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SERO-PREVALENCE OF CHLAMYDIA TRACHOMATIS IN STI PATIENTS

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ABSTRACT: BACKGROUND: 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 prevalence of genital Chlamydia and its association with bacterial flora in STI patients attending STI clinics. **METHOD:** Genital discharge specimens (Endocervical, Vaginal) and Blood from 226 patients were collected as per standard procedures. Isolation and Identification of bacterial flora was carried out by conventional methods. Patients were investigated for the presence of antigen and antibody of chlamydia trachomatis with Immunochromatographic assay (Biomerieux) and ELISA (Novatech, Germany) respectively. **RESULTS:** Of 226 patients, 'Inclusion bodies' were found in 69/226 (30.53%) patients by Giemsa staining. Chlamydia trachomatis was found to be most commonly associated with Candida albicans (29.41%). Of 180 samples, 102/180 (55.66%) were positive for IgG by ELISA. Of 50 samples, 07/50 (14%) were positive for Chlamydia trachomatis antigen by immunochromatographic assay. Results of both the test were evaluated. **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.

BACKGROUND: 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: Gonorrhea – 62 million, Genital chlamydial Infection–92 million, Syphilis–12 million, Chancroid–7 million ¹. 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.² 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 C.trachomatis remain asymptomatic & lesion is often unnoticed & for men rate of asymptomatic chlamydial infection is higher than symptomatic gonorrheal infection. If left untreated, it can lead to irreversible clinical sequelae like pelvic inflammatory disease, ectopic pregnancy, cervicitis, urethritis, tubal infertility, endometritis, abscesses of Bartholin gland etc. in

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Female and Non-gonococcal 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.² 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 prevalence of genital chlamydia and its association with bacterial flora in female STI clinic attendees.

MATERIAL & METHODS: A total of 226 female patients attending STI clinic were included from July 2008 to July 2010.

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.
 - H/o burning micturition.
 - H/o Lower Backache.

EXCLUSION CRITERIA:

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

METHOD OF COLLECTION OF SPECIMEN:

A) SWABS:

Three endocervical swabs were collected. The endocervical region was cleaned with sterile normal saline and any debris present was removed using gauze piece soaked in sterile normal saline. After removing excess mucus from the endocervix, the sterile swab was inserted into endocervical canal and rotated vigorously for 15-20 seconds. The swab was removed without touching the vaginal walls and transported in sterile bottle, having normal saline immediately. Of the three specimens, first was used for microscopy (Wet mount, Gram staining, Geimsa staining).

Second for inoculation on 5% Sheep Blood agar, MacConkey agar, Chocolate agar. Isolation and Identification of bacterial flora was carried out by conventional methods³.

Third swab was collected in sucrose phosphate solution (transport medium) and was refrigerated at 4°C till assayed for chlamydial antigen by Immunochromatographic method. (QuickVue Chlamydia Test, Biomerieux).

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B) BLOOD:

With sterile disposable needle and syringe, 5 ml blood was collected from anticubital vein under strict aseptic precautions in plain sterile test tube. Screening for Chlamydia trachomatis (IgG) antibody was done by ELISA test (Nova Tec, Chlamydia trachomatis IgG ELISA, manufactured by Nova Tec Immunodiagnostics, 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 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. (Figure no.1).

All the total 226 clinical specimens were subjected to microscopy as per conventional methods. On Wet Mount, 18 (07.96%) cases showed Trophozoites of Trichomonas Vaginalis and 34 (15.04%) cases showed budding yeast like cells. On Giemsa staining, 'Cloak Shaped' inclusion bodies of Chlamydia trachomatis were found in 69(30.53%) cases (Figure no.2). On Gram staining, 18 (07.96%) cases showed presence of Clue cells. Out of 226 cases, 104(46.01 %) showed gram negative organisms, 88 (38.93%) showed gram positive organisms, 34 (15.04%) showed gram positive budding yeast like cells & 07 (03.09%) gram negative cocci (Figure no.3). (Table no. 1).

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%. Of the total 102 ELISA positive patients, C.trachomatis was detected maximally along with Candida albicans in 30 (29.41%) subjects, followed by C negative Staphylococcus 19(18.62%), E.coli 17 (16.66%), C+ve Staphylococcus 15 (14.70%), Streptococci species 08 (07.84%), Klebsiella species 05 (04.90%), Pseudomonas species 02 (01.96%), Proteus species 02 (01.96%), Gonococci species 02 (01.96%) and Non-fermenter organism 01(00.98%). C. trachomatis also showed its association with Trichomonas vaginalis only in 01 (00.98%) subject. (Table no. 2). Immunochromatographic test, to detect chlamydial antigen could be carried out on 50 samples only due unavailability of kit. Chlamydial antigen was detected in 07 clinical samples, giving an overall positivity rate of 14%. Chlamydial antigen could not be detected in 43(86%) clinical samples.

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)⁴ 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. Wet mount showed 18/226 (07.96%) of Trichomonas vaginalis and 34/226(15.04%) of budding yeast like cells. Gram staining showed a picture of varied morphology. Direct examination of clinical material on gram stained smear has been used with varying degree of success as an aid in the diagnosis of genital chlamydia. Gram staining revealed clue cells in 18/226(07.96%) cases.

Out of total 226, 97 (42.92%) smears showed Gram negative rods, 88 (38.93%) Gram positive cocci, 34(15.04%) smears showed budding yeast like cells and 07(03.09%) showed Gram negative cocci. In the present study, all the specimens subjected for culture yield positive results. The culture isolates in the present study showed the polymicrobial flora. Giemsa staining was carried over all the

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226 cervical specimens. Of these, 69 (30.53%) were positive for Halberstaedter Prowazek inclusion bodies of *C. trachomatis* and 157 (69.46%) were negative for the same. Similar findings were reported by Savitha S et al (2009)⁵ who detected 20% and 16.67% of inclusion bodies in patients of age group between 20-25 and 25-30 years respectively. 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)⁶ reported a low 10.9% seropositivity in STD cases.

Eckert LO et al (1997)⁷ reported 22.5% chlamydial seropositivity while Joyee AG et al (2007)⁸ 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. In the present study, Co-existence of chlamydia trachomatis with other agents was observed. *C. trachomatis* was detected with various organisms i.e. with *Candida albicans* in 29.41%; *C. negative Staphylococcus* 18.62%; *E. coli* 16.66%; *C. +ve staphylococcus* 14.70%; *Streptococci species* 07.84%; *Klebsiella species* 04.90%; *Pseudomonas species* 01.96%; *Proteus species* 01.96%; *Gonococci* 01.96% and *Non-fermenter and Trichomonas* 00.98% each. This seems to indicate that other organisms along with *C. trachomatis* has certainly played role in etiopathogenesis of STI/RTI's. Of the total 180 clinical specimens 102 (55.66%) had *C. trachomatis* infection, but also co-existently associated with other agents. In 78 (43.33%) clinical specimens who were negative for *C. trachomatis* antibody (IgG), other organisms apart from *C. trachomatis* have played role in causation of STDs. In present study attempt was made to detect chlamydial antigen by immunochromatography.

Because of its high cost, test was carried on only 50 specimens. Of 50 clinical specimens, 07/50(14%) were positive for *C. trachomatis* antigen and 43(86%) were negative for chlamydial antigen. Badrinath S et al (1996)⁹ compared the detection of *C. trachomatis* antigen by using card test "Immunocomb" with the Fluorescent labeled monoclonal antibody test (DFA) on cervical specimens, where he reported 13.3%(4/30) detection of *C. trachomatis* antigen by immunochromatography as contrast to 16.6% (5/30) by DFA method. Isibor JO et al (2005)¹⁰ reported 13.3% (40/300) detection of chlamydial antigen by immunochromatography. Savitha S et al (2009)⁵ reported 8.13 % (7/86) and 12.28 % (14/114) detection of chlamydial antigen among non-pregnant and pregnant women respectively by immunochromatography. Thus, the findings of the present study are in consistent with that reported with above authors.

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

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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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Fig. 1

Wet Mount	Trop. T. Vaginalis.	18 (07.96%)
Giemsa	Inclusion bodies- positive	69 (30.53%)
Gram staining	Clue cells	18 (07.96%)
Gram staining	GNR	97 (42.92%)
	GPC	88 (38.93%)
	Budding Yeast	34 (15.04%)
	GNC	07 (03.09%)

Table 1: Microscopic findings of study subjects (n=226)

Fig. 2: Giemsa Stain: Cloak shaped Inclusion Body

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Fig. 3: Gram negative diplococcic – Gonococci

CT & other agents	Number	Percentage
Candida	30	29.41%
C negative staphylococcus	19	18.62%
E.coli	17	16.66%
C +ve staphylococcus	15	14.70%
Streptococci species	08	07.84%
Klebsiella species	05	04.90%
Pseudomonas species	02	01.96%
Proteus species	02	01.96%
Gonococci	02	01.96%
Non-fermenter	01	00.98%
Trichomonas	01	00.98%
Total	102	100%

Table 2: Co Existence of Chlamydia Trachomatis (IgG) and other agents

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