Detection of *Chlamydia Trachomatis* infection among symptomatic women of reproductive age group attending the gynaecology OPD in a tertiary care centre and risk factors associated with the infection

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Abstract

Chlamydia trachomatis infection is the most common sexually transmitted bacterial infection worldwide, affecting women more than men. Majority being asymptomatic, this infection goes unnoticed and will have a major impact on the reproductive health of women resulting in morbidity. It also increases the risk of HIV.

Objective: To detect the Chlamydial infection in symptomatic women of reproductive age group and to identify the possible risk factors.

Materials and Methods: An endocervical swabs were collected from 100 symptomatic patients of reproductive age group and subjected to direct fluorescent antibody test (DFA).

Results: The C.trachomatis infection was 20%, with a higher positivity in the age group of 26- 30 years. The most presenting complaint was vaginal discharge. A significant past history of PID, coppet T insertion, IUD/still birth was noted. A 20% coinfection with candiadiasis was also observed.

Conclusion: As the occurence of *Chlamydia trachomatis* was substantially high in our study, effective strategies to implement screening programmes should be initiated as a routine diagnostic test for all women of reproductive age group, thereby preventing severe sequelae by providing timely management.

Keywords: *Chlamydia trachomatis*, Percentage, occurrence, Direct fluorescent antibody test, Risk factors.

Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial infection worldwide and women carry the major burden of the disease.[1] It is also a leading preventable cause of Pelvic Inflammatory Disease (PID) and female infertility globally.[2] Majority of the patients are asymptomatic, thereby providing an ongoing reservoir of infection capable of transmitting it to their sexual partners.

These undiagnosed infections often present as PID leading to severe complications like ectopic pregnancy, tubal factor infertility and enhances the risk of transmission of *Human immunodeficiency virus (HIV)* and other sexually transmitted infections (STIs).[3] It is also a risk factor for cervical carcinoma.[4] Although *Chlamydia trachomatis* is an established cause of infertility, there is an increasing prevalence of this infection in developing countries which is attributed to the fact that there is a lack of effective screening programmes, limited resources and cost-effective diagnostic tests. These chlamydial infections are a major health care concern in women more than men since the manifestations and consequences are more detrimental to the reproductive health of women.[5] Worldwide, the magnitude of morbidity associated with sexually transmitted chlamydial infections is enormous.

Chlamydial infections can be easily treated with oral antibiotics, but the percentage of people being symptomatic are very few thereby leading to long term sequelae, especially in women if left untreated. Therefore this study was carried out to evaluate the occurrence of *Chlamydia trachomatis* infection in symptomatic women by using a cost effective and a simple diagnostic procedure like the direct fluorescent assay (DFA).

Materials and Methods

The prospective study was carried out in the Department of Microbiology of a tertiary care teaching hospital, for a period of one and a half years from October 2014 to April 2016 after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all the participants willing to participate in the study. A total of 100 endocervical swabs were collected from all women of reproductive age group (18-40 years) attending the Gynaecology outpatient department (OPD) and in-patients (IP) and sent to the microbiology laboratory for further processing.

Demographic data like age, socioeconomic status, occupation, marital history, presenting complaints, obstetric history, and menstrual history were collected. Relevant clinical details and investigations were also obtained. Only those women of reproductive age group (18-40 years) who are sexually active, presence of active vaginal or cervical discharge, women complaining of infertility, women complaining of pain abdomen with significant menstrual history (menorrhagia/oligomennorhea/irregular menses) were included in the study. All pregnant women, women below < 18 years and above > 40 years and menstruating women were excluded from the study.

A speculum was passed and an endocervical specimen was collected using sterile cotton tipped swab and was placed in a screw capped polypropylene tube. It was then sent to the microbiology laboratory immediately for further evaluation by Direct Fluorescent Antibody Test (DFA).

The cotton swab received at the laboratory was immediately processed. It was rolled onto a sterile glass slide evenly and firmly and left to air dry. The slide was then flooded with 0.5 ml methanol fixative and was allowed to evaporate completely. The process was speeded up by tipping the slide after 5 minutes to drain the excess fixative. The slides were then stored at -20^0 C until staining.

MicroTrak *Chlamydia trachomatis* (Trinity Biotech) direct specimen test kit was used for staining the slides and procedure was followed according to manufacturer instructions. and MicroTrak *Chlamydia trachomatis* direct specimen test control slide pack with positive and negative control slides were also stained each time the specimen slides were stained. Before staining, the reagents, control and specimen slides were brought down to room temperature.The reagent is a fluorescein-isothiocyanate dye conjugated with monoclonal antibodies which is highly specific for the MOMP of *Chlamydia trachomatis*.

The slides were then placed on a staining rack and 30µl of reagent was added to the control and fixed specimen slides and incubated for 15 minutes at room temperature in a humidified chamber. It was then washed with distilled water. Excess water was removed by blotting around the smear with a tissue paper and then was air dried. A drop of mounting fluid was added to the center of the slide and a cover slip was placed on the top of the drop.

Stained slides were then viewed under a fluorescent microscope. 40x was used for screening and 100x was used for confirming the morphology. Specimens were examined for apple green coloured elementary bodies contrasted against the reddish brown background of the counterstained cells. Positive control slides were used as a guide to the size, shape and staining pattern of elementary bodies.

Presence of 10 or more chlamydial elementary bodies was considered positive. When no such structures were seen, the sample was considered negative.

Statistical Analysis

Collected data was analyzed by frequency, percentage, Chi Square test and Fischer Exact test. SPSS version 20.0 was used for all the statistical procedures.

Results

Out of 100 samples processed, 20 were positive for *Chlamydia trachomatis* infection. 10 or more EB's per smear were seen in all the 20 cases. No doubtful positive cases were observed in our study. The highest positivity was seen in the age group 26-30 years 9(45%) followed by >31years 6 (30%) and 20-25 years 5(25%). *Chlamydia* was detected more commonly in women with a parity index of more than one 14(70%) as compared to nulliparous women 6 (30%).

The most common presenting complaint was vaginal discharge. Some presented with significant menstrual disturbances like irregular menses/ menorrhagia/ oligomenorrhea. Table 1 shows presenting complaints of patients. In our study population of 100 women, 2 patients had a history of infertility, out of which only one was detected with *Chlamydia*.

Out of the 20 patients who were positive for *Chlamydia*, 14 patients had a significant past history of PID- single or multiple episodes, copper T insertion, bad obstetric history. Bad obstetric history included abortions seen in 3(15%), intrauterine death in 2(10%) and still birth seen in 1(5%) patient There was no significant history in 6 patients (Table 2). This had a significant p value of 0.036.

The coinfection with *Chlamydia trachomatis* with Candidiasis was seen in 4(20%) cases. Only one patient was positive for HIV.

All the 20 patients that were detected for *Chlamydia* were sexually active. Onset of sexual activity in our study was observed to be higher in the age group of 26-30 years. May be this is the reason for highest positivity in this age group. None gave history of multiple sexual partners.

Table 1: Presenting complaints associated with positive genital *Chlamydia* infection

Presenting complaints	*Chlamydia* positive	
Vaginal discharge	11 (55%)	
Pain abdomen with significant disturbance in menstrual history	8 (40%)	P= 0.724
Primary infertility	1 (5%)	

Table 2: Significant risk factors among women detected positive for *C.trachomatis*

Relevant past history	Chlamydia detected	
PID	4 (20%)	P=0.036
Copper T insertion	4 (20%)	
Bad obstetric history	6 (30%)	

Discussion

Chlamydia trachomatis is a Gram-negative obligate intracellular bacterium with an incubation period of 7 to 21 days and a growth cycle of approximately 48 hours.[5] *C. trachomatis* has a worldwide distribution, affecting both sexes, but has a much greater impact on females than on males. It particularly affects young women and sexually active adolescents. Unfortunately, chlamydial infections may, in many cases, remain silent. Timely detection and effective management of cervical infection due to *Chlamydia trachomatis* in women provides critical interventional opportunities.

The gold standard for diagnosis of *Chlamydia trachomatis* is by culture in cell lines. But as it is highly expensive in addition to its low sensitivity and laborious procedure, a search for better and alternative diagnostic strategies was initiated so that it can be more feasible in a resource restricted setting. Among these was the antigen detection by DFA which has a sensitivity of 90% and 98-99% specificity.[6] This high specificity is due to its dependence on visualization of the brightly stained elementary bodies which implies the presence of an active chlamydial infection. Also the procedure is rapid, making it an ideal test in laboratory set up.

The percentage of *Chlamydia trachomatis* infection in women of reproductive age group attending the Gynaecology department in this study using the DFA technique was found to be 20%. There are various studies which have reported the prevalence in a population. A study conducted in New Delhi revealed a 8.8% prevalence,[7] in contrast to another study conducted in Malhotra M et al in which 19.9% prevalence was observed.[8] A higher prevalence of 24.5% was reported in a study conducted in Chennai, India using DFA technique by Joyee AG et al.[9] A study conducted in Nigeria by Ikeme AC et al, also showed a high prevalence of 29.4%.[10] Study conducted by Sood et al showed a positivity of 11.34% by DFA technique.[11] Another study by Sachadeva et al reported a figure of 22.9% positivity in symptomatic female patients attending the Gynaecology department of a tertiary care center.[12]

We observed detection rate was higher in the age group 26-30 years showing a 45% rate, 30% in the age group >31 years and 25% among women of 20-25 years. Age is the most important risk factor for chlamydial infection. The burden of *Chlamydia* appears higher among women especially those of younger age.

Among women, highest age-specific rates of reported Chlamydia in 2008 were 3,275.8 cases per 100,000 females (3.3%) among 15 to 19 years of age and 3,179.9 cases per 100,000 females (3.2%) among 20 to 24 years of age.[1] CDC recommends routine screening of all sexually active women of <25 years of age[6] as there is a high risk in young women due to behavioral characteristics and also increased exposure of cervical columnar epithelium may enhance the ability of the organism to infect new cells. In UK, it has been reported that 10.3% of women <25 years of age are infected with *Chlamydia*.[7] In a study conducted in Nicaragua by Hermann B et al, the mean age was found to be 27.3 years and majority (30%) were married.[13] In another study the highest rate of chlamydial infection was found to be in the 20-30 years age group.[8] One study conducted in Chennai, India, by Prathiba et al,[14] showed similar results, wherein the highest percentage of *Chlamydia* positive patients was seen in the age group 26-30 years (34.6%), which is almost similar to the data obtained in our study. Also all the women who were detected positive for Chlamydia in this study were married. In a study of 1800 women by Zenilman JM, the prevalence of chlamydial infection among married was 1%.[15] In another study conducted by Sood et al, the mean age was 29.7 years, with 73% in the age group of 15-34 years.[11]

The age of the patients in this study could not be associated with most of the studies because the highest positivity was seen in the age group 26-30 years. Probable explanation could be because of late onset of sexual activity observed in our population. CDC has showed that there is a high prevalence of chlamydial detection rate of 4.7% among sexually active females aged 14-24 years.[6] There is a strong correlation found with age at onset of sexual activity with *C.trachomatis* infection.[14] An inverse trend related age and prevalence of infection was found in this study, but statistically no significance was associated.

The most common presenting complaint by women in our study was vaginal discharge, seen in 55% of the patients. A study by Sood et al, India, showed vaginal discharge to be the most common presenting complaint, seen in 91.75% patients.[11] Nessa et al[16] showed major cases 49.6% had excessive vaginal discharge. Hermann B et al,[13] in Nicaragua, showed 64% women complaining with vaginal discharge. Mohan DG et al,[17] showed 33.3% women complaining of vaginal discharge who were detected positive for *Chlamydia*. The strongest association was found with vaginal

discharge, thus supporting the use of this symptom in the syndromic algorithm for the management of vaginal discharge that is recommended by WHO.

The next complaint was lower abdominal pain associated with significant disturbance in menstrual history (menorrhagia/irregular bleeding/oligomenorrhea) seen in 40% of the patients. Nessa et al showed 48.4% of the patients complaining with lower abdominal pain, which is closer to our study.[16] A study conducted in North India by Dhawan et al, showed 10.5% came with a complaint of abdominal pain.[18] Study done in Nicaragua by Hermann B et al, showed a rate of 55%.[13]

Chlamydia was detected only in 1 patient (5%) presenting with primary infertility. She had a history of multiple episodes of PID which could be attributed to the development of infertility. A study by Dhawan et al, conducted only in infertile female patients, showed a chlamydial detection rate of 9%.[18]

Out of the 20 patients that were detected positive for *Chlamydia*, 14 had a significant past history. Majority had a history of PID (20%); with one or multiple episodes in the past, showing that probably untreated chlamydial infections in the past lead to the development of PID which if left untreated might go into further complications leading to infertility.[19] 20% had a history of copper T insertion, which again is a major risk factor for chlamydial infection. Insertion of intra uterine devices when not done under aseptic precautions might also lead to PID. Another study by Herman B, et al, showed a prevalence rate of 1.8% in women with intra uterine devices.[13] 30 % had bad obstetric history wherein which, 15% had a history of unexplained abortions in the first trimester. Study by Visnovsky J et al,[20] showed a significant correlation between chlamydial positivity and first trimester abortions of about 15.2% which is similar to what we obtained in our study. Dhawan et al[18] showed a history of abortions in 39% of the women who were detected positive for *Chlamydia.*

10% of patients in our study had a history of intrauterine death (IUD) and 5% had history of still birth, implicating the need to screen women in pregnancy to prevent such disasters in the future pregnancies. There has been very limited data regarding these factors in association with chlamydial positivity in other studies. As there are no factors explaining the cause of IUD/ still birth in these patients, *Chlamydia* can be a significant risk factor. One study conducted in Assam, India by Mohan DG et al,[17] has taken these factors under consideration but showed no positivity in the group.

Co-infection of *Chlamydia trachomatis* with other STI's was seen in 25% of the cases. 20% had co infection with *Candida albicans* and 5% had co-infection with *HIV*. In a study reported in North India by Malhotra M et al,[8] showed co-infection with Candidiasis in 10.9% of the cases. Study by Joyee AG et al[9] Chennai, showed *HIV* co-infection with *C.trachomatis* in 29.5% cases. However, our study has shown a low co-infection rate with *HIV*. This probably might be because *HIV* was not routinely tested on all the patients in our study group. There is however, significant literature which prove the high rate of *HIV* co-infection. Therefore, controlling and treating *Chlamydia* by screening women at an early age might be able to bring down the burden and transmission rate of *HIV*.

Co-infection with *N.gonnorheae* was not noticed in this study. Individuals infected with both *C.trachomatis* and *N.gonnorheae* shed larger number of *C.trachomatis* than those infected with *Chlamydia* alone. Co-infection between the two has been reported to range between 1.1 to 67%.[21]

Co-infection with other STIs highlights the importance of early laboratory diagnosis and specific treatment of the condition as they increase the risk many folds when infections exist together.

Primary prevention through life style modifications and health education can prevent both exposure and acquisition of genital chlamydial infections but has not gained popularity in the developing world. Secondary prevention through screening of sexually active women can detect chlamydial infections at an early stage and prevent the drastic sequelae of chlamydial infection. Also this study shows a high percentage of chlamydial infections which brings about the need to implement effective screening strategies, as this infection can be easily diagnosed with the latest noninvasive and rapid turnaround time tests like DFA. Tertiary prevention of chlamydial infection of the upper genital tract has largely failed because by the time the patient becomes symptomatic, substantial damage to the tubes would have occurred, leading to infertility.

Limitations of the study

There are certain limitations to our study. Ours is a hospital based study involving only the symptomatic group .Due to lack of accessthe test could not be compared with other specific tests like nucleic acid amplication tests or culture of the bacteria. But most of our laboratories lack these facilities except few elite laboratories situated in urban areas .Evaluation with larger sample size and for a longer duration is required to know the exact magnitude of the problem.

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