

**STUDY OF EPIDEMIOLOGICAL MARKERS AND SEROPREVALENCE OF CHLAMYDIA TRACHOMATIS IN FEMALE STI ATTENDEES**Atul R. Rukadikar<sup>1</sup>, Sharmila S. Raut<sup>2</sup>, Supriya S. Tankhiwale<sup>3</sup>, Kiran R. Munne<sup>4</sup>, S. G. Joshi<sup>5</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: INTRODUCTION:** Chlamydia trachomatis is the leading cause of sexually transmitted infections (STIs). Chlamydial infections, if undiagnosed and untreated can result in irreversible sequelae. Infected patients serve as a reservoir of infection to their partners. The present study was designed to determine the epidemiological markers and seroprevalence of genital Chlamydia in female STI patients attending STI clinics. **METHODS:** A total of 226 clinically suspected cases of Sexually Transmitted Infection (STI) patients attending STI clinic were studied for seroprevalence of chlamydia trachomatis along with age, marital status, history of sexual contact and contraception use. Patients were investigated for the presence of IgG antibody of chlamydia trachomatis with ELISA (Novatech, Germany). **RESULTS:** Seroprevalence of chlamydia trachomatis was found to be 55.66% for IgG by ELISA. Genital Chlamydia was more commonly seen in sexually active group (21-30 years). Highest prevalence was seen in married patients (53.75%), in patients who had history of sexual contact (61.25%) and who were using oral contraceptive pills (63.93%) as a contraceptive method. **CONCLUSION:** Though tissue culture is gold standard, serological assays are much simpler, sensitive and rapid methods for detection of chlamydia trachomatis. Co-infection of Chlamydia with other STIs highlights the importance of early laboratory diagnosis and specific treatment.

**KEYWORDS:** Chlamydia trachomatis, STI patients.

**INTRODUCTION:** The sexually transmitted diseases are the group of communicable diseases that are transmitted by sexual contact and caused by a wide range of bacterial, viral, protozoal, fungal agents and ectoparasites. During the past two decades, STDs have undergone a dramatic transformation, first the change in name from venereal diseases to STD indicate this change. Minimal estimates of yearly incidence for four major bacterial STD are, Bacterial STD: Gonorrhoea – 62 million, Genital chlamydial Infection – 92 million, Syphilis – 12 million, Chancroid – 7 million<sup>1</sup>. Among the bacterial causes, Chlamydia trachomatis have currently emerged as most prevalent among bacterial STDs causing genital infection. Despite increased awareness of the importance of infections with chlamydia trachomatis little is known about its prevalence.<sup>2</sup> Worldwide, it is estimated that there are more than 50 million new cases of chlamydia trachomatis infection annually. The prevalence of chlamydia trachomatis infection in sexually active adolescent women population, considered most at risk, generally exceeds 10% and in some adolescent and STD clinic population of women, the prevalence reached 40%.

Most women infected with chlamydia trachomatis remain asymptomatic & lesion is often unnoticed & for men rate of asymptomatic chlamydial infection is higher than symptomatic gonorrhoeal infection. If left untreated, it can lead to irreversible clinical sequelae like pelvic inflammatory disease, ectopic pregnancy, cervicitis, urethritis, tubal infertility, endometritis,

## ORIGINAL ARTICLE

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abscesses of Bartholin gland etc. in Female and Nongonococcal urethritis, epididymitis, Reiter's Syndrome etc. in Male and neonatal conjunctivitis, pneumonia etc. in infants.

Early diagnosis and treatment of affected individuals are type of strategies necessary to prevent development of irreversible sequel and to reduce transmission. The diversity of laboratory procedures and the controversies which surround some of them are bewildering to those who are contemplating a laboratory service for the identification of *C. trachomatis* infection. The choice of methods will depend on the level of diagnostic service required and on the laboratory resources which are available.<sup>2</sup> However, simpler procedures for the isolation or detection of chlamydia trachomatis are needed. With this background, attempt was made in the present study, to determine the seroprevalence of chlamydia trachomatis and its association with various epidemiological markers.

**MATERIAL & METHODS:** A total of 226 female patients attending STI clinic were included in the study. Detailed history including age, occupation, marital status, sexual history, contraceptive methods was taken. A through general, systemic and local examination of all suspected STI patients was done.

### INCLUSION CRITERIA:

- 1) Patient's willingness to participate after written informed consent.
- 2) Patient's between age group of 15 – 40 years.
- 3) Patient with responsible accompanying person (guardian).
- 4) Patient with following complaints:
  - H/o vaginal discharge.
  - H/o urethral discharge.
  - H/o fever, rash
  - H/o burning micturition.
  - H/o lower backache.

### EXCLUSION CRITERIA:

- Non willingness of patients.
- Age less than 15 yrs. & more than 40 yrs.
- Patients on antibiotic therapy.

### METHOD OF COLLECTION OF BLOOD SAMPLE:

#### A) BLOOD:

After brief counseling and informed written consent, 5 ml blood was withdrawn from antecubital vein under strict aseptic precautions in plain sterile test tube. Serum was separated and stored at -20°C. Serum samples were processed for Chlamydia trachomatis (IgG) antibody by using ELISA test (Nova Tec, Chlamydia trachomatis IgG ELISA, manufactured by Nova Tec Immunodiagnostica, Germany), as per manufacturer's instructions.

**RESULTS:** Out of 226 female patients studied, 104 (46.01%) were in 26-30 yrs. Seventy six (33.62%) were in 21-25 yrs. age group, 32 (14.15%) in 31-35 yrs. age group, 11 (4.86%) in 15-20yrs age group

## ORIGINAL ARTICLE

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and 3 (1.32%) belonged to 36-40 yrs. age group. The minimum age of the patient studied was 15 years and maximum age was 40 years. (Table no.1).

In the study group, Genital chlamydia 103(45.57%) and Bacterial vaginosis 54(23.89%) accounted for the majority of STI. These were followed by Candidiasis 34(15.04%), Trichomoniasis 18 (07.96%) and Gonorrhoea 07(03.09%) respectively. HIV and Syphilis were diagnosed only in 06(02.65%) and 04(01.76%) cases respectively. (Table no.2)

Of the total 226, 201 (88.93%) were married while the group of unmarried comprised of 25 (11.06%). No study subject belonged to category of widow, divorced or separated. The entire study subjects were non-pregnant. (Table no.3)

The ELISA test, to detect chlamydial antibody (IgG) was carried out over a sample of 180. Chlamydial antibody (IgG) was detected in 102 clinical samples, giving an overall positivity rate of 55.66%. C.trachomatis antibody could not be detected in 78 (43.33%) clinical samples. (Table no: 4)

Of the total 180 samples, 10 in the age group of 15-20 years 07 (70%) were positive and 03 (30%) were negative for C.trachomatis. In the age group 21 -25 years 39 (62.90%) were positive of the total 62 subjects. The remaining 23 (37.09%) were negative. Of the total 78 in the age group 26 -30 years, 45 (57.69%) and 33 (42.30%) were positive and negative respectively. Of the total 29 in the age group 31-35 years, 10 (34.48%) were positive and 19 (65.51%) were negative. The only one subject (0.55%) in the age group 36 -40 years was positive for C.trachomatis. The decreasing frequency of C.trachomatis detection by ELISA test is evident as the age advances. The difference between frequency of C.trachomatis below and above 25 years of age shows statistical significance, p value being less than 0.05. (Table no: 5)

Of the total 180 study subjects, whose clinical samples were subjected to ELISA test, 160 (88.88%) were married and 20 (11.11%) were unmarried. Of the total 160, 86 clinical samples were positive for ELISA test, giving an overall positivity rate of 53.75%. In 74 (46.25%) samples C.trachomatis antibody could not be detected and hence were negative by ELISA test. Of the 20, 16 (80%) and 04 (20%) were positive and negative for C.trachomatis antibody (IgG) respectively. Thus the frequency of C.trachomatis in married and unmarried patients shows statistical significance in the present study, p value being less than 0.05. (Table no: 6)

The relationship between the history of sexual contact of the study subjects and ELISA results were evaluated. Of the 180 study subjects, whose clinical specimens were subjected to ELISA test, 160 (88.88%) had history of sexual contact either with single or multiple partners and 20 (11.11%) did not report the same. Of the 160, 98 (61.25%) were positive by ELISA test and 62(38.75%) could not reveal C.trachomatis antibody and hence were negative by ELISA test. Of the 20, who did not have history of any sexual contact, only 04 (20%) were positive and 16 (80%) were negative for ELISA (IgG) test. When the results were subjected to statistical treatment, this difference was found to be statistically significant, p value being less than 0.05. (Table no: 7)

Of the total 180 subjects, 78 (43.33%) did not use any contraceptive method while 102 (56.66%) used one or other methods of contraception. Of the total 78, who did not use any contraception 49 (62.82%), had C.trachomatis infection. Out of 61 study subjects who used oral contraception 39 (63.93%) had C.trachomatis infection. C.trachomatis could be detected in 12 (34.28%) of 35 study subjects who used IUCD. In the small subsamples of 06 subjects who used barrier methods only 02 (33.33%) had C.trachomatis infection. No statistical significance was found between the subjects who used and not used contraceptive methods. (Table no: 8)

## ORIGINAL ARTICLE

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**DISCUSSION:** In the present study, majority of the patients 104(46.01%) were belonged to the age group 26-30 years followed by 76(33.62%) in the age group of 21-25 years. Westorm L and Mardh PA (1983)<sup>3</sup> has discussed age as an important epidemiological factor. Estimates during 1960's and 1970's in Europe and in U.S.A agreed upon higher annual incidence in the age group 15-30 years. The findings of the present study are in consistent with that reported in literature. Thus the younger sexually active age group is one of the important factors for acquiring infection.

In the present study, 180/226 specimens were subjected to ELISA (IgG). Of 180 samples, 102(55.66%) were positive for *C. trachomatis* antibody (IgG) by ELISA test. Malhotra M et al (2008)<sup>4</sup> reported a low 10.9% seropositivity in STD cases. Eckert LO et al (1997)<sup>5</sup> reported 22.5% chlamydial seropositivity while Joyee AG et al (2007)<sup>6</sup> reported 58.7% in STD cases. Thus, wide range of chlamydial seropositivity has been reported in the literature. This noted difference could be because of the differences in the antigen used in the kits. Of 102 ELISA positive samples, 62.90% belong to age group 21-25years, while 57.69% belong to age group 26-30years, 34.48% belong to age group 31-35years. The decreasing frequency of chlamydia trachomatis detection by ELISA is evident as the age advances. The difference between frequency of chlamydia trachomatis below and above 25years are statistically significant ( $p < 0.05$ ). The present study findings are concurrent with the other studies reported in literature.

The frequency of chlamydia trachomatis detection in married and unmarried patients differed in the present study, giving an overall positivity of 53.75% in married and 80% in unmarried patients.

Magder LS et al (1988)<sup>7</sup> has stated that marital status was not a significant predictor of chlamydial isolation in men but in women, those who were married were significantly less likely to be positive.

In the present study, of the 160 who had history of sexual contact 98(61.25%) were ELISA (IgG) positive and 62 (38.75%) were ELISA negative for chlamydia trachomatis.

From the present study observation the role of sexual activity was found to be statistically significant ( $p < 0.05$ ) in giving higher incidence of chlamydia trachomatis infection.

Chlamydia trachomatis is isolated from cervical material from the female sexual partner of men with chlamydia positive NSU and only rarely from the sexual contacts of men with chlamydia negative NSU. The role of sexual activity in giving higher incidence of chlamydia trachomatis infection is well documented in literature.

In the present study there was significant difference between the rate of cervical chlamydial detection in women who used oral contraceptive and no use of any contraceptive method compared with women who used IUCD and barrier methods. The overall positivity rate of 63.93% and 62.82% was observed in oral contraceptive users and who used no method of contraception. 34.28% of IUCD users had chlamydia trachomatis infection while 33.33% barrier method users had chlamydia trachomatis infection. Magder LS (1988)<sup>7</sup> has assessed the contraceptive use in relation to genital chlamydia trachomatis. In their study none of 77 women who used diaphragm were positive for chlamydia trachomatis at the cervix compared with 20% of those used no method of contraception. There was no significant difference between the rates of cervical chlamydial isolation in women who used oral contraception compared with women who used no contraceptives. Hilton AL et al (1974)<sup>8</sup> have reported that hormonal factors may influence the chlamydial isolation rate, since it was greater in patients using oral contraception than those who did not. Harrison RH et al (1985)<sup>9</sup> have found

## ORIGINAL ARTICLE

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that, women who used IUCD or barrier methods had less infection (2%) than women who used oral contraception (14%) or none at all (10.7%). McCormack WM et al (1985)<sup>10</sup> have found in their study that sexually experience women who used barrier methods of contraception were less likely to be infected (1%) than those who used other contraception measures or those who did not use the contraception (7.2%). Women who used barrier methods of contraception also were less likely to have local chlamydial antibody.

**CONCLUSION:** Co infection of chlamydia with other STI/RTI's highlights the importance of early laboratory diagnosis and specific treatment of the condition as they increase the risk many folds when the infections exist together. The high seroprevalence observed in the present study indicated that the exposure rate to chlamydial infection in STD patients is very high. In view of potential clinical sequelae and subsequent morbidity associated with chlamydial infection, it may be ideal to employ non-invasive serological tests to identify chlamydial etiology and to initiate treatment in patients, particularly in clinical settings where cell culture and costly molecular methods are not feasible. The observations of the current study thus, reinforce the importance of routine screening for *C. trachomatis* as a necessary intervention to decrease the burden of chlamydial disease and to reduce the risk of its spread.

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## ORIGINAL ARTICLE

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| Age (Yrs.) | No of patients. | Percentage |
|------------|-----------------|------------|
| 15-20      | 11              | 4.86%      |
| 21-25      | 76              | 33.62%     |
| 26-30      | 104             | 46.01%     |
| 31-35      | 32              | 14.15%     |
| 36-40      | 03              | 1.32%      |
| Total.     | 226             | 100%       |

**Table No. 1: Age distribution of study subjects (n=226)**

| Clinical Diagnosis  | Number | Percentage |
|---------------------|--------|------------|
| Chlamydiasis        | 103    | 45.57%     |
| Bacterial Vaginosis | 54     | 23.89%     |
| Candidiasis         | 34     | 15.04%     |
| Trichomoniasis      | 18     | 07.96%     |
| Gonorrhea           | 07     | 03.09%     |
| HIV                 | 06     | 02.65%     |
| Syphilis            | 04     | 01.76%     |
| Total               | 226    | 100%       |

**Table No. 2: STI in study group according to clinical diagnosis (n=226)**

| Marital status | No. of the patients | Percentage |
|----------------|---------------------|------------|
| Married        | 201                 | 88.93%     |
| Unmarried      | 25                  | 11.06%     |
| Total          | 226                 | 100%       |

**Table No. 3: Marital status of the patients (n=226)**

| ELISA    | No. of patients | Percentage |
|----------|-----------------|------------|
| Positive | 102             | 55.66%     |
| Negative | 78              | 43.33%     |
| TOTAL    | 180             | 100%       |

**Table No. 4: Results of ELISA (IgG)**

## ORIGINAL ARTICLE

| Age group            | ELISA   |             | Total       |
|----------------------|---|-------------|-------------|
|                      | Positive  | Negative    |             |
| 15-20                | 07 (70%)  | 03 (30%)    | 10 (05.55%) |
| 21-25                | 39 (62.90%)   | 23 (37.09%) | 62 (34.44%) |
| 26-30                | 45 (57.69%)   | 33 (42.30%) | 78 (43.33%) |
| 31-35`               | 10 (34.48%)   | 19 (65.51%) | 29 (16.11%) |
| 36 - 40              | 01 (100%)   | 00 (0%)     | 01 (0.55%)  |
| Total                | 102 (56.66%)  | 78 (43.33%) | 180 (100%)  |
| Test of significance | Odds Ratio (OR) = 2.66 (1.11- 6.49),<br>Chi Square ( $\chi^2$ ) = 5.86, p value < 0.05 (significant). |             |             |

**Table No. 5: Age distribution & ELISA Results**

| Marital status       | ELISA  |             | Total        |
|----------------------|--|-------------|--------------|
|                      | Positive   | Negative    |              |
| Married              | 86 (53.75%)  | 74 (46.25%) | 160 (88.88%) |
| Unmarried            | 16 (80%)   | 04 (20%)    | 20 (11.11%)  |
| Total                | 102 (56.66%)   | 78 (43.33%) | 180 (100%)   |
| Test of significance | Odds Ratio(OR) = 0.29 (0.08-0.98),<br>Chi Square ( $\chi^2$ )=4.99, p value < 0.05 (significant) |             |              |

**Table No. 6: Marital Status & ELISA (IgG)**

| H/O sexual contact   | ELISA   |             | Total        |
|----------------------|---|-------------|--------------|
|                      | Positive  | Negative    |              |
| Present              | 98 (61.25%)   | 62 (38.75%) | 160 (88.88%) |
| Absent               | 04 (20%)  | 16 (80%)    | 20 (11.11%)  |
| Total                | 102 (56.66%)  | 78 (43.33%) | 180 (100%)   |
| Test of significance | Odds Ratio (OR)= 6.32 (1.86-23.57),<br>Chi square( $\chi^2$ ) = 12.32, p value < 0.05 (significant) |             |              |

**Table No. 7: History of Sexual contact & ELISA results**

| Contraceptive   | Number   | CT-Positive | Percentage |
|-----------------|--|-------------|------------|
| O.C.Pills       | 61   | 39          | 63.93%     |
| IUCD            | 35   | 12          | 34.28%     |
| Barrier Methods | 06   | 02          | 33.33%     |
| No Use          | 78   | 49          | 62.82%     |
| Total           | 180  | 102         | 56.66%     |
| Test            | Odds ratio (OR)=1.56 (0.82-2.98), Chi Square( $\chi^2$ ) =2.12,<br>p value = 0.14 (Not significant). |             |            |

**Table No. 8: Contraceptive use & Chlamydia Trachomatis ELISA (IgG)**

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