50 -year-old man presented with hoarseness of voice, cough with expectoration for one month and severe dyspnoea from 15 days. C T Scan of the neck revealed soft tissue lesion circumferentially involving supra-glottic region with effacement of bilateral pyriform fossa predominantly on left side. Extralaryngeal spread to adjacent paralaryngeal space was also seen on left side. Marked airway luminal compromise was seen at the level of false vocal cord . Primary diagnosis of laryngeal malignancy was considered on findings revealed by CT scan neck. Direct laryngoscopy revealed erythema and ulcer with white pus discharge on left false vocal cord. A swab was taken from ulcer on left false vocal cord and sent for microbiological examination. The microbiological findings confirmed Mycobacterium tuberculosis with Candida albicans.. **CONCLUSION:** Primary candidiasis with tuberculosis in the laryngeal mucosa is a rare entity.
The clinical and pathological presentations of laryngeal candidiasis and tuberculosis might be confused with those for malignant lesions if extralaryngeal spread is seen. Potential pitfalls in diagnosis and the importance of microbiological examination in rare case of dual superimposed primary laryngeal infections has been outlined.
KEYWORDS: Primary Laryngeal Candidiasis, Tuberculosis, mimicking, Laryngeal malignancy

INTRODUCTION: Primary candidiasis with tuberculosis in the laryngeal mucosa is a rare entity. The clinical and pathological presentations of laryngeal candidiasis and tuberculosis might be confused with those for benign or malignant lesions. We are presenting a case in which primary diagnosis of laryngeal malignancy was considered by findings revealed on CT scan neck but laryngoscopic and microbiological evaluation revealed and confirmed laryngeal infection i.e Primary Laryngeal Candidiasis with Tuberculosis. We have outlined potential pitfalls in diagnosis, and highlight the importance of evaluating microbiologically in rare case of dual superimposed primary laryngeal infections.

CASE REPORT: A 50 -year-old man presented with hoarseness of voice, cough with expectoration for one month and severe dyspnoea from 15 days. The patient has medical

history of hypertension. Physical examination was otherwise unremarkable. Chest x-ray showed no evidence of pulmonary nodules, consolidation, or infiltrates.

Soft tissue computed tomography of the neck revealed soft tissue lesion circumferentially involving supra-glottic region with effacement of bilateral pyriform fossa predominantly on left side. Extralaryngeal spread to adjacent paralaryngeal space was also seen on left side(Fig1). Marked airway luminal compromise was seen at the level of false vocal cord (Fig 2). Few sub centimeter lymph nodes were seen in bilateral submandibular region.

Direct laryngoscopy revealed erythema and ulcer with white pus discharge on left false vocal cord(Fig 3). Swab was taken from ulcer on left false vocal cord and sent for microbiological culture and sensitivity. On Gram staining, Gram positive budding Yeast cells of Candida albicans were noted. On Ziehl Neelsen staining red coloured acid fast bacilli against blue background with budding yeast cells were seen with 20% Sulphuric acid (Fig 4). These microbiological findings confirmed Mycobacterium tuberculosis with Candida albicans.

Patient received a course of 6 weeks fluconazole antifungal with 6 months of antitubercular therapy. Hoarseness resolved, and there has been no evidence of recurrence during the subsequent 24 months.

DISCUSSION: Primary laryngeal candidiasis along with tuberculosis is rare entity and can seldom be diagnosed clinically or radiologically . However, infective differential diagnosis may be kept if diffuse submucosal involvement is seen on soft tissue CT scan. Mycotic infections of the larynx are frequently seen in patients with immune insufficiency although they have also been reported in individuals with normal immune status[1,2,3]. Candidiasis is frequently seen in patients with immune deficiency syndromes or diabetes mellitus or those receiving immunosuppressive treatment[3,4] and in individuals whose mucosal barriers are affected by excessive use of antibiotic or inhaled corticosteroids[5,6] radiation therapy, smoking, trauma, gastro esophageal reflux or chemical–thermal damage[4]. Laryngeal candidiasis can present with various symptoms, such as odynophagia, dysphagia, hoarseness, respiratory distress and stridor[7,8].Similarly laryngeal tuberculosis is almost always associated with pulmonary tuberculosis[9,10,11].

In our patient soft tissue computed tomography of the neck showed soft tissue lesion circumferentially involving supra-glottic region. Effacement of bilateral pyriform fossa with predominant involvement on left side was noticed. Extralaryngeal spread to adjacent paralaryngeal space was seen on left side. Marked airway luminal compromise was seen. Laryngeal malignancy was considered as primary diagnosis as per findings.

The radiological findings of laryngeal tuberculosis depend on the stage and extension of lesion .In the infiltrative stage, there is focal thickening. In the ulcerative stage, the ulceration is not deep and rarely reaches the paraglottic spaces and the cartilage. Perichondritis is sometimes noted (epiglottis, arytenoids), but calcifications are not common and the para-laryngeal fat spaces are usually spared. The last stage is characterized by sclerosis. Various radiological findings that have been described include edema alone, an ulcero-infiltrative mass, infiltrative and pseudo-tumoral appearance (66%); sub-glottic laryngitis (isolated swelling of the aryepiglottic fold or even massive cartilaginous ulceration and, sometimes, chondritis or perichondritis); diffuse form; and tuberculoma (enormous ventricular vegetation with a large base that elevates the ventricular strip)[12,13].

Previous studies showed that sparing of paralaryngeal spaces were suggestive of infective etiology[14,15]. However , in our case despite of infectious etiology paralaryngeal involvement was seen .

Laryngeal erythema and ulcer with white pus discharge on left false vocal cord was seen in our patient on laryngoscopy. The laryngoscopic appearance of candidiasis and tuberculosis generally includes oedema, erythema, ulceration, white plaque and pseudomembranous formations[5].

SUMMARY: Characteristic CT findings of laryngeal tuberculosis include bilateral involvement, thickening of the free margin of the epiglottis, and extensive mucosal involvement. By comparison, laryngeal carcinoma present as unilateral involvement, infiltration of the pre epiglottic and paralaryngeal fat spaces by a submucosal mass, cartilage destruction, and extralaryngeal invasion. So due to paralaryngeal fat involvement malignancy was considered primary diagnosis in our case. Further evaluation is must before reaching to final diagnosis and starting treatment. In our case provisional diagnosis of malignancy was made as per radiological findings but confirmatory diagnosis was made after microbiological evaluation and patient was treated accordingly.

Conflicting Interests: The authors have no conflicting interests.

REFERENCES:

1. Makitie AA, Back L, Aaltonen LM, et al: Fungal infection of the epiglottis simulating a clinical malignancy. Arch Otolaryngol Head Neck Surg 2003;129: 124 – 126.
2. Pabuççuog˘lu U, Tuncer C, Sengiz S: Histo-Pathology of candidal hyperplastic lesions of the larynx. Pathol Res Pract 2002; 198: 675 – 678.
3. Haberman RS, Becker ME, Ford CN: Candida epiglottitis. Arch Otolaryngol 1983; 109: 770 – 771.
4. Forrest LA, Weed H: Candida laryngitis appearing as leukoplakia and GERD. J Voice 1998; 12: 91 – 95.
5. DelGaudio JM: Steroid inhaler laryngitis: Dysphonia caused by inhaled fluticasone therapy. Arch Otolaryngol Head Neck Surg 2002;128: 677 – 681.
6. Gallivan GJ, Gallivan KH, Gallivan HK: Inhaled corticosteroids: Hazardous effects on voice—An update. J Voice 2007; 21: 101 – 111.
7. Tashjian LS, Peacock JE: Laryngeal candidiasis. Arch Otolaryngol 1984; 110: 806 – 809.
8. Ganesan S, Harar RP, Dawkins RS, et al: Invasive laryngeal candidiasis: A cause of stridor in the previously irradiated patient. J Laryngol Otol 1998; 112: 575 – 578.
9. Sá LC, Meirelles RC, Atherino CC, Fernandes JR, Ferraz FR: Laryngo-pharyngeal Tuberculosis. Braz J Otorhinolaryngol 2007;73:862–866.
10. Kozakiewicz J, Dec M, Gorczyca-Tarnowska J: The rare case of primary isolated tuberculosis in a 19 year-old patient. Otolaryngol Pol. 2006;60: 607–709.
11. Loehrl TA, Smith TL: Inflammatory and granulomatous lesions of the larynx and pharynx. Am J Med. 2001;111:113–117.
12. Aspestrand F, Kolbenstvedt A, Boysen M: CT findings in benign expansions of the larynx. J Comput Assist Tomogr. 1989;13:222–225.

13. Jan A: Primary laryngeal tuberculosis (a case report) J Laryngol Otol. 1986;100:605–606.
14. Kim MD, Kim DI, Yune HY, Lee BH, Sung KJ, Chung TS, Kim SY: CT Findings of Laryngeal Tuberculosis: Comparison to Laryngeal Carcinoma. Journal of Computer Assisted Tomography. 1997; 21 : 29-34.
15. Jae-YL, Kwang-MK, Eun CC, Young-HK, Han SK ,Hong-SC: Current clinical propensity of laryngeal tuberculosis: review of 60 cases . European Archives of Oto-Rhino-Laryngology .2010;263:269.

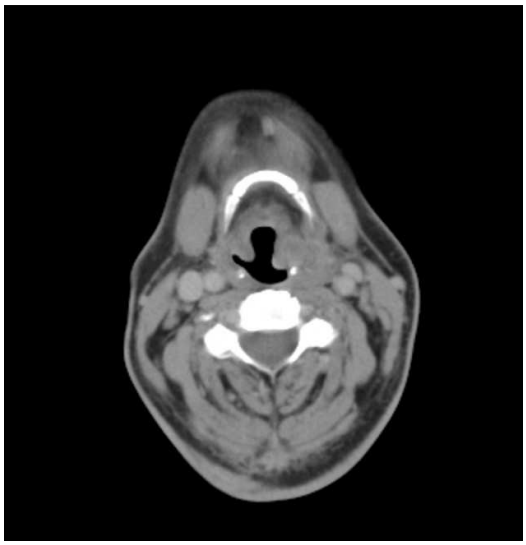

Figure 1: CT Scan neck shows soft tissue lesion circumferentially involving supra glottic region. Effacement of bilateral pyriform fossa with predominant involvement on left side noticed. Extralaryngeal spread to adjacent paralaryngeal space seen on left side.

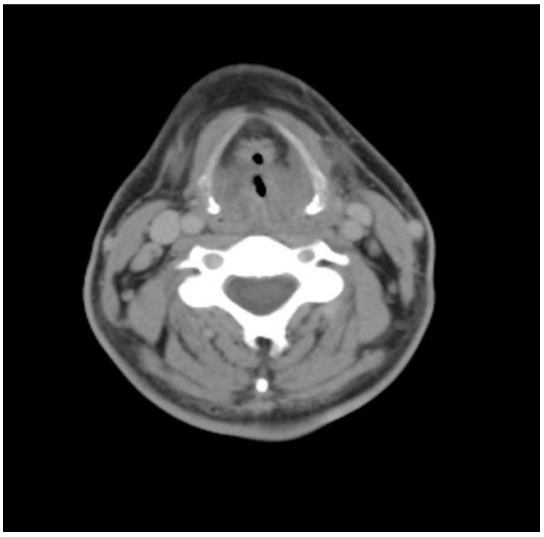

Figure 2: CT neck shows circumferential soft tissue infiltration at the level of false vocal cord. Marked airway luminal compromise also seen.

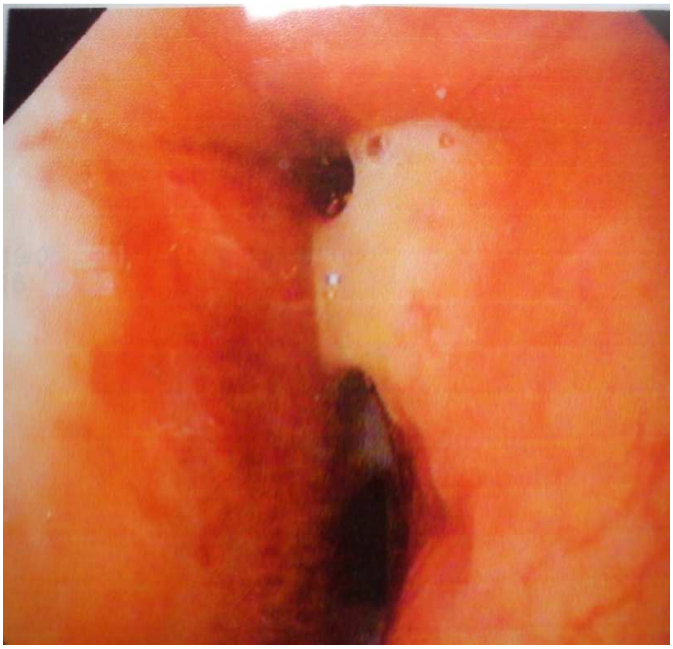

Figure 3: Direct laryngoscopy showing erythema and ulcer with white pus discharge on left false vocal cord.

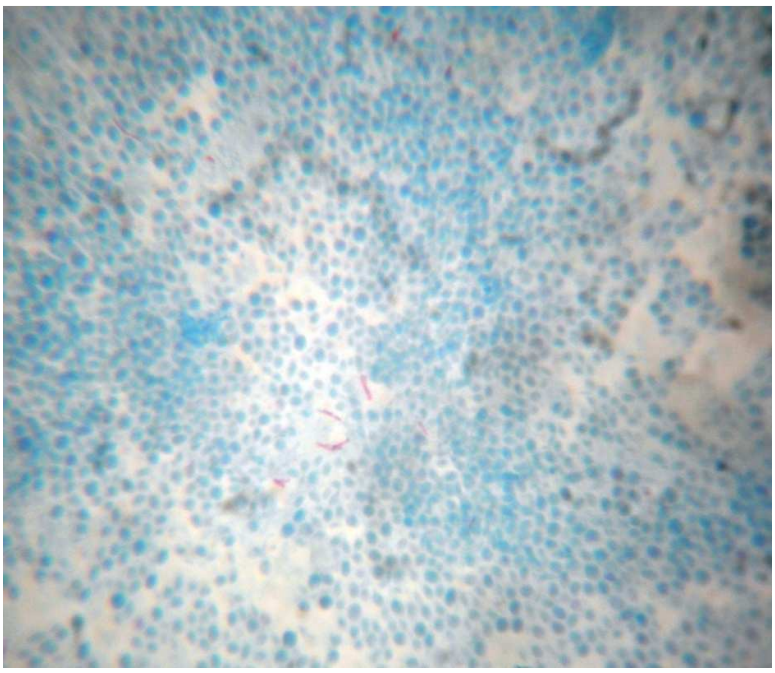

Figure 4: Ziehl Neelsen Staining (100x) : Acid Fast Bacilli with budding yeast cells.

DENGUE IN AND AROUND NAGPUR- CENTRAL INDIA

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ABSTRACT: Dengue infection has been known to be endemic in India for over the centuries as a benign and self limiting disease. Dengue epidemics had been regularly reported from north India but no reports are available from central India. Here we report the data of dengue epidemics from March 2008- June 2010 from Nagpur region (Central India). A total of 289 serum samples from clinically suspected cases of dengue fever admitted in GMCH, Nagpur, over a period of 3 years (Mar 2008-June 2010) were tested for dengue specific IgM antibodies using IgM capture ELISA (MAC ELISA). Amongst a total of 289 serum samples tested for dengue IgM antibodies, 85(29.41%) were positive for dengue IgM antibodies. Children <10 yrs were most commonly affected with male predominance. Maximum number of cases occurred in the year 2009. Although the epidemiology of dengue is changing with dengue serotype 3 as an emerging serotype, dengue infections are seen every year, thus making it as an epidemic disease in Central India also.

KEY WORDS: Dengue, IgM capture ELISA, DF (Dengue fever), DHF (Dengue haemorrhagic fever).

INTRODUCTION: Dengue infection has been known to be endemic in India for over the centuries as a benign and self limiting disease.[1] In recent years, the disease has changed its course manifesting in severe form as dengue hemorrhagic fever and with increasing frequency of outbreaks.[3] Dengue is emerging as a major health problem in India. Since the first epidemic in Kolkata during 1963-64, many places in India have experienced dengue infection. Delhi has experienced seven outbreaks of dengue virus since 1967 with the last reported in 2003. All four serotypes are circulating and cause epidemics. Earlier serotype 2 was implicated as the etiology of major epidemics in Delhi in the year 1996 and Gwalior in 2001[2] .The implication of dengue serotype 3 as an etiology of DHF epidemic recently confirms re-emergence of serotype 3 as a dominant form on the Indian subcontinent [4]. Similar outbreak of dengue in Mumbai in 2003[5] has been reported. Dengue epidemics had also occurred in central India. Here we report the data of dengue epidemics from March 2008- June 2010 from Nagpur region (Central India).

MATERIAL AND METHODS: A total of 289 serum samples from clinically suspected cases of dengue fever classified as undifferentiated fever/ dengue fever/ DHF (WHO classification

) admitted in GMCH, Nagpur, over a period of 3 years (Mar 2008- June 2010) were tested / for dengue specific IgM antibodies.

The samples were screened for the presence of IgM antibodies using IgM capture ELISA (MAC ELISA) supplied by NIV Pune. OD was measured at 450nm using ELISA reader.

RESULTS: During the study period (2008-2010), a total of 289 serum samples were tested for dengue IgM antibodies, by ELISA test.

Year wise distribution of samples being 52 in 2008, 223 in 2009 and 14 in 2010. Of these 85(29.41%) were positive for dengue IgM antibodies. Year wise distribution of dengue IgM positive over 3 years period is shown in table 1.

Maximum number of samples were received in the year 2009. There was an increase in the number of samples in the monsoon season (August to November). Overall males predominated over females (1.65:1). Age wise distribution of IgM positive cases in all three year clearly indicates that children (<10 yrs) were more commonly affected (table 2). Fever was the commonest presentation (98%). 30 % of the patients had hemorrhages & 4% had altered sensorium. In 76% of patients, the platelet count was between 5,0000 /mm^3-10,0000/ mm^3, while only in 24% of patients it was less than 5,0000/mm^3.

DISCUSSION: Dengue is emerging as a great burden in our country. All 4 types of dengue virus have been isolated from affected Indian population. Cyclical epidemics of dengue are becoming more frequent. The outbreak in 1996 was the largest one to occur in Delhi [6] following which vigorous steps were taken to prevent and control DF/ DHF. Most of the patients in the present study were children (<10 years) as compared to the older age group (>10Yrs). This observation is quite in accordance to the studies reported from south India[5, 7] . However studies from Delhi[6,8] found out the adults to be more susceptible to infection than children. Erythematous rash was found in 8% of the patients. This percentage is lower than previously reported dengue fever epidemics in Delhi[9]. Bleeding from various sites /hemorrhage was found in 30% of cases. A similar high percentage of patients with bleeding manifestations was found during 1998 and also in the year 2005. The causes of bleeding in DF are not well established, but could be due to thrombocytopenia, consumption coagulopathy , capillary fragility or platelet dysfunction. Although 24% of cases shows significant thrombocytopenia (<50000/mm^3). No correlation could be established between the platelet count or bleeding manifestations and hence it indicated other features contributing to the bleeding diatheses. Since no platelet function tests or coagulation profile was available, the exact cause could not be elucidated. Dengue specific IgM antibodies were found in 29.41% which is comparable with other studies [2,10]. The sensitivity of this test depends on the duration of prodromal illness. The disadvantage of MAC ELISA is the delayed appearance of antibodies from 5-10 days after the onset of illness in case of primary dengue virus infection and 4-5 days in secondary infection. The requirement of paired sera, subsequently in convalescent phase, if negative in acute phase also delays diagnosis. Detection of NS1 antigen assays hold promise in early diagnosis in dengue infection [11] .

CONCLUSION: Although the epidemiology of dengue is changing with dengue serotype 3 as emerging serotype, dengue infection are seen every year, thus making it as an epidemic disease in central India also. Appropriate investigation, strict monitoring and

prompt supportive management can reduce mortality of dengue. Detection of NS1 antigen assays hold promise. Also prevention of transmission by mosquito control & maintaining water sanitation is required to effectively control dengue epidemic.

REFERENCES:

1. Broor S, dar L, Sengupta S, Chakraborty M, Wali J P, Biswas A, Kabra S K, Jain Y, Seth P. Recent dengue epidemic in Delhi, India. In Factors in the emergence of arbovirus diseases, edited by: saluzzo JE, Dode B, Paris : Elsevier 1997, 123-27.
2. Bandopadhyay S, Jain D C, Datta K K. Reported incidence of Dengue/ Dengue hemorraghic fever in India 19991-95. Dengue Bulletin 1996, 20:33-34.
3. Dar l, Broor S, Sengupta S, Xess I, Seth P. The first major outbreak of dengue hemorrhagic fever in Delhi, India. Emerg Infect Dis 1999, 5:589-90.
4. Dash R K, Saxena P, Abhyankar A, Bhargava R, Jona A M. Emergence of dengue virus type 3 in northern India. Southeast Asian J Trop Med Public Health, 2005 36:370-77.
5. Shah I, G C Deshpande, P N Tardeja. Outbreak of dengue in Mumbai and predictive markers for dengue shock syndrome. Journal of Tropical Paediatrics 2004, 50:301-05.
6. Anuradha S, Singh N P, Rizvi S N, Agrawal S K, Gur R, Mathur MD. The 1996 outbreak of Dengue hemorraghic fever in India. Southeast Asian J Trop Med Public Health 1998, 29: 503-06.
7. Vijaykumar T S, Chandy S, Sathish N Abraham M, Abrabham P, Sridharan G. Is dengue emerging as a major health problem?. Indian J Med Res 2005, 121:100-07.
8. Singh N P, Raja Jhamb, S K Agrawal, M Gaiha, Richa Diwan, M K Daga, anita Chakravarti, Satish Kumar. The 2005 outbreak of dengue fever in Delhi, India. Southeast Asian J Trop Med Public Health,2005,Sep;36(5):1174-78.
9. Sharma S, Sharma S K, Mohan. Clinical profile of dengue haemorragic fever in adults during 1996 outbreak in Delhi ,India, Dengue Bull 1998;22:20-27.
10. Gera C, Singh A, Jose W, Dhanoa J. Dengue outbreak 2006 in Ludhiana, Punjab. The Indian Practitioner 2 009; 62:703-09.
11. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. IJMM 2010; 28:107-10.

Table 1 YEAR WISE DISTRIBUTION OF DENGUE CASES

Year	Total cases	Positive
2008	52 (17.99%)	04 (7.69%)
2009	223 (77.16%)	80 (35.87%)
2010	14 (04.85%)	01 (07.14%)
Total	289	85

Table 2 AGE WISE DISTRIBUTION OF MALES AND FEMALES

Age	Male	Female	Positive male	Positive female
0-10 yrs	107	83	42 (39.25%)	28 (35.8%)
11-20 yrs	27	17	06 (22.22%)	04 (23.5%)
21-30 yrs	16	03	02 (12.50%)	01 (33.3%)
31-40 yrs	20	03	01 (05%)	00
41-50 yrs	07	02	01 (14.29%)	00
>51 yrs	03	01	00	00
Total	180	109	52	33

EMERGING TRENDS IN THYROID DISEASES IN TSUNAMI HIT COASTAL AREAS OF PUDUCHERRY AND CUDDALORE, INDIA.

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ABSTRACT: BACKGROUND: Thyroid diseases are major global health problem but the incidence and prevalence of this varies from place to place. The government of India has started universal iodization of salt owing to the huge burden of thyroid diseases in the country. In 2004, the coastal regions of Puducherry and Cuddalore were flooded by tsunami. **AIMS:** The aim of this study is to determine the prevalence of thyroid diseases in coastal regions of Puducherry and Cuddalore. We also studied the trends in prevalence of thyroid diseases after tsunami. **MATERIALS AND METHODS:** All the patients undergoing thyroidectomy in a tertiary care teaching hospital in South India were included in our study. Histopathological examination of thyroidectomy specimens was performed. **RESULTS:** A total of 342 thyroidectomy specimens were studied. Of the 342 cases, 30 were males and 312 were females with a ratio of 1: 10.4. Out of 342 cases, 169 (49.42 %) were non neoplastic lesions, 173 (50.58%) were neoplastic. Of the 342 cases, 118 (34.5%) cases were simple goiter, 98 (28.66%) were adenoma, 70 (20.47%) were papillary carcinoma, 42 (12.28%) were thyroiditis, 9 (2.63%) were toxic goiter and 5 (1.46%) were follicular carcinoma. We observed an increasing trend in the prevalence of papillary carcinoma and thyroiditis after tsunami in 2004. **CONCLUSIONS:** There is an urgent need to establish quality assurance system to monitor iodine content in the soil, food, water and salt in the tsunami affected coastal areas. It is also necessary to study the other possible causes for papillary carcinoma and take measures to prevent it.

KEY WORDS: Thyroid, Emerging trends, Histopathology, Tsunami

INTRODUCTION: Thyroid diseases are a major health problem both in developed and developing countries. Their incidence varies from 4 – 7% of the population.[1-4] They are more in hilly terrain and away from the sea coast.[2,5-7] The prevalence of thyroid disease is 1 – 7% of females in UK and 4% of the population in US.[8,9] In India, thyroid diseases are endemic in many parts of Goa, Gujarat, Kerala, and the Himalayan region. The principal diseases of thyroid are simple goiter (diffuse and nodular), hyperthyroidism, hypothyroidism, thyroiditis (Hashimoto thyroiditis, granulomatous, sub-acute lymphocytic thyroiditis) and neoplasms.[2] Colloid goiter affects more than 200 million individuals throughout the world.[1, 2] FNAC is the

most cost effective method for diagnosis of thyroid disease.[4,9-11] Histopathology is used for the final diagnosis when FNAC is inconclusive.[4,9]

Thyroid diseases cause concern because 5 – 10% of them are malignant.[1, 3, 9] Exact causes of thyroid neoplasms are not well known. Exposure to radiation and high dietary intake of iodine are the risk factors for papillary carcinoma while iodine deficiency is a risk factor for follicular carcinoma and colloid goiter.[12,13] Viral infections are predisposing factors for autoimmune thyroiditis. The government of India has started universal iodization of salt owing to the huge burden of thyroid diseases in the country. However, in view of the recent occurrence of tsunami in 2004 in the coastal areas of South India which is likely to have increased the iodine levels in the adjacent regions, there is need to know if the practice of consuming iodized salt is still necessary in the tsunami hit coastal areas.

We therefore performed this study to determine the prevalence of thyroid disease and the ongoing trends in the occurrence of various thyroid diseases in the tsunami hit coastal areas of Puducherry and Cuddalore in South India. This is the first study conducted in the tsunami hit coastal areas to observe the changing trend in the occurrence of thyroid diseases.

MATERIALS AND METHODS: This study was conducted for a period of 9 years from January 2002 to July 2010 at a tertiary care teaching hospital in Puducherry, which caters to the health care needs of the people living in the adjacent coastal areas of Puducherry and Cuddalore. We received a total of 16,979 surgical specimens, of which 354 were thyroid specimens. Of these 354 thyroid specimens received in our lab, 12 had incomplete clinical data and were therefore excluded from the study. The remaining 342 thyroid specimens obtained by total or partial thyroidectomy from clinically diagnosed thyroid cases, irrespective of age, gender, race and demographic habitat were included in the study. This study was approved by the Institute Ethics Committee.

Clinical history, examination findings and all the investigation results were noted. Careful gross examination of thyroidectomy specimen and lymph nodes (in malignancy with secondaries) was performed. Tissue bits were processed after fixation in 10% formalin, embedded in paraffin wax, thin section of 3-5 microns were taken on albuminized slide, kept at 60° C for 30 minutes and immersed in Xylene. These sections were stained by H & E and mounted with DPX.

The sections were examined by a team of experienced Pathologists. The slides were grouped according to the different thyroid lesions, age, sex and other demographic features. The results were analyzed with available data and compared with other studies. The ongoing trends in thyroid disease are also analyzed.

Statistical significances were calculated by Chi square test and Fisher's exact test. *P* values < 0.05 were considered statistically significant. Statistics software GraphPad InStat version 3.06 for Windows, USA was used for calculation of *P* values.

RESULTS: We received 2.01% (342) thyroidectomy (total as well as subtotal) specimens out of total 16,979 surgical specimens during study period. The results were analyzed with available data and compared with other studies.

Of the total 342 cases studied 312 (91.23%) were female and only 30 (8.77%) cases were male. The distribution of thyroid disease in relation to sex is shown in Table 1. Highest incidence (19.82%) of the thyroid disease was seen in the age group 26 – 30 years. The age

distribution of the thyroid diseases is shown in Figure 1. Overall 67.54% of thyroid diseases were in the age group of 21 to 40 years.

Of the 342 cases, 118 (34.5%) were simple colloid goiter and 98 (28.66%) were adenomas. Malignancy was observed in 75 (21.93%) cases. Thyroiditis and toxic goiter accounted for 42 (12.28%) and 9 (2.63%) cases respectively. The most common thyroid disease in female patients was nodular colloid goiter (110/312), while in male patients it was adenoma (10/30). Year wise follow up shows there is mild decrease in the trend of simple goiter and adenomas (Figure 2). Similar follow up for inflammatory thyroid diseases shows there is significant increase (Figure 3). But most important is there is striking increasing in trend of papillary carcinoma since last 6 years (after 2004 tsunami) (Figure 3). Follicular carcinoma and toxic goiter show mild change in trend and as their sample size small, it is difficult to give any opinion on them (Figure 2).

The comparison of the distribution of thyroid diseases in different studies is shown in Table 2. Of the 42 cases of thyroiditis observed in our study, 25 were identified as Hashimoto thyroiditis and the remaining 17 were classified as lymphocytic thyroiditis. Among the 75 cases of malignant neoplasms, 70 were morphologically sub-classified as papillary carcinoma, while the other 5 were classified as follicular carcinoma. The relative predominance of papillary carcinoma among thyroid malignancies observed in different studies is summarized in Table 3.

DISCUSSION: Thyroidectomy specimens accounted for 2.08% of the total surgical specimens received during the study period. This shows a low frequency of thyroid diseases as compared to Tsegaye B & Ergete W, but is similar to that of Arora R.[2,14] This could be due to the fact that people from the coastal regions of Puducherry and Goa consume lot of sea food. In addition, the soil in coastal areas is rich in iodine and consequently the food and water also have a high content of iodine.

In our study the thyroid diseases showed a very high female preponderance with female to male ratio of 10.4: 1. This relatively very high female preponderance observed in our study in comparison to other similar studies is found to be statistically very significant.[1-3, 10,11] However, the exact reason for this difference between our study and other studies is not known. It could probably be due to severe nutritional deficiency. There is scope for further study to know the reason

In the present study, high prevalence of thyroid diseases was seen in relatively younger age group (26 – 30 years). In a study from Ethiopia, majority of the thyroid diseases occurred in the age group 30 – 39 yrs.[2] Similarly, in two different studies from Kolkata (India) and Malaysia, the age group 41 – 60 yrs was observed to be commonly affected by thyroid diseases.[3, 10] The reason for the increased occurrence of thyroid diseases in younger age group in our study is not clear.

The presence of goitrogens in foods of coastal places is often considered as a reason for the predominance of simple colloid goiter in these areas. Although simple colloid goiter was the most common thyroid disorder in our study, the proportion of patients with this condition was relatively less in our study compared to other similar studies. This can be attributed to the fact that the patients from Puducherry and Cuddalore consume iodized salt and sea food rich in iodine. There is mild decrease in the trend of this simple goiter. This could be due to people's awareness about thyroid diseases and practice of preventive measures like using sea foods and iodized salt.

In a study from Malaysia only 203 of 820 (25%) thyroid specimens were noted to have a neoplastic condition.[3] Similarly, in a study from Bahrain 43 of 110 (39%) thyroid specimens had neoplasms.[1] However, in our study 173 of 342 patients (50.59%) had thyroid neoplasms (adenomas, papillary carcinoma and follicular carcinoma). The increased occurrence of thyroid malignancies in our study in comparison to the above two studies was statistically very significant (*P* value < 0.0001 vs. Malaysia study and *P* value 0.0275 vs. Bahrain study).

The present study shows an increasing trend in the prevalence of neoplasm especially thyroid malignancy. The increasing incidence of malignancy is everyone's concern. It is necessary to find the cause and take preventive measures for the same. Compared to other studies, statistically significant increase in the occurrence of papillary carcinoma was observed in our study. This increasing incidence of papillary carcinoma in the present study is alarming.

There was also a striking increase in the trend of papillary carcinoma in the present study. The risk factors for papillary carcinoma are radiation, high intake of iodine. The flooding of the coastal regions of Puducherry and Cuddalore during the tsunami in 2004 could have increased the iodine content of the soil. Despite the increased iodine levels in the soil due to tsunami the people still continue to consume iodized salt, which probably could be the reason for increased occurrence of papillary carcinoma in our population. There is an urgent need to establish quality assurance system to monitor iodine content in the soil, food, water and salt in the tsunami affected coastal areas. It is also necessary to study the other possible causes for papillary carcinoma and take measures to prevent it. Another common lesion encountered in our study was thyroiditis. It is comparatively higher than the incidence in different studies, but is less than the study by Richard et al.[1-4,9,15,16] In our study we observed an increase in the incidence of inflammatory thyroid diseases almost similar to papillary carcinoma. So, we suspect that the increased occurrence of these two conditions might be interrelated.

Thyrotoxicosis was rare in our study and was observed in only 1.95% of the cases. It is similar to studies done in Pakistan and Malaysia, but is lower than studies done in Bahrain.[1,4,11] The incidence of thyrotoxicosis is not showing much change in its trend. The incidence of follicular carcinoma was decreasing. But the sample sizes of these two conditions were too small to observe any significant trend.

In conclusion, our Histopathological study emphasizes the fact that there was a higher predominance of thyroid disease in female as compared with other studies. The thyroid diseases were seen in relatively younger age group. Neoplasms are the commonest thyroid lesions necessitating thyroidectomy. There was higher incidence of papillary carcinoma and thyroiditis as compared with other studies. There was also a striking increase in the trend of papillary carcinoma after tsunami. However, further studies in the other tsunami affected areas are necessary to confirm this increased incidence as there is a paucity of similar data from other tsunami hit coastal areas. There is an urgent need to establish quality assurance system to monitor iodine content in the soil, food, water and salt in the tsunami affected coastal areas. It is also necessary to study the other possible causes for papillary carcinoma and take measures to prevent it.

REFERENCES:

1. Darwish AH, Al-Sindi KA, El-Kafsi J. Pattern of thyroid disease – a histopathological study. Bahrain Med Bull 2006;28:1-6

2. Tsegaye B, Ergete W. Histopathologic pattern of thyroid disease. East African Medical journal 2003;80:525-8
3. Htwe TT, Hamdi MM, Swethadri GK, Wong JOL, Soe MM, Abdullah MS. Incidence of thyroid malignancy among goitrous thyroid lesion from Sarawak General Hospital 2000 – 2004. Singapore Med J 2009;50:724 -8
4. Nurismah MI, Sharifah NA, Usama AE, Rohaizak M, Ibrahim N. Fine needle aspiration (FNA) cytology of the thyroid: a cyto-histopathological study of 361 cases in Hospital Universiti Kebangsaan Malaysia. Med and Health 2007;2:58-65
5. Kumar V, Abbas AK, Fausto N. Robbins and cotran Pathologic basis of disease. 7th ed. Saunders: Elsevier Inc, 2004
6. Park K. Iodine deficiency disorders. In: Park's text book of Preventive and social Medicine. 19th ed. Jabalpur: Banarsidas Bhanot, 2007. p. 510-1
7. Das S. The Thyroid and Parathyroids. In: A concise textbook of Surgery. 4th ed. New Delhi: Das S, 2006. p.644-78
8. Walker BR, Strachan MWJ. Endocrine disease. In: Boon NA, Colledge NR, Walker BR, Hunter JAA editors. Davidson's principles and practice of Medicine. 20th ed. Edinburgh: Churchill Livingstone, 2006. p.744-62
9. Prinz RA, O'Morchoe PJ, Barbato AL, Braithwaite SS, Brooks MH, Emanuele MA, et al. Fine needle aspiration biopsy of thyroid nodules. Ann Surg 1983;198:70-3
10. Guhamallick M, Sengupta S, Bhattacharya NK, Basu N, Roy S, Ghosh AK, Chowdhury M. Cytodiagnosis of thyroid lesions – usefulness and pitfalls: a study of 288 cases. J Cytol. January 2008;25:6-9
11. Kumar S, Aqil S, Dahar A. Role of fine needle aspiration cytology in thyroid diseases. Journal of Surgery Pakistan (International) 2008;13:22-5
12. Krukowski ZH. The Thyroid gland and the thyroglossal tract. In: Russell RCG, Williams NS, Bulstrode CJK editors. Bailey and love's short practice of surgery 23rd ed. London: Hodder Arnold, 2008. p. 771-806
13. Dorairajan N, Pandiarajan R, Yuvaraja S. A Descriptive study of papillary carcinoma in a teaching hospital in Chennai, India. Asian Journal of Surgery 2002;25:300-4
14. Arora R, Dias A. Iodine and thyroid cancer in Goa. Online J Health Allied Scs 2006; 5:1-3
15. Perros P. Thyrotoxicosis and Pregnancy. PLoS Med 2005;2:e370
16. Desailloud R, Hober D. Viruses and thyroiditis: an update. Virology Journal 2009;6:5

Table 1. Sex distribution of thyroid disease by different studies

Place (Reference no.)	Female (%)	Male (%)	No. of patients studied	*P* value*
Puducherry (Present study)	312 (91.23)	30 (08.7)	342	-----
Kolkata (10)	173 (69.5)	76 (30.5)	249	< 0.0001
Malaysia (3)	677 (82.6)	143 (17.4)	820	0.0002
Bahrain (1)	84 (76.4)	26 (23.6)	110	< 0.0001
Ethiopia (2)	628 (80.5)	152 (19.5)	780	< 0.0001
Pakistan (11)	60 (67.4)	29 (32.6)	89	< 0.0001

*** - *P* value with reference to the present study**

Table 2. Comparison of the distribution of thyroid diseases in different studies

Place (Reference no.)	Simple Colloid goitre (%)	Toxic goitre (%)	Thyroiditis (%)	Benign (Adenoma) (%)	Malignant (%)
Puducherry (Present study)	34.5	2.63	12.28	28.65	21.93
Malaysia (4)	71.0	0.3	0.3	11.0	17.4
Malaysia (3)	70.4	4.0	0.9	18.1	6.7
Bahrain (1)	45.5	8.0	7.0	15.5	24.0
Ethiopia (2)	76.9	0	2.1	12.8	8.2
Kolkata (10)	79.1			10.8	10.0
Goa (14)	89.4				10.6
Pakistan (11)	90.0				10

Table 3. Comparison of the predominance of papillary carcinoma among thyroid malignancies in different studies

S. No.	Place of Study (Reference no.)	No. of papillary carcinoma	Total no. of thyroid malignancies	% of papillary carcinoma	P value *
1.	Puducherry (Present study)	70	75	93.3	-----
2.	Ethiopia (2)	49	64	76.6	0.0103
3.	Malaysia (3)	39	55	70.9	0.0014
4.	Goa (14)	36	66	54.5	< 0.0001

* - *P* value with reference to the present study

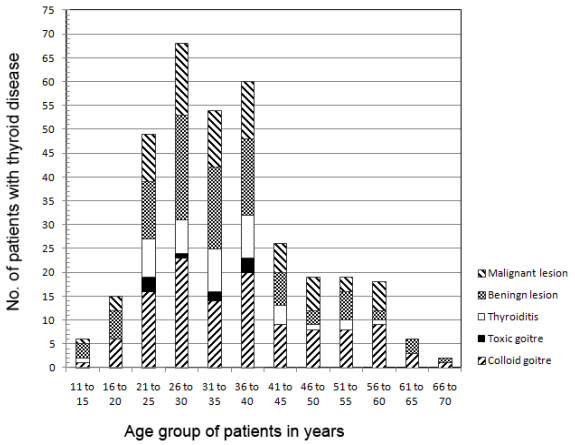

Figure 1. Age distribution of different thyroid diseases in our study

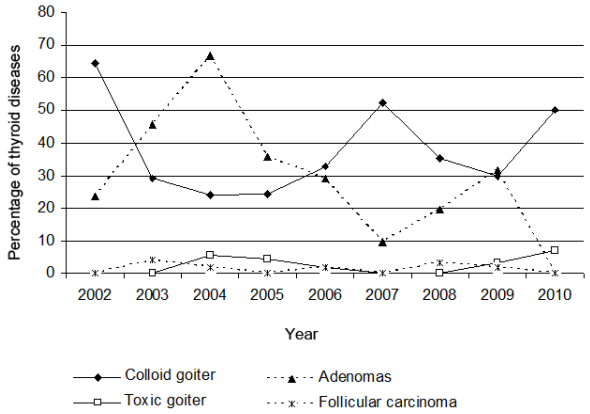

Figure 2. Trend in occurrence of colloid goiter, toxic goiter, adenomas and follicular carcinoma

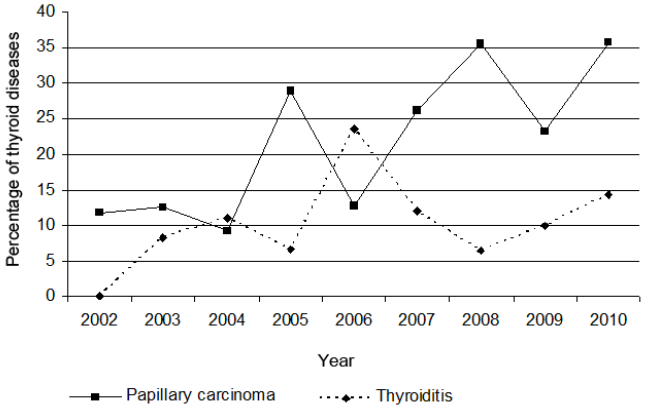

Figure 3. Trend in occurrence of thyroiditis and papillary carcinoma

"EFFECT OF OCIMUM IN CHEMOTHERAPY INDUCED OXIDATIVE STRESS" A STUDY CONDUCTED IN DAKSHINA KANNADA DISTRICT, KARNATAKA, INDIA

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ABSTRACT: Adriamycin (ADR) belongs to anthracycline group of antibiotics, which is widely used for the complete remission of solid tumours. In carcinoma breast, non Hodgkin's lymphoma and Hodgkin's lymphoma, ADR is considered as most useful chemotherapeutic medication. Anthracycline by virtue of their quinone group generates free radicals in solution in both normal and malignant cells. The Ocimum leaf extract as well as their flavonoids orientin and vicenin have strong antioxidant activity in vitro and antilipid peroxidative effect in vivo, and strongly suggest free radical scavenging as a major mechanism by which Ocimum products protect against cellular damage and tumour induction. However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants in vitro has been a subject of several studies in the past years. The antioxidant efficacy of flavonoids present in Ocimum sanctum in vivo is less documented, presumably because of the limited knowledge on their uptake in tumours. Inspite of limitation of the knowledge of exact mechanism of uptake of flavonoids we have studied antioxidant efficacy of Ocimum sanctum aqueous extract (140mg) with placebo control in carcinoma breast, non Hodgkin's lymphoma, Hodgkin's lymphoma cases along with chemotherapy particularly during third cycle. Statistical analysis has been done by Mann Whitney's test which shows no significant difference of antioxidant enzymes between study (n=19) and control (n=16) group before chemotherapy. There is a significant increase of all scavenging enzymes in study group (n=19) compared to control group (n=16) after chemotherapy. Significant increase in the Haemoglobin content and enhanced activities of Superoxide dismutase (SOD) and Catalase (CAT) in the study group which was given Ocimum.

KEY WORDS: Ocimum sanctum, Adriamycin, Antioxidants, Free radicals, Superoxide dismutase, Catalase

INTRODUCTION: In last 25 years, a large number of studies have shown that free radical mediated reactions are responsible for a wide range of chemotherapy induced side effects, and that antioxidants are able to protect non malignant cells against some of the damaging effect of cytotoxic drugs(1).Inspite of many available natural and synthetic antioxidants, a few number have been studied in clinical trials. Beta carotene, Vitamin C and E, azelastine, co-enzyme Q10,

selenium have been studied for their chemotherapeutic effects and found to be effective in ameliorating the side effects of anticancer drugs. Natural products like silymarin(1), ginseng(2), ashwagandha(3) are found to be beneficial adjuncts to chemotherapy. There is a concern that potent antioxidants may interfere with the anticancer efficacy too, which however appears to be a theoretical concern since many studies done using both chemotherapy and antioxidants have shown just the opposite. Antioxidant supplements have shown to protect the normal cells while making the cancer cells more vulnerable to cytotoxicity of chemotherapy and radiation therapy(1).The unpretentious little backyard plant plays on essential role in the folk medicine of South Asia named "Tulsi" (Ocimum sanctum) in Sanskrit, is a gentle therapeutic herb belonging to the family Basil. Conventionally, it is supposed to guard against stress and modern research now corroborates the concept (4).In laboratory animals, Ocimum sanctum has been shown to prevent cancer and to protect against radiation damage. Study established that hamsters were protected from developing cancer of oral cavity by Ocimum leaf extract(5).Mice survived radiation exposure when they have given with the herb(6,7,8).Its historical use as immuno-stimulant has now been demonstrated(4).Two flavonoids have been identified in Ocimum leaves, Orientin and Vicenin(6). In vivo and vitro studies have shown that, in general, flavonoids have antioxidant (9), antitumour promoter (10), antimetastatic (11) and anti-proliferative (12) properties. Flavonoids may act as antioxidants either by directly scavenging the free radicals or by modulating the free radical scavenging enzymes in the body(4).The direct free radical scavenging action of Ocimum aqueous extract(13) and indirectly by modulating Superoxide dismutase, Catalase and other antioxidant enzymes like Glutathione peroxidase(14).in its protective role in radiation induced damage in mice ,have been established .In human beings, Ocimum has been studied for its efficacy in non Insulin dependent Diabetes Mellitus (NIDDM)(15) but not for its chemotherapeutic effects. However, Ocimum aqueous extract is undergoing clinical trial currently for its radiation protective effect at Tata Memorial Hospital, Mumbai.

Our aim of study is to evaluate the safety and efficacy of Ocimum aqueous extract in modifying the side effects of Adriamycin (ADR) based chemoschedules in the treatment of post operative breast cancer, non Hodgkin's lymphoma and Hodgkin's lymphoma.

MATERIALS AND METHODS: This is a simple randomized single blind placebo control study, where the study group was having 19 and control group includes 16 cases of post operative female breast cancer, non Hodgkin's lymphoma, Hodgkins lymphoma receiving Adriamycin based chemoschedules and Statistical analysis has been done by Mann Whitney's test which shows no significant difference of antioxidant enzymes between study (n=19) and control (n=16) group before chemotherapy, but after chemotherapy it was significant . The study was undertaken in Department of Biochemistry in association with Department of Radiotherapy and Radiobiology in Kasturba Medical College, Mangalore, India.

INCLUSION CRITERIA: Post operative breast cancer in women, non Hodgkin's and Hodgkin's lymphoma in either gender, cancer diagnosed on both clinical and histological basis; age below 55 years, normal laboratory safety profile range, normal Electrocardiogram(ECG),general well being as assessed by Karnofsky's performance status scale more than 70;willing to give the written informed consent.

EXCLUSION CRITERIA: Haemoglobin less than 10 g/dl, WBC less than 3,000/ml, Platelet count less than 80,000/ml grossly damaged Liver and renal function tests more than thrice of the normal range, congestive cardiac failure, hypertension, uncontrolled diabetes mellitus.

STUDY DRUGS: Placebo capsule contains lactose.
Ocimum capsule contains 140mg dried aqueous extract of Ocimum leaves. The fresh leaves of Ocimum shades dried and powdered. The aqueous extract was prepared by refluxing with distilled water and was vacuum dried in a speed vac system prepared in Radiobiology Department, KMC, Manipal. The dose has been selected on the basis of the animal experiments done in same department.

TREATMENT PLAN AND ASSESSMENT OF ENDPOINT MEASURES: Patients who are willing to give written informed consent were enrolled for the study, before which the clinical examination and laboratory assessment of organ function tests were done to rule out the exclusion criteria. Patients were randomized to either of the groups to receive the placebo or Ocimum capsules. The study medication was given during 3rd cycle, 24 hours after chemotherapy for six consecutive days. Superoxide dismutase (SOD), Catalase (CAT) and Haemoglobin (Hb) was estimated before chemotherapy and 24 hours after the end of Ocimum / Placebo adjuvant therapy of the same cycle on 7th day. Estimation of Haemoglobin was done by cyanmethaemoglobin method (16,17).Superoxide dismutase (SOD) was determined by Mccord and Fridovich method (18) and Catalase (CAT) activity was assessed by Brannan *et al* (19) method.

RESULTS: The baseline SOD values of the two groups are not significantly different. The SOD levels in the study group and control group were 6814.66 ± 3540.04 and 9321.83 ± 5887.23. However the control group had a higher mean value of SOD than the study group. The values of SOD levels and the change in SOD levels obtained after 3rd cycle of chemotherapy are given in Table 3.

The mean value of SOD in the control group was 6422.94 ± 3854.10 U/g Hb while study groups showed a value of 9892.46 ± 4227.60 U/ g Hb. There was a significant difference in the change in SOD after chemotherapy between the two groups. The mean change in SOD in the study group was 3077.79 while the control group showed a fall in SOD value with the mean change in the value of -2898.89.

Figure 1 and 2 depicts the mean values of SOD obtained before and after 3rd cycle of chemotherapy in the study and control groups.

The mean values of CAT levels estimated before 3rd cycle of chemotherapy are presented in table 4

There is no significant difference in the baseline Catalase values of the two groups. However the study group showed an apparently higher mean value of Catalase in comparison with with control group (Figure 3).Values of CAT and the change in CAT observed after 3rd cycle of chemotherapy are given in table 5

A significant increase in the Catalase levels was observed in the study group with the mean value of 49,727.72 ± 18,264.80. The control group showed a significant decrease and the mean value was 10,016.83 ± 7547.10.There was a significant difference in the change in Catalase values after chemotherapy between the two groups. Study group showed a difference of 28,826.69 and the control group showed a mean value of -3477.19. Figure 3 and 4 depicts the

mean values of CAT obtained before and after 3rd cycle of chemotherapy in the study and control groups

Table 1 Karnofsky's Performance Status (KPS)

KPS	Description of the general condition of the patients
100%	Normal,no complaints,no evidence of disease
90%	Able to carry on normal activity;minor signs or symptoms of disease
80%	Nornal activity with effort;some signs and symptoms of disease
70%	Cares for self;unable to carry on normal activityor to do active work
60%	Requires occational assistance,but is mostly able to carefor himself
50%	Requires considerable assistance and frequent medical care
40%	Disabled,requires special care and assistance
30%	Severely disabled,hospitalization indicated;death not imminent
20%	Very sick,hospitalization necessary active supportive treatment
10%	Moribund,fatal processes,progressing rapidly
0%	Dead

Table 2 : SOD levels before 3rd cycle of chemotherapy

	n	Mean ± SD(Unit/g Hb)
Study Group	19	6814.66 ± 3540.04
Control Group	16	9321.83 ± 5887.23

Table 3 : SOD levels after 3rd cycle of chemotherapy

	n	Mean ± SD (U/gHb)	Change in SOD
Study Group	19	9892.46 ± 4227.60	3077.79 ± 2325.68 **
Control Group	16	6422.94 ± 3854.10	- 2898.89 ± 3183.50

Table 4 : CAT levels before 3rd cycle of chemotherapy

	n	Mean ± SD(U/g Hb)
Study Group	19	20.901.03 ± 9901.13
Control Group	16	13494.03 ± 7476.94

Table 5 : CAT levels after 3rd cycle of chemotherapy

	n	Mean ± SD (U/g Hb)	Change in Catalase
Study Group	19	49,727.27±18264.80**	28,826.69±25536.24***
Control Group	16	10,016.83±7547.10	-3477.19±2946.16

n = number of subjects

**p value 0.002

***p value 0.001

Figure 1 : Comparison of SOD(Unit/gHb) levels between study and control group before 3rd cycle of chemotherapy

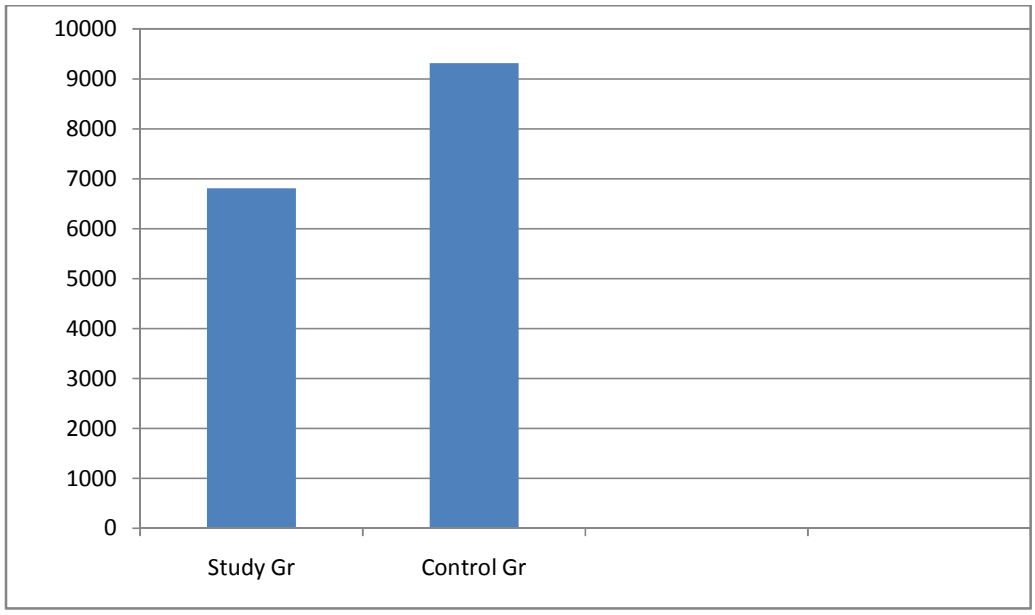


Figure 2 : Comparison of SOD(Unit/gHb) levels between study and control group after 3rd cycle of chemotherapy

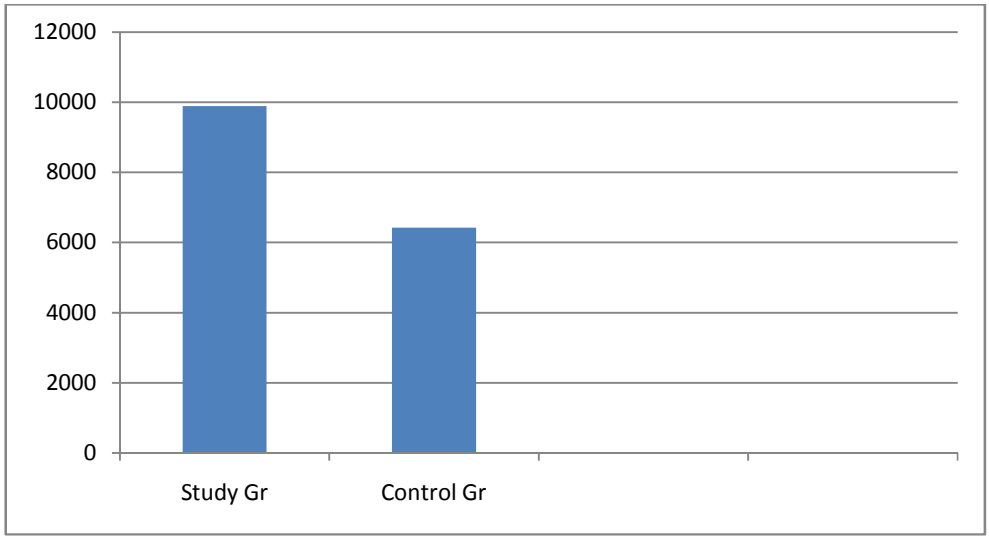


Figure 3 : Comparison of CAT(Unit/gHb) levels between study and control group before 3rd cycle of chemotherapy

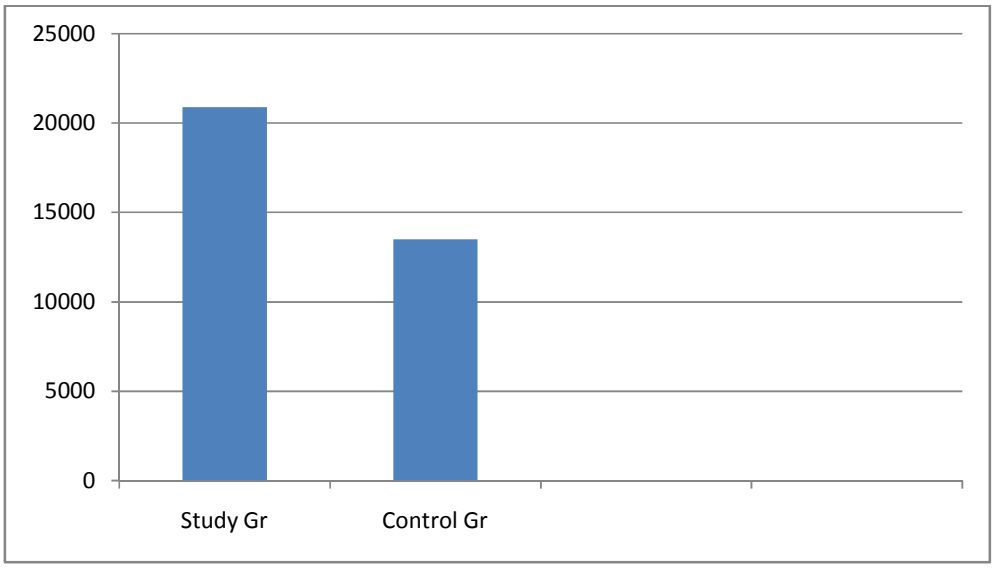
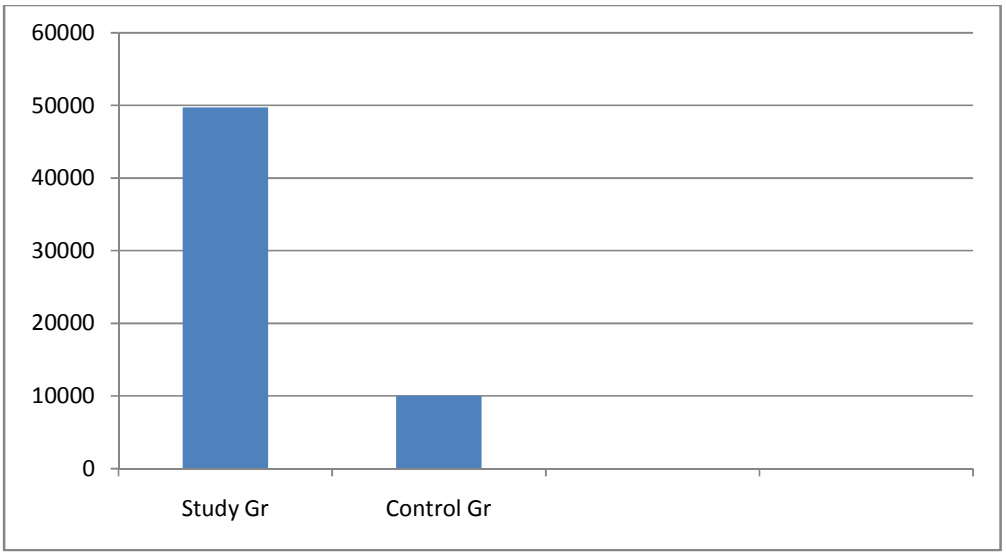


Figure 4 : Comparison of CAT(Unit/gHb) levels between study and control group after 3rd cycle of chemotherapy



DISCUSSION: Chemical toxins produce biological damage by forming reactive oxygen species like singlet oxygen, superoxides, hydroxyl, hydroperoxy, hydrogen peroxides (20).Ikeda *et al.*(21) have shown that anthracycline by virtue of their quinone groups generate free radicals in solution in both normal and malignant cells. In addition generation of lipid peroxide nitric oxide and other destructive radicals are also produced from semiquinone intermediates of anthracyclines. Under normal conditions the inherent defence system including SOD,CAT and other antioxidant enzymes protect against free radical mediated oxidative stress.

Cu-Zn SOD serves as a major antioxidant enzyme in erythrocytes where superoxide radicals are continuously generated by auto oxidation of Haemoglobin. Several anticancer drugs lead to a generation of reactive oxygen species which partly explains the cytotoxic effect of cancer therapy.

Increase in SOD activity was 44% in the study group treated with Ocimum extract. However placebo treated group showed a 31% reduction in SOD activity suggesting an efficient scavenging of free radicals by Ocimum extract.

Uma devi *et al* (22) have shown that significant decrease in SOD activity after radiotherapy,but the activity increases following administration of Ocimum extract.

Our result is in agreement with the above study. Significant increase in SOD observed in Ocimum treated patients suggests that the Ocimum extract facilitates the removal of superoxide anions produced by chemotherapy.

Mammalian erythrocytes are endowed with extraordinarily high activities of Catalase. Cohen *et al* (23) have suggested that under physiological conditions practically all hydrogen peroxides encountered by erythrocytes are detoxified by glutathione peroxidase and that Catalase has a vital role in the clearance of hydrogen peroxide. Jacob *et al* (24) have reported that humans lacking erythrocyte Catalase activity are not even susceptible to hemolysis induced by oxidant drugs. Nihal *et al* (25) have reported protection by Catalase against exogenous oxidant challenge. A 2.3 fold increase in Catalase activity was observed in the study group treated with Ocimum extract where as placebo group showed 1.3 fold reduction in activity. A significant increase in Catalase activity observed (p value 0.001) suggests a brisk generation of superoxides and therefore hydrogen peroxide in the present circumstances. Flavinoids orientin and vicenin in Ocimum seems to have induced the activity of Catalase. Thus providing protection against the toxicity of anthracyclines.

Doroshow *et al* 1986(26) have shown that anthracycline induced cytotoxicity is ameliorated by adding SOD and CAT to the culture medium.

No significant change in Haemoglobin content observed in the two groups after chemotherapy. However the decrease in SOD, CAT observed in control group without any appreciable alteration in the Haemoglobin levels.

A significant increase in the SOD,CAT levels after chemotherapy along with Ocimum extract is an important observation of this study. This suggests that absorbed flavonoids of Ocimum may play an important role to promote the body enzymatic (SOD, CAT) defence system to combat the free radicals produced during ADR based chemotherapy. Thus Ocimum sanctum could be a beneficial adjuvant for the same.

The possibility of developing new chemoprotective for application from Ocimum sanctum should be further explored. In addition, its impressive antioxidant property and stimulatory effect on the cellular antioxidants can be exploited for adjuvant therapy against a number of human ailments such as cancer and other stress related disorders.

CONCLUSION: The results of present study indicate a significant elevation in the antioxidant enzymes viz.SOD and Catalase after chemotherapy along with Ocimum as an adjuvant.

Increased antioxidant activity brought about by Ocimum may be responsible to combat the damage in normal healthy tissues with little or no protection to the tumour following chemotherapy. Hence Ocimum can be a beneficial adjuvant with ADR based chemotherapy.

REFERENCES:

1. Weji NL,Cleton FL,Osanto S.Free radicals and antioxidants in chemotherapy induced toxicity.Cancer Treat Rev 1997;23:209-240
2. Torosian MH.Metabolic abnormalities and conditionally essential nutrients in the cancer patient. Adjuvant Nutrition in Cancer Treatment 1992;173-233.

3. Ziauddin N,et al.Studies on the immunomodulatoryeffects of ashwagandha.J of Ethnopharm, 1996;50(2):69-76
4. Tirtha,Sadashiva,Swami.The Ayurveda Encyclopedia 1998;106.
5. Kartkeyan K,Ravichandran P,Govindaswami S.Chemoprotective effectof ocimum sanctum on DMBA- Inducedhamster buccal pouch carcinogenesis.Oral Oncl 1999;35(1):112-119
6. Umadevi P,Ganasoundari A,Rao BS,Srinivasan KK.In vivo radioprotection by ocimum flavonoids:survival of mouse.Radiat res 1999;151(1):74-78
7. Umadevi P,Bisht KS,Vinitha MA. Comparative study of radioprotection by ocimum flavonoids and Synthetic aminothiol protectors in the mouse.Br.J Radiol 1998;71(847):782-784
8. Ganasoundari A,Mahamood Zare S,Uma Devi.Modification of bone marrow sensitivity by Medicinal Plant Extracts.The Br.J Radiol 1997;70:599-602
9. Alan L,Muller MD.Antioxidant Flavonoids:Structure,function and clinical usage.Alt Med Rev 1996;1(2):103-111
10. Fujiki H et al. Inhibition of tumour promotion by flavonoids.In plant flavonoids in biology and medicine. Biochemical,pharmacologicaland structure activity relationships Alan R.Liss.New York.1986;429-440
11. Bracke ME et al.Flavonoids inhibit malignant tumours invasion in vitro.In plant flavonoids in Biology and Medicine.II.Biochemical,Cellular and medicinal properties.Alan R.Liss.New York 1988;219-233
12. Kandaswamy C et al.Antiproliferative effects of citrus flavonoids on a human squamous cell carcinoma in vitro.Cancer Lett 1991;56:147-152
13. Ganasoundari A,Uma Devi P,Rao MNA.Protection against radiation induced chromosome damage in Mouse bone marrow by ocimum sanctum.Mutat Res 1997;373:271-276
14. Uma Devi P,Ganasoundari A.Modulation of glutathione and antioxidant enzymes by ocimum sanctum and its protection against radiation injury.Indian J Exp Biol 1999;Mar 37:262-268
15. Agarwal P,Rai V,Singh RB.Randomized,placebo controlled,single blind trial of holy ocimum leaves in Patientswith non insulin dependent diabetes mellitus.Int J Clin Pharmacol Ther 1996;Sept 34:406-409
16. Drabkin DL et al.J Biol Chem 1932;98:719
17. Zijlstra NC.Clinical Chem Acta 1960;5:719
18. McCord JM,Fridovich I.Superoxide dismutase :An enzymatic function for erythrocuprein.J.Biol Chem 1969;24:6049-6055
19. Brannan TS,Maker HO,Raes JP.Regional distribution of catalase in the adult rat brain.J Neurochem 1981; 86:307-309
20. Von Sonntag C.The chemical basis for radiation Biology (Taylor and Francis,London).1987
21. Ikeda K,Kajiwara K,Tanabi E,Tokumaru S.Kishida E,Masuzawa Y and Kojo S.Involvement of hydrogen peroxide and hydroxyl radical in chemically induced apoptosis of HL -60 cells.Biochem Pharmacol 1999;57:1361-1365
22. Younes Mand Siegers CP.Planta Medica 1981;43:240
23. Cohen,G and P.Hochstein.Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes.Biochemistry:1963;2:1420-1428
24. Jacob,H.S.,S.H.Ingbar and J.H.Jandl.Oxidative hemolysis and erythrocyte metabolism in hereditary acatalasia.J.Clin.Invest.1965;44:1187-1199
25. Nihal S Agar,S.M.H.Sadrzadeh,Philip E.Hallaway and John W Eaton.Erythrocyte Catalase a somatic oxidant defence.J Clin Invest.1985;319-321
26. Doroshow JH,Role of hydrogen peroxide and hydroxyl radical formation in killing of Ehrlich tumour Cells by anticancer quinines,Proc Natl Acad Sci USA,1986;83:4514-4518

LINGUAL THYROID IN CHILDREN

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INTRODUCTION: Lingual thyroid is a rare embryological anomaly, the incidence being 1/100000 population, that originates from failure of the thyroid gland to descend from the foramen caecum to its normal prelaryngeal site. The ectopic gland, located at the base of the tongue is often asymptomatic, but may cause local symptoms such as dysphagia, dysphonia, stomatologia, upper airway obstruction and haemorrhage, often with hypothyroidism. This infrequent congenital anomaly is often asymptomatic, until a pathologic stress such as systemic disease or physiologic stress such as puberty causes enlargement of ectopic tissue, leading to dysphagia, dysphonia stomatologia. A six year old girl child presented with complaints of mass over the base of tongue, noticed since six months, with no other symptoms in this case report the presentation, diagnosis and management of this condition are highlighted.

CASE REPORT: A six year old female child presented with the complaints of swelling over the base of the tongue, with no other complaints/difficulties such as dysphagia, dysphonia, stomatologia or difficulty in breathing. The swelling was noticed by the parents six months back. The swelling is of the same size since then. There was insignificant medical history and no history of dysphagia, dysphonia, stomatologia, delayed developmental milestones and mental retardation. She weighed 16 kgs and her height was 90 cms. Physical examination revealed a solid pink spherical mass covered with intact mucosa, located at the base of the tongue measuring 1.8cms X1.4cmsX 2cms, obstructing the visualization of larynx, which was nontender, fixed and firm in consistency (figure-1)

The clinical examination revealed no palpable thyroid gland, in the normal pretrecheal position and no cervical lymphadenopathy. Ultrasonic scan of the neck showed nonvisualsation of thyroid in its normal position. X-ray of the chest was normal. CT(computerized tomography) of the neck revealed hyperdense area of 110-130 HU ,measuring about 1.8X1.4 cms noted at the base of the tongue in midline. Normal thyroid tissue was not made out in the neck region. Thyroid function tests revealed thyronormalcy, other laboratory tests also were within normal range. FNAC of the swelling under general anesthesia was done. Smear prepared showed colloid

and follicular epithelial cells, arranged in small clusters and in follicular pattern. Numerous pigment laden histiocytes and occasional multi nucleated histiocytes were noted. No malignant cells were seen. Pathologist gave an opinion that cytological features were suggestive of lingual thyroid. She was diagnosed as a case of ectopic lingual thyroid with euthyroid state. As the lingual thyroid was causing no difficulty of whatever sort, to the child and being in euthyroid state, parents were assured that no intervention was presently required .Parents were also emphasized about the need for regular follow up in due course.

DISCUSSION: Embryologically, the thyroid gland develops as the first pharyngeal derivative by an endodermal diverticulum, in the midline of the ventral pharynx between the first and second pharyngeal pouches. A diverticulum descends caudally into the loose prepharyngeal connective tissue and passes anterior to the developing hyoid bone and forms most of the thyroid parenchyma. However, parafollicular C-cells reach the thyroid by ultimobranchial bodies, which are the product of the fourth and fifth branchial pouches and form 1-30% of the thyroid weight. [1] Failure of descent of either the medial anlage of the thyroid, or the ultimobranchial bodies, and the incomplete obliteration of its vertical tract, lead to ectopic thyroid development. The ectopic thyroids are usually located in the midline from the base of tongue to the diaphragm, but can be also be present laterally. Lingual thyroid is not a very common lesion; carcinomatous change in it is very rare. The majority of carcinomas observed in the lingual thyroid are reported to be follicular. [2] Lingual thyroid is the most frequent ectopic location of the thyroid gland, although its prevalence varies between 1: 100 000 and 1 : 300 000 and its clinical incidence is reported to range from 1 : 4000 to 1 : 10000.

Ectopic thyroid tissue can also occur between the geniohyoid and mylohyoid muscles (sublingual thyroid), above the hyoid bone (prelaryngeal thyroid) and in other rare sites such as the mediastinum, precardial sac, heart, breast, pharynx, oesophagus, trachea, lung, duodenum, and mesentery of the small intestine, adrenal gland. [3] CT (computerized tomography) or MR (magnetic resonance) scans show a midline mass extending from the midline mucosal surface of the tongue base into the medial sublingual space that can resemble a thyroglossal cyst. However, nuclear scans are better to demonstrate the location of ectopic glands. [4] Thyroid scan can also reveal whether there are other sites of thyroid tissue; in approximately 75% of patients the ectopic tissue is the only functioning thyroid tissue in the body. [5] Management of lingual thyroid is still controversial. No treatment is required when the lingual thyroid is asymptomatic and the patient is in a euthyroid state; the patient has to be followed to be aware of development of complications. Malignant transformation has been described [1],[2] and, for this reason, some authors consider complete surgical removal of the gland as an appropriate treatment. [6],[7] For patients with no or only mild clinical symptoms and elevated TSH concentration, substitutive therapy with thyroid hormone may be successful, producing a slow reduction of the mass. Ablative radioiodine therapy is an alternative approach recommended in older patients or patients who are deemed unfit for surgery. This treatment should be avoided in children and young adults since the systemic doses required have potentially damaging effects on the gonads or other organs. [8] Surgical excision or radioiodine therapies are effective treatments for lingual thyroid, but no treatment should be attempted until a radioisotope scan has determined that there is adequate thyroid tissue in the neck. In patients, those with lacking thyroid tissue in the neck, the lingual thyroid can be excised and autotransplanted to the muscles of the neck. If emergency surgery is not necessary, suppression therapy should be tried first in order to decrease the dimensions of the mass. The general conditions of the patient, the

size of the lesion, and presence of local symptoms or complications, such as hemorrhage, cystic degeneration, or malignancies, are the most important conditions for planning the choice of treatment. [9]

REFERENCES:

1. Collins P. Embryology and development. In: Williams PL, Bannister LH, Berry MM, Gray H. editors. Gray's Anatomy - The Anatomical Basis of Medicine and Surgery. 38 th ed. New York: Churchill Livingstone; 1995. p. 174-99.
2. Gooder P. Follicular carcinoma in a lingual thyroid. J Laryngol Otol 1980;94:437-9.
3. Di Benedetto V. Ectopic thyroid gland in the submandibular region simulating a thyroglossal duct cyst: A case report. J Pediatr Surg 1997; 32:1745-6.
4. Elprana D, Manni JJ, Smals AG. Lingual thyroid. ORL J Otorhinolaryngol Relat Spec 1984;46: 147-52.
5. Baik SH, Choi JH, Lee HM. Dual ectopic thyroid. Eur Arch Otorhinolaryngol 2002;259: 105-7.
6. Galizia G, Lieto E, Ferrara A, Castellano P, Pelosio L, Imperatore V, et al. Ectopic thyroid: Report of a case. G Chir 2001;22:85-8.
7. Shah BC, Ravichand CS, Juluri S, Agarwal A, Pramesh CS, Mistry RC. Ectopic thyroid cancer. Ann Thorac Cardiovasc Surg 2007;13:122-4.
8. Alderson DJ, Lannigan FJ. Lingual thyroid presenting after previous thyroglossal cyst excision. J Laryngol Otol 1994;108:341-3.
9. Toso A, Colombani F, Averono G, Aluffi P, Pia F. Lingual thyroid causing dysphagia and dyspnoea. Case reports and review of the literature. Acta Otorhinolaryngologica ital 2009;29:213-7.

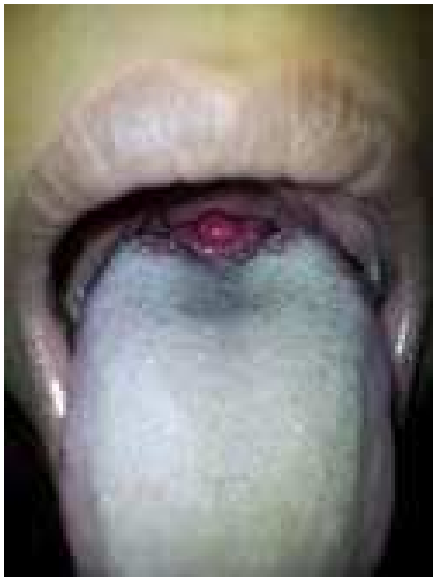

Figure 1

INCREASING PREVALENCE OF HEPATITIS AMONG TRANSFUSION TRANSMITTED INFECTIONS: A TERTIARY CARE CENTRE EXPERIENCE

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ABSTRACT: Increasing Prevalence of Hepatitis among Transfusion Transmitted Infections: A tertiary care centre experience. **BACKGROUND:** Blood transfusion despite being a lifesaving intervention may result in acute or delayed complications and carries the risk of transfusion–transmissible infections (TTI's). There has been an alarming rise of hepatitis (B&C) infection among blood donors and not much related to prevention or prophylaxis has been done so as to reduce the risk of transmission. **AIMS OF THE STUDY:** The study was done to find out the prevalence of Hepatitis B and Hepatitis C among transfusion transmitted infections in a healthy blood donor population **METHODOLOGY:** A six years retrospective study of sero-reactive cases of TTD's among the blood donors was done (2004-2009) in Blood Transfusion Unit, Christian Medical College & Hospital, Ludhiana. The data was retrieved from the blood bank records with special emphasis on hepatitis infection among the blood donors. **RESULTS:** Over all 64,528 donors were screened for the Transfusion Transmitted infections (HBV, HCV, HIV1&2,Malarial Parasite, and VDRL) of which majority were males. Overall seroprevelance for the transfusion transmitted infections was 2.72%. Seropositivity for Hepatitis per se was 2.4%. Majority of the donors were reactive for HCV infection (1.4%) followed by HBV (1.0%), HIV1&2(0.2%), VDRL (0.1%) and Malaria parasite infection (0.02%). **CONCLUSION:** There has been an alarming rise of hepatitis cases, especially HCV among the blood donor population which need to be looked upon and special preventive measures need to be taken at the national level to combat this problem.

KEY WORDS: Blood donors, Transfusion-transmitted infections, hepatitis

INTRODUCTION: Transfusion of blood and blood products is a life saving measure and saves millions of lives each year globally, but at the same time it also carries a significant risk of transmission of many blood transfusion transmitted infections (TTI's). Despite stringent donor screening and testing practices, safe blood free from transfusion-transmitted infections (TTI's) continues to be a threat to safe transfusion practices. [1]

TTI's can exist as asymptomatic diseases (silent killers) in the hosts, and acquisition of infections during the window period from such blood donors can be a serious threat to the safety of the collected donations. [2]

Among the various TTI's, the most dreaded and less sought after infection is by Hepatitis B (HBV) and Hepatitis C (HCV) viruses and are considered two established causes of post transfusion hepatitis. Many Blood donors are carriers, not realizing that are infected with this disease. Individuals with chronic infection have a high risk of developing liver cirrhosis and hepatocellular carcinoma leading to serious mortality and morbidity. [3] There are about 5.7 million cases of HIV in India, second highest pool of patients in the world. Syphilis is less often transmitted by blood and the prevalence is low in most studies reported. [4]

We did this study to find the prevalence of TTI's with emphasis on hepatitis infection among blood donors in our hospital.

MATERIAL & METHODS: This six years retrospective study was done in the Transfusion Medicine Unit, Christian Medical College & Hospital, Ludhiana, a tertiary care centre in North India over a period of 6 years from 2004-2009. Average donor numbers is about 10,000-12,000 per year.

The donor's details were noted down from the donor consent forms kept in the blood bank records. The donor blood samples were later screened for mandatory tests [Malaria, Venereal Disease and Research Laboratory (V.D.R.L), Hepatitis B antigen (HBsAg), Anti HCV & Anti HIV 1&2)].

Testing for malaria parasite was done by card test based on malarial (pLDH) antigen based principle. VDRL testing was based on TPHA (Treponema Palladium Haemagglutination Antibody) based principle. Testing for Hepatitis B, Hepatitis C and HIV 1&2 were based on 3rd generation ELISA techniques (Ortho Clinical Diagnostics, Vitros ECiQ-based on chemiluminiscense technology). The serological results were retrieved from the records available in the department

RESULT AND ANALYSIS: A total of 64,528 donors were screened over the 6 year study period. Majority (94.7%) of them were replacement donors. Male donors (97.8%) outnumbered females (2.2%). Out of the potential donors screened, 1755 (2.72%) of the blood donors were reactive for various Transfusion Transmitted Diseases (TTD's). The details are shown in Fig 1. Majority (71.8%) of the donor's positive for TTD's (HBsAg, HCV and HIV) were in 18-35 years age group (Fig 2).

Prevalence of hepatitis infection formed the majority (2.4%) of the total TTI's over the 6 year period (Fig 3) of which HCV reactive donors were (1.4%) and HBsAg were 1%.Prevalence of HIV, VDRL, and MP was 0.2%. 0.1% and .02% respectively. Hepatitis infection formed majority both among males and females. The detail regarding various TTI's among males and females are given in Table 1.

Co-infection was seen in 1.2 % of the total TTD positive donors of which 14 donors had both HBsAg and HCV positivity. The detail is given in Table-2

DISCUSSION: Blood transfusion even though is a lifesaving procedure is associated with acute and delayed complications and carries the risk of transmitting TTI's. Despite stringent donor screening and testing practices, safe blood free from transfusion-transmitted infections (TTI's) remains an elusive goal. [1] Although technological advancements have led to the development of

more sensitive methods to detect markers of TTI's, the problems of 'window period', false-negative results, prevalence of asymptomatic carriers, genetic variability in viral strains and technical errors remain. [5]

Among the various TTI's, hepatitis has become an issue of global importance. Hepatitis B and C are highly infectious and pose major public health problem in developing countries and are the commonest cause of chronic liver disease in several regions of the world. [2] Hepatitis B is one of the most common diseases transmitted by blood and has infected two million people worldwide including an estimated 400 million chronically infected cases. Individuals with chronic infection have a high risk of developing liver cirrhosis and hepatocellular carcinoma. [6] Hepatitis C virus (HCV) infection is another common chronic blood born infection with an estimated 3.9 million persons infected by the virus and a high rate of development of liver cirrhosis. Infection by HBV and HCV causes serious mortality and morbidity. [7]

The present study showed higher seroprevelance of HBV and HCV among the blood donors as compared to other TTI's, rather it formed the majority of the infections (2.4% of the total 2.7% of reactive TTI's).

The prevalence of HBV reactive blood donors is different in various countries. It is as low as 0.1-0.5% in a healthy population in United States and Western Europe, whereas it ranges from 5-20% in far Eastern and some Tropical countries.[8] Seroprevelance of HBsAg in various Indian studies has shown to range between1.86%-4% [9,10,11] ,which was comparable to our study showing the incidence of HBsAg to be 1%.

HCV is transmitted primarily through blood exposure. In contrast to HBV, about 20 to 40% of HCV cases are acute and majority of them progress to chronic infection. The long term risk of developing cirrhosis and hepatocellular carcinoma is greater in HCV infected individuals than in those infected with HBV. [12] There is wide variation globally in the seroprevelance of HCV with the studies showing lowest prevalence in United States (0.1%)[8] and highest in Egypt (24.8%). [9]Various other Indian studies indicate seroprevelance of HCV ranging from 0.4%-1.09%. [9,10,13,14]

Most of the above mentioned studies have shown increase prevalence of HBV as compared to HCV. The seroprevelance of HCV was highest (1.4%) among all other TTD's in the present study which was comparable to the study done in Pakistan where HCV prevalence was 2.06%. [15]

Majority of the sero-positive donors were between 18-35 years age group in the present study, which is alarming, considering the fact that this is the age during which a person is considered healthy and can give maximum number of blood donation. Few other studies also showed increase prevalence of TTI's in 18-35 years of age group.[16, 17]

Dual infection was seen in 1.2% of the total TTI's in our study of which most common co-infection was HBV and HCV(14 out of 23), which was similar to study done by Rodenas et al. [17] Few other studies showed HIV to be the commonest co-infection associated with hepatitis infection.[1,18]

The results from our study as well as various studies mentioned, showed the endemicity and the rising prevalence of both HBsAg and HCV in an apparently healthy population as compared to HIV. There is consequent risk of transmission of these viruses through blood/blood products, albeit unknowingly. So far, most of the published data worldwide is on HIV/AIDS and much is being done regarding its prevention and cure by various government organizations as well as World Health Organization.

Despite various studies suggesting an increase in the hepatitis infection not much has been done for its prevention. The emphasis must be given to increase the knowledge and to change the attitude of the people. It was shown that the risk of HIV infection in SanFrancisco declined substantially, to about 0.2 per cent as a direct result of efforts to educate at risk individuals to avoid donation [14].

RECOMMENDATIONS: Various measures like strict donor selection criteria, use of sensitive tests like Nucleic Acid Testing (NAT) and judicious use of blood and blood components have been taken to reduce the risk of TTI's per se. Pre-donation counseling, and self-exclusion of donors is a rare phenomenon seen in our set up. National agencies like National Aids Control Organization (NACO) and World Health Organization(WHO) should join hands along with various other voluntary organizations in doing community based study for Hepatitis to identify at risk and non- risk subjects. At risk subjects should be identified and preventive strategies should be made at the national level, as the risk factors might vary from one geographical area to another.

To achieve all this, strong political commitment, multi-sectorial engagement along with public awareness, educational and motivational programmes and mass immunization (for Hepatitis B) should be done to substantially decrease the prevalence of hepatitis in an otherwise healthy population.

CONCLUSION: From our study it has been clear that prevalence of hepatitis is on rise especially infection with Hepatitis C in healthy population without them being aware of it ("silent carriers") and carries the risk of transmitting infection albeit unknowingly.

BIBLIOGRAPHY:

1. Kaur G, Basu S, Kaur R, Kaur P, Garg S. Patterns of infections among blood donors in a tertiary care centre: A retrospective study. Natl Med J India 2010; 23:147–9
2. Kalepoto G.N., Bhally H.S., Khaliq G. Epidemiology of blood-borne viruses. A study of health blood donors in southern Pakistan. J. Pak. Med. Assoc 1996; 27: 703-6.
3. Karki S, Ghimire P, Tiwari BR, Maharjan A, Rajkarnikar M. Trends in hepatitis B and hepatitis C seroprevalence among Nepalese blood donors. Ind Jl Infec Dis 2008; 61: 324-326.
4. Bhattacharya P, Chandra PK, Datta S, Banerjee A, Chakraborty S, Rajendran K, Basu SK, Bhattacharya SK, Chakravarty R. Significant increase in HBV, HCV, HIV and syphilis infections among blood donors in West Bengal, eastern India, 2004-2005. World Journal of Gastroenterology 200; 13 :3730-3733.
5. Singh B, Kataria SP, Gupta R. Infectious markers in blood donors of East Delhi: Prevalence and trends. Indian J Pathol Microbiol 2004;47:477–479.
6. Karki S, Ghimire P, Tiwari BR, Maharjan A, Rajkarnikar M, Trends in hepatitis B and hepatitis C seroprevalence among Nepalese blood donors. Indian Journal of Infectious Diseases 2008; 61:324-326.
7. Bhawani Y, Rao PR, Sudhakar V. Seroprevalence of transfusion transmissible infections among blood donors in a tertiary care hospital of Andhra Pradesh. Biology and Medicine, 2010 ;l 2 : 45-48

8. Dienstag J.L., Isselbacher K.J. Acute viral hepatitis In Braunwald E. Fauji A.S., Kasper D.I. et al. 15th ed. Harrison's principles and practice of internal medicine. New York McGraw Hill 2001: 1721-37.
9. Chandrasekaran S, Palaniappan N, Krishnan V, Mohan G, Chandrasekaran N: Relative prevalence of hepatitis B viral markers and hepatitis C virus antibodies (antiHCV) in Madurai, South India. Indian Journal of Medical Sciences 2000;547:270-273.
10. Srikrishna A, Sitalakshmi S, Damodar P: How safe are our safe donors?. Indian Journal of Pathology and Microbiology, 1999;424:411-416.
11. Choudhury N, Ramesh V, Saraswat S, Naik S: Effectiveness of mandatory transmissible diseases screening in Indian blood donors. Indian Journal of Medical Research, 1995;101:229-232.
12. WHO hepatitis C. WHO fact sheet No164, October 2000b.Available from URL.http:/www.who.int/mediacentre/factsheets/fs164/en/print.htm. [Cited on 2007 May 14]
13. Sharma RR, Cheema R, Vajpayee M, Rao U, Kumar S, Marwaha N, Agnihotri SK: Prevalence of markers of transfusion transmissible diseases in voluntary and replacement blood donors. The National Medical Journal of India, 2004;171:19-21.
14. Gupta N, Kumar V, Kaur A: Seroprevalence of HIV, HBV, HCV and syphilis in voluntary blood donors. Ind Jl of Med Sci, 2004;58:255-257.
15. Nazar H, Nadia N, Shazia A, Zulfiqar, Farhat A. Prevalenc of hepatitis B and C in blood donors in Karachi. Biomedica 2008;24:116-117
16. Shrestha AC, Ghimre P, Tiwari BR, RajKarnikar M. Transfusion- Transmissible infections among blood donors in Kathmandu, Nepal. J Infect Dev Ctries 2009;3:794-797
17. Rodenas JG, Bacasen LC, Que ER. The prevalence of HBsAg (+) anti HCV(+) among healthy blood donors at East Avenue Medical Centre, Quezon City. Phil J Gastroenterol 2006;2:64-70
18. Kapur S, Mittal A. Incidence of HIV infection and its predictors in blood donors in Delhi. Indian J Med Res 1998;108:45–50.

Table 1 showing trend of TTI reactivity among males and females

TTD Reactive(n=1755)	**Male**	**Female**
HCV	879	18
HBV	611	15
HIV 1&2	142	1
VDRL	73	2
MP	14	-
Total	**1719**	**36**

Table 2 showing dual reactivity among Blood Donors

TTD.s(Dual reactivity) n=23	**Number**
HBV+ HCV	14
HIV1&2 + HCV	06
HBV+ HIV1&2	01
HBV + VDRL	01
HCV+ VDRL	01

Fig 1 shows distribution of Transfusion Transmitted diseases (n=1755) among blood donors

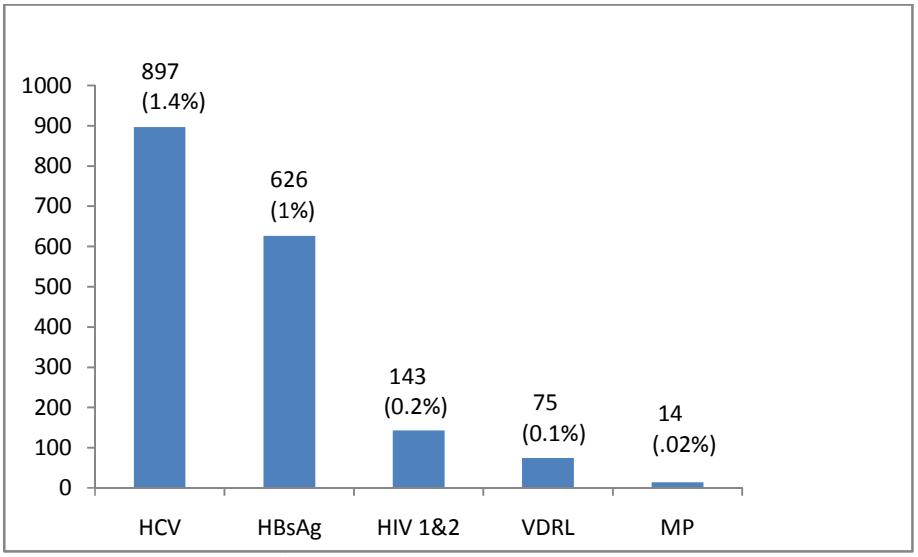


Fig 2 showing age groups among various TTD reactive blood donors

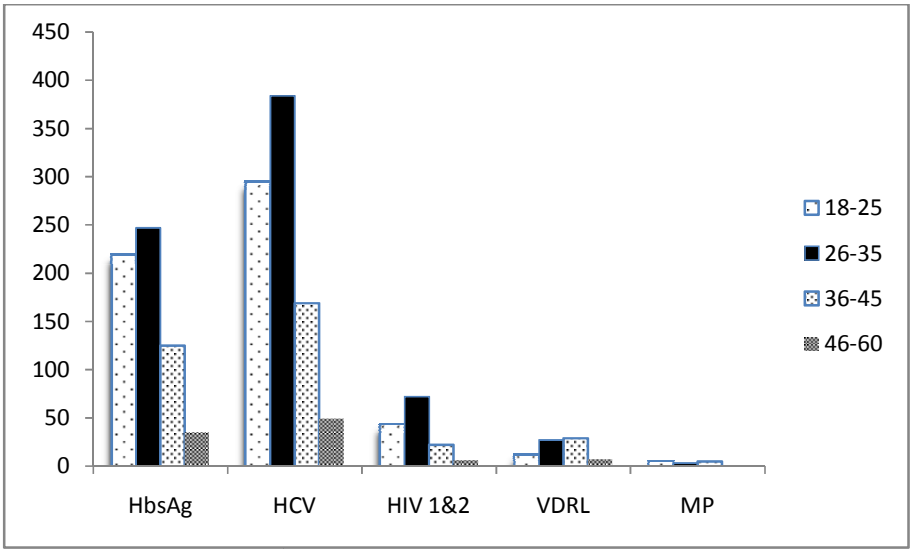
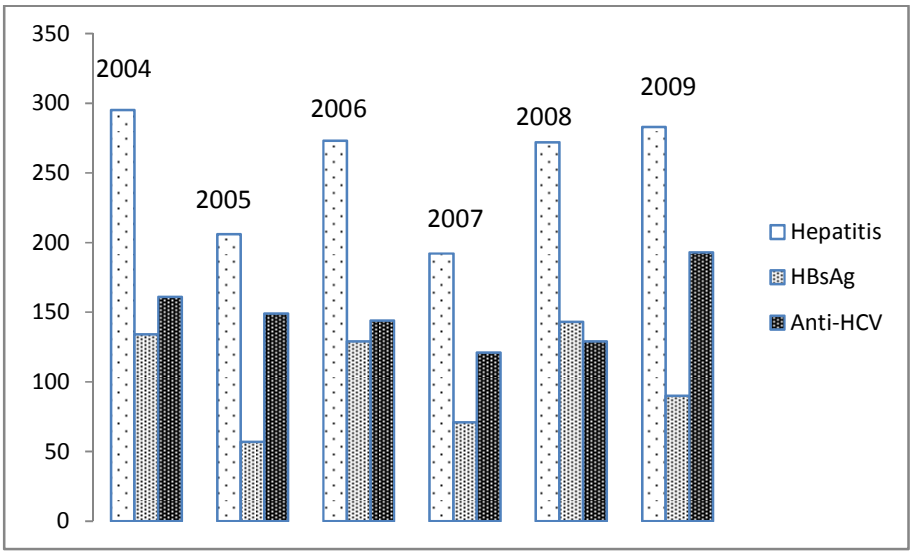


Fig 3 showing prevalence of Hepatitis among blood donors



DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE) IN HOSPITALIZED PATIENTS AND COMPARISION OF KIRBY-BAUER DISC DIFFUSION AND VANCOMYCIN SCREEN AGAR METHOD.

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ABSTRACT: The increasing occurrence of *Enterococcus* species, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE). The appearance of VRE has limited the therapeutic options available for clinicians. VRE infection is the most common type of infection acquired by patients while hospitalized. Patients at risk for VRE are those who are already ill, and hospitalized, including individuals with diabetes, elderly, ICU patients, kidney failure patients, or patients requiring catheters.

Present study was undertaken to detect vancomycin resistance in enterococcal isolates from hospitalized patients and the comparison of Kirby-Bauer disc diffusion method and vancomycin agar screen method to screen for vancomycin resistance.

A total of 45 enterococcal isolates from various samples of hospitalized patients were speciated by standard biochemical reactions and screened to detect vancomycin resistance by Kirby-Bauer disc diffusion method and vancomycin agar screen method. Out of 45 enterococcal isolates, 26 (57.77%) were Enterococcus faecalis, 18 (40%) were Enrerococcus faecium and 1(3.33%) was Enterococcus avium. Resistance to vancomycin was 29% (13 isolates) where as 71% (32 isolates) were sensitive. By Kirby-Bauer disc diffusion method, 1 isolate (2%) showed resistance to vancomycin whereas by vancomycin screen agar, 13 (29%) showed resistance.

The study highlights that vancomycin screen agar method is more sensitive than Kirby-Bauer disc diffusion method for detecting vancomycin resistance in enterococcal isolates. This study also signals the emergence of VRE in the hospital and highlights the importance of screening for VRE in enterococcal isolates from various samples.

KEY-WORDS: Vancomycin resistant Enterococci, Kirby-Bauer disc diffusion, vancomycin screen agar

INTRODUCTION: The increasing occurrence of Enterococcus species, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE).[1] VRE has also been reported from some parts of India. [2-3] The appearance of VRE has limited the therapeutic options available for clinicians.VRE infection is the most common type of infection acquired by patients while they are hospitalized.[4] Patients at risk for VRE are those

who are already ill, and hospitalized, including individuals with diabetes, elderly, ICU patients, kidney failure patients, or patients requiring catheters. [4]

Present study was undertaken to detect Vancomycin resistance in enterococcal isolates from hospitalized patients and comparison of Kirby-Bauer disc diffusion method and Vancomycin agar screen method for Vancomycin resistance screening.

MATERIALS AND METHODS: A total of 45 enterococcal isolates, 28 from urine, 6 from brain abscess, 4 from CSF, 2 from blood, 2 from wound swab, 2 from ear swab, and 1 from tracheal secretion, were included in the study. Refer Table1 for details. They were identified and speciated by standard biochemical tests.[5]

Susceptibility to Vancomycin was performed by Kirby-Bauer Disc Diffusion Method (KBDDM) [6] on Mueller Hinton Agar by using 30µg Vancomycin disc (HiMedia).

Vancomycin resistance was also determined by Vancomycin agar screen method using 6µg/ml of Vancomycin incorporated in Brain Heart Infusion (BHI) agar.[6] BHI agar with 6 µg of Vancomycin was inoculated from a direct colony suspension equivalent to a 0.5 McFarland standard using a swab. The plates were incubated at 35 ± 2 °C for 24 hours and later examined carefully for evidence of small colonies (>1 colony) or a film of growth, indicating Vancomycin resistance.[6]

RESULTS: Out of the 45 enterococcal isolates, 57.77% (26) were Enterococcus faecalis, 40% (18) were Enterococcus faecium and 3.33% (1) were Enterococcus avium. Refer Table2 for details.

29% (13 isolates) were resistant to Vancomycin where as 71% (32 isolates) were sensitive. Refer Table3 for details.

One of the isolates (2%) showed resistance to Vancomycin by Kirby-Bauer disc diffusion method (Figure 1). By Vancomycin screen agar, 13 isolates showed growth, giving an overall VRE positivity of 29% (Figure 2).

In this study, all the 13 VRE isolates were Enterococcus faecium.

In this study, the Vancomycin screen agar method was found to be more sensitive in comparison to Kirby-Bauer disc diffusion method for detecting Vancomycin resistance in enterococcal isolates (Figure 3).

DISCUSSION: Vancomycin-resistant Enterococci (VRE) infection is the most common type of infection acquired by patients while hospitalized.[4] Patients at risk for VRE are those who are already ill, and hospitalized, including individuals with diabetes, elderly, ICU patients, kidney failure patients, or patients requiring catheters. [4]

Enterococci are currently ascendant nosocomial pathogens, having become the second most common organisms recovered from nosocomial urinary tract and wound infections and the third most common cause of nosocomial bacteremia in the United States.[7]

There are the two types of Vancomycin resistance in Enterococci. The first type is intrinsic resistance. Isolates of Enterococcus gallinarum and E. casseliflavus/E. flavescens demonstrate an inherent, low-level resistance to Vancomycin. [5]

The second type of Vancomycin resistance in Enterococci is acquired resistance. Enterococci can become resistant to Vancomycin by acquisition of genetic information from another organism. Most commonly, this resistance is seen in E. faecium and E. faecalis, but also

has been recognized in E. raffinosus, E. avium, E. durans, and several other Enterococcal species.[5]

Several genes, including vanA, vanB, vanC, vanD, and vanE, contribute to resistance to Vancomycin in Enterococci.[5]

Vancomycin resistance in enterococci has coincided with the increasing incidence of high-level enterococcal resistance to penicillin and amino glycosides, thus presenting a challenge for physicians who treat patients who have infections caused by these microorganisms[8,9]

Transmission of VRE by health care workers whose hands become transiently contaminated with the organism while caring for affected patients is probably the most common mode of nosocomial transmission. Transmission of VRE may also occur by way of contaminated medical equipments, although this is probably much less important than transmission by the hands of personnel.[10]

In this study, out of 45 enterococcal isolates, 57.77% (26) were Enterococcus faecalis, 40% (18) were Enterococcus faecium and 3.33% (1) were Enterococcus avium. This is in comparison with other Indian studies.[2,3]

De A.et al reported out of the 200 Enterococcus species, 55% (110) were Enterococcus faecium, 31% (62) were Enterococcus fecalis and 14% (28) were other Enterococcus species.[11]

In this study, 29% (13 isolates) were resistance to Vancomycin where as 71% (32isolate) were sensitive. De A.et al reported 1.5% (3 isolates) were resistance to Vancomycin.[11]

In this study, one isolate (2%) was showed resistance to Vancomycin by Kirby-Bauer disc diffusion method and by Vancomycin screen agar, 13 were showed growth, giving an overall VRE positivity of 29%. De A.et al reported two isolates (1%) were resistant to Vancomycin by Kirby-Bauer disc diffusion method and by Vancomycin agar screen method, three isolates showed growth, giving an overall VRE positivity of 1.5%.

In this study, Enterococcus faecium was the commonest amongst VRE. De A.et al also reported Enterococcus faecium was the commonest amongst VRE.

This study showed Vancomycin screen agar method is more sensitive in comparison to Kirby-Bauer disc diffusion method for detecting Vancomycin resistance in enterococcal isolates

CONCLUSION: The study highlights that Vancomycin screen agar method is more sensitive than Kirby-Bauer disc diffusion method for detecting Vancomycin resistance in enterococcal isolates. This study also signals the emergence of VRE in the hospital and highlights the importance of screening for VRE in enterococcal isolates from various samples.

All laboratories should have effective detection methods for Vancomycin resistance, which will be helpful in reducing the morbidity and mortality due to VRE in hospitalized patients.

REFERENCES:

1. Zhanel GG, Laing NM, Nichol KA, Palatnick LP, Noreddin A, Hisanaga T, et al . Antibiotic activity against urinary tract infection (UTI) isolates of Vancomycin resistant Enterococci. J Antimicrob Chemother 2003; 52:382-88
2. Mathur P, Kapil A, Chandra R, Sharma P, Das B. Antimicrobial resistance in Enterococcus fecalis at a tertiary care center of Northern India. Indian J Med Res 2003; 118:25-28.

3. Taneja N, Rani P, Emmanuel R, Sharma M. Significance of Vancomycin resistant enterococci from urinary specimens at a tertiary care center in Northern India. Indian J Med Res 2004;119:72-74.
4. Murray BE. Vancomycin-resistant enterococcal infections. N Eng J Med 2000; 342: 710-21.
5. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 5 th Ed. Lippincott, Philadelphia. 1997; pp577-650.
6. Performance Standards for Antimicrobial Susceptibility Testing. Seventeenth Informational Supplement. Clinical Laboratory Standards Institute. 2007; M100-S17;27(1):p53.
7. Schaberg, D. R., D. H. Culver, and R. P. Gaynes. 1991. Major trends in the microbial etiology of nosocomial infection. Am. J. Med. 91(Suppl. 3B)**:**72S–75S.
8. 8.Handwerger S, Raucher B, Altarac D, et al. Nosocomial outbreak due to Enterococcus faecium highly resistant to Vancomycin, penicillin, and gentamicin. Clin Infect Dis 1993; 16:750-55.
9. CDC. Nosocomial enterococci resistant to Vancomycin -- United States, 1989-1993. MMWR 1993; 42:597-99.
10. Tornieporth, N. G., R. B. Roberts, J. John, A. Hafnier, and L. W. Riley**.** 1996. Risk factors associated with Vancomycin-resistant Enterococcus faecium infection or colonization in 145 matched case patients and control patients. Clin. Infect. Dis. 23**:**767–772.
11. De A, Bindlish A, Kumar S, Mathur M. Vancomycin resistant enterococci in a tertiary care hospital in Mumbai. Indian J Med Microbiol 2009; 27:375-76

Table 1: Enterococcal isolates from various specimens

Sl no.	Specimen	Number
1	Urine	28
2	Brain abscess	6
3	CSF	4
4	Blood	2
5	Wound swab	2
6	Ear swab	2
7	Tracheal secretions	1
	Total	45

Table 2: Distribution of enterococcal isolates

Sl.no	Isolate	Number of isolates	Percentage
1	E. feacalis	26	57.77%
2	E. faecium	18	40%
3	E. avium	1	2.22%
	Total	45	100

Table 3: Vancomycin susceptibility pattern of enterococcal isolates

Total number of isolates	Vancomycin susceptibility	
	S	R
45	32 (71%)	13 (29%)

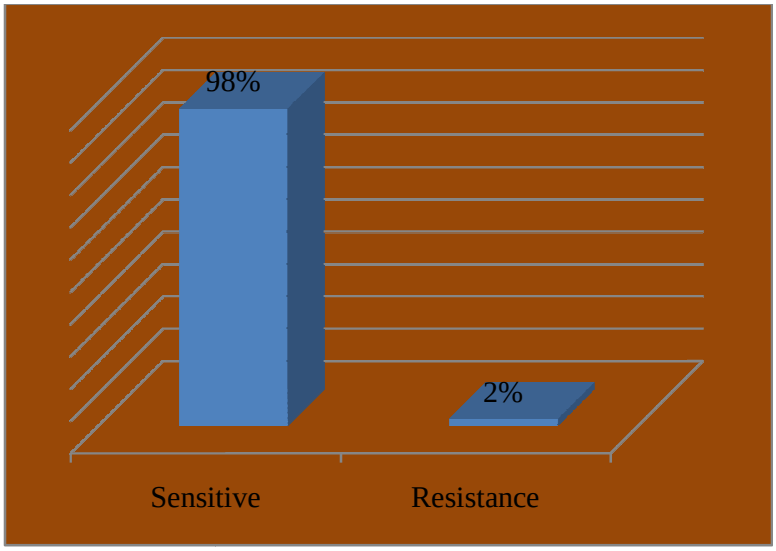

Figure1: Schematic representation of vancomycin susceptibility pattern of enterococcal isolates by disc diffusion method

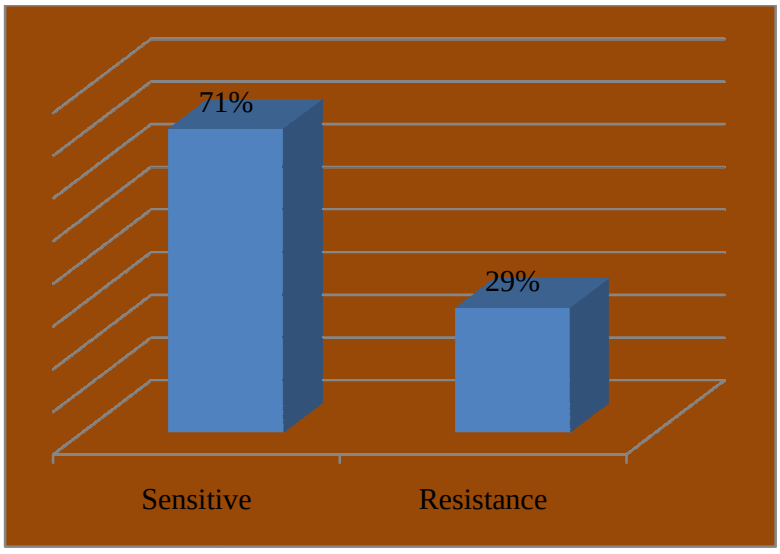

Figure2: Schematic representation of vancomycin susceptibility pattern of enterococcal isolates by vancomycin screen agar

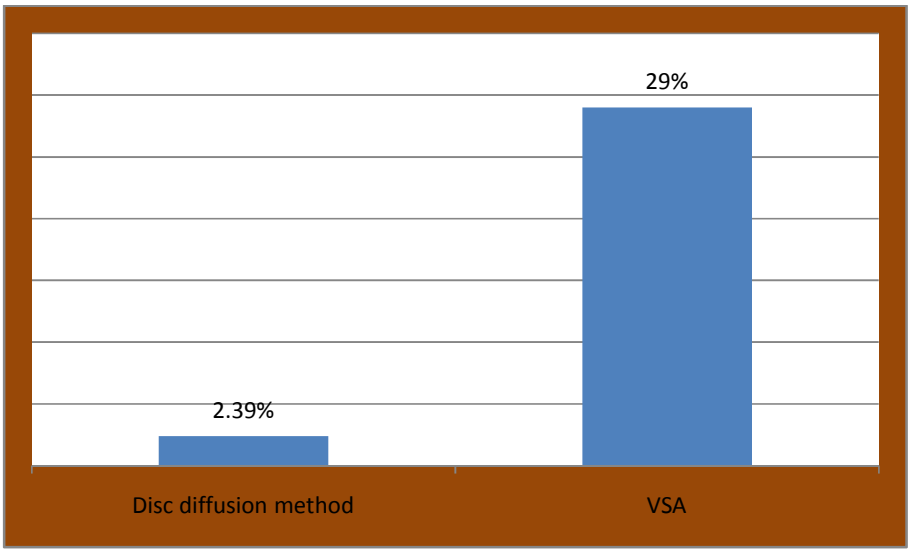

Figure 3: Comparison of enterococcal resistance by disc diffusion method and vancomycin screen agar

CAECAL PERFORATION SECONDARY TO IDIOPATHIC CAECAL DILATATION– AN INTERESTING CASE

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ABSTRACT: Acute pseudo-obstruction of the colon(Ogilvie's syndrome) involves acute colonic distension without mechanical obstruction or stercoroma in a previously healthy colon. Colonic pseudo-obstruction can be diffuse or segmental. The cause for idiopathic colon dilatation is not known. Caecal rupture due to colonic ileus is rare and has a mortality rate of 43 per cent. We report a case of idiopathic caecal dilatation causing caecal perforation. On emergency laparotomy, caecal perforation secondary to segmental caecal dilatation without organic obstruction was found in this patient.

KEYWORDS: Acute pseudo-obstruction of the colon; Idiopathic caecal dilatation; Caecal perforation; Emergency laparotomy

MESH TERMS: Colonic Pseudo-Obstruction; Intestinal Pseudo-Obstruction, Idiopathic; Intestinal Perforation; Laparotomy

INTRODUCTION: Colonic pseudo-obstruction is a type of adynamic ileus. It can be "idiopathic" or can complicate other diseases or surgical procedures (urological and gynaecological procedures mostly). Conservative treatment is the method of choice but when the caecal diameter is more than 12 cm (impending perforation), when the colon is perforated or when medical measures are unsuccessful, surgical procedure is compulsory. Even with a proper management, the prognosis is severe and the mortality rate is high (3-50%).

We report this case of idiopathic caecal dilatation causing caecal perforation as it can be often misdiagnosed and has a high mortality rate. Hence, always consider colonic pseudo-obstruction as a differential diagnosis for any case of subacute or acute large bowel obstruction.

CASE REPORT: A 25 year old female patient was admitted with abdominal pain, abdominal distension, fever and vomiting of 3days duration. Patient was never admitted for any complaints in the past nor has undergone any abdominal surgeries.

General examination revealed a pulse rate of 72/min, blood pressure of 120/80mmhg with normal temperature. On systemic examination, abdomen was soft and distended, shifting dullness was present. No guarding or rigidity. Bowel sounds were sluggish. All blood investigations were within normal limits. Erect x ray abdomen showed dilated large bowel loops with few air fluid levels but no air under diaphragm (Fig.1). Ultrasound abdomen showed features of subacute intestinal obstruction with ascites.

Patient was put on conservative treatment. She improved symptomatically but the symptoms recurred after a period of 10days. Clinically, patient became sick with a palpable mass in the right iliac fossa. CECT abdomen revealed grossly dilated caecum and multiple loculated collections in pelvis with thickened omentum and peritoneum (Fig.2).

Patient underwent emergency laparotomy. Grossly dilated caecum of >10cm diameter with 0.5x0.5cm caecal perforation was noted (Fig.3). Caecum was not mobile. No obstruction in the distal colon noted. Appendix was normal and found adhered to the caecum retrocaecally. Omental and peritoneal thickening with pelvic exudates were noted.

First caecum was decompressed. Then, Right hemicolectomy with side to side ileo-transverse anastomosis was done. Peritoneal lavage done and bilateral pelvic drains placed. Patient was given total parenteral nutrition for 1week and her general condition was improved. Patient postop recovery was uneventful and was discharged on postop day 14. Histopathological examination of the specimen revealed no obvious pathology responsible for the segmental large bowel dilatation and caecal perforation.

DISCUSSION: Pseudoobstruction of the colon is a specific variety of adynamic ileus. The clinical and radiologic picture closely resembles mechanical obstruction of the large bowel (1).

The pathophysiology of the syndrome is still unknown. Ogilvie, who first described the syndrome in 1948, suggested an imbalance between the sympathetic and parasympathetic innervation of the colon (2).

The most relevant clinical finding in Ogilvie's syndrome is abdominal distension, which arises suddenly, has a progressive course and reaches massive levels. The first-line diagnostic investigation is plain abdominal radiography which shows extreme colon dilation without air-fluid levels of the small intestine (3).

The bowel distension in colonic pseudo-obstruction is from swallowed air so stop oral intake and decompress the stomach with nasogastric tube. Goal is to prevent colonic perforation as acute pseudo-obstruction of colon is self-limiting (4). Caecal rupture due to colonic ileus is rare and has a mortality rate of 43 per cent. The disease has always occurred in association with another illness, has usually afflicted patients over the age of 55 and has only resulted when the caecum was at least 9 cm in diameter (5).

The acute colonic pseudo-obstruction, Ogilvie's Syndrome, most often appears as a complication of other clinical conditions like restrictive respiratory dysfunction (6). Principal associated diseases are cardiopulmonary insufficiencies, postoperative conditions, and systemic disorders (7).

There are many other conditions that mimic colonic pseudo-obstruction which are vascular lesions of gut, lead poisoning, porphyria, acute pancreatitis, acute cholecystitis, acute appendicitis, acute dysentery and morphine overdosage (8).

Pseudo-obstruction of the colon is a potentially lethal condition. The diagnosis should be suspected in a patient with derangement of a major extra-abdominal organ system in whom abdominal distension develops (9). The most severe complication is cecal rupture, which may occur at a diameter of 12 cm or more and which has a lethality rate of 40% (10).

In 1978, authors reported a new case of caecal perforation complicating acute dilatation of the colon without organic obstruction (Ogilvie's syndrome). They recall the two characteristics of this syndrome : abdominal distension due to colonic ileus, without any organic cause, and the constant coexistence of an associated pathological condition (traumatic, post-

operative, infective, cardio-vascular, respiratory or neurological). The pathogenesis of Ogilvie's syndrome remains mysterious (11).

Diagnosis is generally made on the basis of the plain roentgenogram of the abdomen and a barium enema of the colon (12). Endoscopic decompression and tube placement is effective and safe for acute colonic pseudo-obstruction not responding to 24 hour conservative treatment (13). Surgery should only be envisaged when there are setbacks, due to the seriousness of the operation and the possibility of postoperative complications (14).

In reviewing 750 cases of acute colonic pseudo-obstruction from the literature, the most commonly associated disorders are listed and the therapeutical management is critically discussed. Ogilvie's syndrome has been used synonymously with acute colonic pseudo-obstruction (ACPO) of the colon, first defined by Sir Heneage Ogilvie in 1948. If inappropriately managed, the massive colonic dilatation may lead to caecal ischaemia and perforation with a high mortality rate. Conservative treatment is indicated if the caecum is less than 12 cm in diameter. If there is a progressive increase in diameter or no improvement is seen, the colon should be decompressed without further delay. The indications for surgery are failure of conservative treatment and colonoscopy, signs for ceacal ischaemia or perforation. The choice of procedure, caecostomy or resection, is dictated by the state of the caecum. Due to a high mortality rate (up to 50%) if the caecum is perforated, an aggressive therapeutical management should be applied (15).

REFERENCES:

1. Bachulis BL, Smith PE. Pseudoobstruction of the colon. Am J Surg. 1978 Jul;136(1):66–72. [PubMed]
2. Nanni G, Garbini A, Luchetti P, Nanni G, Ronconi P, Castagneto M. Ogilvie's syndrome (acute colonic pseudo-obstruction): review of the literature (October 1948 to March 1980) and report of four additional cases. Dis Colon Rectum. 1982 Mar; 25(2):157-66. Review. PubMed PMID: 7039994.
3. Grassi R, Cappabianca S, Porto A, Sacco M, Montemarano E, Quarantelli M, Di Mizio R, De Rosa R. Ogilvie's syndrome (acute colonic pseudo-obstruction): review of the literature and report of 6 additional cases. Radiol Med. 2005 Apr; 109(4):370-5. Review. English, Italian. PubMed PMID: 15883521.
4. Nivatvongs S, Vermeulen FD, Fang DT. Colonoscopic decompression of acute pseudo-obstruction of the colon. Ann Surg. 1982 Nov;196(5):598–600. [PMC free article] [PubMed]
5. Gierson ED, Storm FK, Shaw W, Coyne SK. Caecal rupture due to colonic ileus. Br J Surg. 1975 May;62(5):383–386. [PubMed]
6. Georgescu S, Dubei L, Zaharia M, Cîrdei C, Crumpei F, Cijevschi-Prelipcean C, Stoian M. Ogilvie's syndrome--acute colonic pseudo-obstruction. Case report and review of the literature. Rom J Gastroenterol. 2003 Mar;12(1):51-5. Review. PubMed PMID: 12673381.
7. Wegener M, Börsch G. Acute colonic pseudo-obstruction (Ogilvie's syndrome).Presentation of 14 of our own cases and analysis of 1027 cases reported in the literature. Surg Endosc. 1987;1(3):169-74. Review. PubMed PMID: 3332478.
8. Stephens FO. Syndrome of Intestinal Pseudo-obstruction. Br Med J. 1962 May 5;1(5287):1248–1238.2. [PMC free article] [PubMed]
9. Melzig EP, Terz JJ. Pseudo-obstruction of the colon. Arch Surg. 1978 Oct;113(10):1186–1190. [PubMed]

10. Ritschard T, Filippini L. [Acute pseudo-obstruction of the colon (Ogilvie's syndrome). Internal medicine aspects]. Schweiz Med Wochenschr. 1985 May 4;115(18):614-9. German. PubMed PMID: 3839093.
11. Leborgne J, Pannier M, Le Neel JC. [Cecum perforation, complication of an acute idiopathic dilatation of the colon, or Ogilvie's syndrome. Practical interest of various pathogenic data. Apropos of one case]. Sem Hop. 1978 Jun;54(17-20):665-8. French. PubMed PMID: 211619.
12. Schippers E, Raguse T, Brenner P, Dyballa G. [Pseudo-obstruction of the colon]. Zentralbl Chir. 1983;108(20):1249-62. Review. German. PubMed PMID: 6359773.
13. Naef M, Maurer CA, Scheurer U, Seidel K, Langen HP, Sell F, Büchler MW. [Idiopathic dilatation of the large intestine (Ogilvie syndrome—acute pseudo-obstruction)]. Zentralbl Chir. 1998;123(12):1360-4. German. PubMed PMID: 10063545.
14. Pinta P, Offenstadt G, Guidet B, Senouci M. [Syndrome of acute colonic pseudo-obstruction (Ogilvie's syndrome). Study of 13 cases]. Ann Gastroenterol Hepatol (Paris). 1986 Mar-Apr;22(2):69-76. French. PubMed PMID: 3717887
15. Meier C, Di Lazzaro M, Decurtins M. [Ogilvie syndrome with cecal perforation. A rare complication after isolated thoracic trauma. Case report and current literature review]. Swiss Surg. 2000;6(4):184-91. Review. German. PubMed PMID: 10967947.

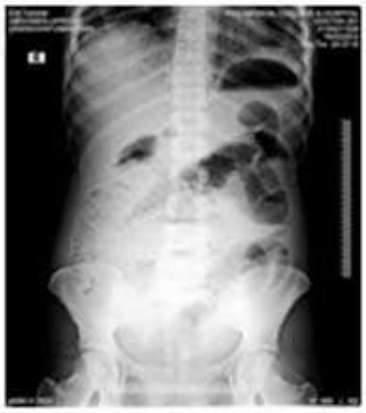

Fig.1 Dilated large bowel loops with distended Caecum and Right colon

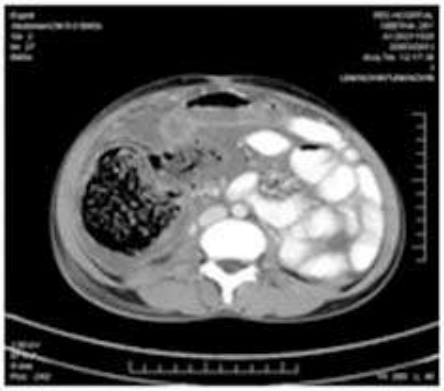

Fig.2 Grossly dilated caecum of>10cm diameter

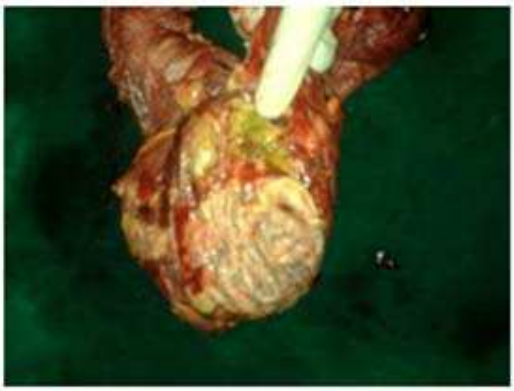

Fig. 3 Right hemicolectomy specimen showing decompressed caecum with perforation