**Chlamydia trachomatis** infection & female infertility

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Background & objectives: *Chlamydia trachomatis* is a well recognized sexually transmitted pathogen. Besides its potential to produce genital tract infection, *C. trachomatis* is increasingly being associated with long-term complications like infertility. The present study was undertaken to assess the role of *C. trachomatis* in female infertility as such data are lacking.

Methods: Women of primary and secondary infertility (n=110) and 30 healthy term pregnant women as control group were enrolled in the study. Detailed clinical history of each patient was recorded. Hysterosalpingography was performed in all patients. Endocervical swabs were collected for culture on cycloheximide treated McCoy cell line and for antigen detection by ELISA.

Results: *C. trachomatis* was detected in 31 (28.1%) of the 110 infertile women while one (3.3%) in control group was positive for *C. trachomatis* (*P*<0.01). Cell culture alone identified 25 (22.72%) patients suffering from chlamydial infection while *C. trachomatis* antigen was detected by ELISA in 18 (16.37%) patients. The one control case was positive for *Chlamydia* antigen by ELISA and not by cell culture. Chlamydial positivity was seen in 20 of the 74 (27%) women with primary infertility and in 11 of the 36 (30.6%) with secondary infertility. Of the 58 asymptomatic women, 21 (36.2%) had chlamydia infection while among the 52 symptomatic cases 10 (19.2%) were infected; 38 per cent women with chlamydial infection also had tubal occlusion.

Interpretation & conclusion: A significantly high rate of *C. trachomatis* infection was found in infertile women and more so in asymptomatic females and in secondary infertility cases. Lack of symptoms make clinical diagnosis of chlamydial infection difficult. Screening of infertile women for *C. trachomatis* is therefore recommended so far early therapeutic interventions.

Key words Antigen ELISA - cell culture - *Chlamydia trachomatis* - infertility

*Chlamydia trachomatis* has currently emerged as the most common sexually transmitted pathogen. Chlamydial infection produces less severe symptoms than other sexually transmitted diseases. These deceptively mild symptoms allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease lead not only to significant morbidity but far more importantly to infertility. Infertility due to *C. trachomatis* represents a
preventable type of infertility, if detected early. However, data pertaining to infertility attributed to *C. trachomatis* infection is very limited in India particularly in northern India, thus preventing any policy from being formulated regarding screening of patients with infertility.

The present study aims to evaluate chlamydial infection in women suffering from infertility attending a tertiary care hospital in north India by a two pronged approach: isolation of *C. trachomatis* on McCoy cell line and antigen detection by ELISA. The association of *C. trachomatis* infection in primary and secondary infertility was also assessed.

**Material & Methods**

Infertile women of reproductive age attending Obstetrics and Gynaecology outpatient department of Jawaharlal Nehru Medical College, Aligarh Muslim University (AMU), Aligarh, during May 2003 to June 2004 were included in the study. Thirty healthy term pregnant women of similar age during the study period attending the antenatal clinic constituted the control group. Infertility was defined as inability to conceive for more than a year despite regular unprotected intercourse. Primary infertility was defined as those cases in whom conception had never occurred whereas the term secondary infertility was used to define those cases where there was inability to conceive after a previous successful conception. Infertile women who had normal montoux test, normal X-ray chest, no specific findings in endometrial biopsy and husbands having normal semenogram were enrolled in the study. Patients with history of antibiotic treatment in the previous two months were excluded from the study. Detailed history and clinical features were recorded and all relevant investigations were performed. Hysterosalpingography (HSG) was done in all cases. Tubal infertility was said to be present if hydrosalpinx was seen on HSG. Study group comprised of 110 infertile women. They were further categorized on the basis of primary (74 cases) and secondary infertility (36 cases) and whether they presented with symptoms (52 patients) or were asymptomatic (58 cases).

**Specimen collection:** The endocervix was first cleaned with a sterile cotton swab to remove mucous and exudate after which endocervical specimens were collected in triplicate from all women.

**For cell culture:** Two Dacron swabs on a plastic shaft were used. The samples were collected by inserting the swab into the cervical canal up to a depth of 1-2 cm close to the endocervix and rotated through an angle of 15°-30° to collect the specimen from the squamocolumnar junction. Care was taken to prevent the swab from touching the vaginal mucosa. They were transported to the microbiology laboratory immediately in 2SP transport medium. The swabs were processed and cultured immediately.

**For antigen detection:** Chlamydia swab collection kit (Biorad, USA) was used for sample collection. One small Dacron swab on a stainless steel/plastic shaft for collecting specimen and one tube containing phosphate buffered saline (PBS) for transporting specimen to the laboratory was provided with the kit. Specimens, after collection, were stored at -20°C till use.

**Tissue culture technique:** McCoy cell line used for the isolation of *C. trachomatis* was obtained from National Centre for Cell Science, Pune. The cell line was maintained in the laboratory according to standard technique. *C. trachomatis* was cultured on cycloheximide treated McCoy cell lines. One ml suspension of 1,00,000 McCoy cells/ml of growth medium was seeded in Leighton tubes containing cover slips. The tubes were incubated at 37°C in a stationary position for 2-3 days for adequate growth to appear after which minimum essential medium (MEM) was aspirated from the vials and 0.1 ml from each 2SP specimen extract was inoculated into two tubes, one each for iodine and Giemsa staining. Tubes were centrifuged at
2500-3000 g for 1 h after adding 1 ml of MEM containing 1µg/ml cycloheximide, the tubes were incubated at 37°C for 48-72 h. Inclusion bodies were detected by Giemsa and iodine staining2,3. Fig. 1 shows cycloheximide treated uninoculated McCoy cell line, and inclusion bodies stained by Giemsa are shown in Fig. 2. The inclusion forming units (IFU) were graded from 1-4 according to the number of inclusions seen. The grading was as follows:

In grade 1, 5-9 IFU/ high power field (HPF); 10-20 IFU/ high power field (HPF) in grade 2; 1-10 IFU/HPF in grade 3, and >10 IFU/HPF in grade 4.

Antigen detection: C. trachomatis antigen was detected by enzyme linked immunosorbent assay (Biorad, USA). Chlamydia microplate EIA is a monoclonal antibody test based on qualitative enzyme immunoassay for the direct detection of chlamydial organisms in adult urogenital specimens. The samples were processed weekly. The same kit was used throughout the period of study. The EIA was performed and interpreted as per the manufacturer’s instructions.

The study was approved by the Institutional Ethics Committee and a written informed consent was obtained from each patient.

Statistical methods: The data were analysed using statistical software SPSS for windows version 10.0. Chi square test, Fisher’s exact test and McNemar’s chi square test were used for significance analysis.

Results

The mean age of the 110 women enrolled in this study was 26.5±4.34 yr while the mean age of the control group was 25.4±2.31. Among the infertile cases 52(47.29%) were in the 21-25 yr age group followed by 40 (36.36%) in the 26-30 yr age group. Of the remaining 18 women, 2 were in the 18-20 yr bracket, 10 in the 31-35 yr age group and 6 were more than 36 yr of age. The healthy term pregnant control women were free of all signs and symptoms and their age distribution was similar to the study group.

Asymptomatic cases (n=58, 52.7%) slightly predominated in the study. Majority of the asymptomatic cases (n=46, 79.3%) had primary infertility while 12 (20.7%) had secondary infertility. Among the 52(47.3%) symptomatic cases there

Fig.1. Cycloheximide treated uninoculated McCoy cell line.

Fig.2. Giemsa stain showing inclusion bodies on McCoy cell line.
were 28 (54%) cases of primary infertility and 24 (46%) cases of secondary infertility. By hysterosalpingography 20 (18%) women had tubal occlusion, 7 of these had primary infertility and 13 had secondary infertility.

The overall chlamydial positivity in the infertile women was found in 31 (28.1%; 95% CI: 19.8%-36.6%) cases who were positive for one or both chlamydial markers while 1(3.3%; 95% CI: 0-9.75%) healthy at term control women was found positive for *C. trachomatis* (*P*<0.01). Among the infertile cases, 25 (22.72%) were culture positive while none of the controls were positive for *Chlamydia* by cell culture (*P*<0.01). On comparing positivity of Giemsa and iodine, Giemsa was more sensitive. In grade I, 5 positive cases were seen. In grade 2 there were 7 positive cases, while 9 and 4 positive cases were present in grades 3 and 4 respectively.

*C. trachomatis* antigen was detected in 18 (16.37%) infertile cases while one woman in the control group was also positive for chlamydia antigen by EIA.

Among the total 31 infertile chlamydia positive cases, *C. trachomatis* was detected by both cell culture and EIA, in 12 (38.7%), 13 cases (41.9%) were positive for *C. trachomatis* by cell culture alone and in six (19.3%) only antigen could be detected. Taking culture as gold standard, in infertile females the sensitivity of antigen detection was 48 per cent while specificity was 92.9 per cent. The positive predictive value was 66.6 per cent and negative predictive value was 85.8 per cent.

The accuracy of antigen detection by EIA was 82.7 per cent. The results of both cell culture and EIA for antigen detection were found to be equivalent by McNemar’s chi square test (*χ²=2.57*).

A majority 24(77.4%) of the infertile women who were positive for *C. trachomatis*, were in the 21-30 yr age group, 5(16.1%) were in the 31-40 yr age group while 2(6.4%) were less than 20 yr of age. The duration of infertility was 2-4 yr in 23 cases, (74.2%), in the remaining eight (25.8%), the infertility was of > 4 yr duration.

The results of cell culture and antigen detection in women with primary and secondary infertility are shown in Table I. Of the 31 women who tested positive for *C. trachomatis* infection, 21(67.7%) were asymptomatic while remaining 10(32.3%) were symptomatic (*P*<0.01). A significant number of women with chlamydia infection (14, 45%) had bad obstetric history as against 6(7.5%) chlamydia negative cases (*P*<0.01). Pelvic inflammatory disease was seen in 15(48.3%) chlamydia positive cases in comparison to 21(26.5%) who did not have chlamydia infection but the association was not statistically significant.

Twelve (38%) chlamydia positive cases had tubal occlusion, 5 of these had primary infertility and 7 had secondary infertility. Majority of them (10, 83%) were asymptomatic. Of the 52 symptomatic women, 20 (18.4%) had vaginal discharge (Table III). Bleeding per vaginum (on touch) and vaginal discharge were found to be significantly associated (*P*<0.02) with chlamydial infection.

<table>
<thead>
<tr>
<th>Type of infertility (N)</th>
<th>Total no. infected</th>
<th>Chlamydia detected by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Both cell culture and antigen</td>
</tr>
<tr>
<td>Primary (74)</td>
<td>20 (27)</td>
<td>7(35)</td>
</tr>
<tr>
<td>Secondary (36)</td>
<td>11 (30.6)</td>
<td>5(45.4)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.


**Table II.** Comparison of cell culture and antigen detection of *C. trachomatis* in symptomatic and asymptomatic females

<table>
<thead>
<tr>
<th>Clinical profile</th>
<th>Total no. infected (N)</th>
<th>Chlamydia detected by Both cell culture and antigen</th>
<th>Cell culture alone</th>
<th>Antigen alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic women</td>
<td>10 (19.2)</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>0 (0)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asymptomatic women</td>
<td>21 (36.2)</td>
<td>6 (28.57)</td>
<td>9 (42.85)</td>
<td>6 (28.57)</td>
</tr>
</tbody>
</table>

Figure in parentheses denote percentages

**Table III.** Clinical profile of symptomatic infertile women in relation to *Chlamydia* positivity

<table>
<thead>
<tr>
<th>Presentation</th>
<th>No of cases n=52</th>
<th>Patients infected with <em>Chlamydia</em> (n=10)</th>
<th>Patients not infected with <em>Chlamydia</em> (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding per vaginum (on touch)</td>
<td>4 (3.6)</td>
<td>02 (20)</td>
<td>2 (4.76)</td>
</tr>
<tr>
<td>Vaginum discharge</td>
<td>20 (18.4)</td>
<td>06 (60)</td>
<td>14 (33.3)</td>
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<td>Chronic cervicitis</td>
<td>1 (0.9)</td>
<td>0</td>
<td>1 (2.38)</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>1 (0.9)</td>
<td>0</td>
<td>1 (2.38)</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>1 (0.9)</td>
<td>0</td>
<td>1 (2.38)</td>
</tr>
<tr>
<td>Scanty menses</td>
<td>5 (4.5)</td>
<td>01(10)</td>
<td>4 (9.5)</td>
</tr>
<tr>
<td>Vaginismus</td>
<td>3 (2.7)</td>
<td>0</td>
<td>3 (7.1)</td>
</tr>
<tr>
<td>Burning micturition</td>
<td>3 (2.7)</td>
<td>0</td>
<td>3 (7.1)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages

**Discussion**

Infertility is becoming an emerging health problem in many countries of the world including India. The increase appears to coincide with the growing role played by *C. trachomatis* as a sexually transmitted disease. In our study, *C. trachomatis* infection was found in 28 per cent of the infertile females which is quite high. A WHO study reported the current chlamydial infection in infertile women to be 18-20 per cent.

The duration of infertility in the chlamydia positive cases in our study was approximately 2-4 yr which corresponds well with other reports. A large number of the infected infertile women were asymptomatic. This highlights clinical inadequacy in diagnosing *C. trachomatis*. Other reports have also concluded the same. The incidence of *C. trachomatis* infection was more common in women with secondary infertility. This increased susceptibility could be due to their longer period of active sexual life thus enhancing their exposure to chlamydial infection. Secondary infertility associated with higher rates of chlamydial infection have been reported earlier by others. Bleeding per vaginum (on touch) and vaginal discharge were found to be more common clinical presentations in symptomatic chlamydia positive cases.

A surprisingly high percentage (38%) of the women positive for *C. trachomatis* had tubal infertility. Majority of these women had no
history of symptoms suggestive of previous upper genital tract infection. This is consistent with other reports. Prevalence of *C. trachomatis* varies with the population under study and the sensitivity of the laboratory method used. Our study suggests that all infertile women should be screened for *C. trachomatis*. The index of suspicion should be higher in asymptomatic women in whom our study revealed a larger chlamydial positivity. In the absence of requisite infrastructure and skills for culture and for direct fluorescent assay, ELISA can play a significant role in screening for *C. trachomatis* in infertile women. Screening of infertile women for *C. trachomatis* is recommended in the first year of infertility itself so that early therapeutic intervention can be instituted to allow women to conceive naturally. Studies with larger sample size should further elucidate the extent of infertility caused by *C. trachomatis* in India.

**References**


