Need for specific & routine strategy for the diagnosis of genital chlamydial infection among patients with sexually transmitted diseases in India

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Background & objectives: With increasing burden of human immunodeficiency virus (HIV) infection acquired immunodeficiency syndrome (AIDS) in India, documentation on the epidemiology of genital chlamydial infections in high-risk patients with sexually transmitted diseases (STD) is of significant public health value. Specific diagnosis is essential to prevent the morbidity due to the chlamydial infection and to reduce the risk of acquiring HIV infection. The present study was undertaken to analyse the usefulness of culture and antigen detection by direct fluorescent antibody (DFA) test for assessing the rate of \textit{Chlamydia trachomatis} infection in symptomatic patients and feasibility of these tests for routine adoption in Indian setting.

Methods: Clinically diagnosed patients of both sex (n=143) attending the Institute of Sexually Transmitted Diseases, Government General Hospital, Chennai who consented for the study, were enrolled. Clinical and demographic details were recorded on a stratified proforma. Genital swab specimens collected from them were subjected for culture using McCoy cell line and for antigen detection by DFA testing.

Results: \textit{C. trachomatis} was isolated in 27 of the total 143 patients (18.9%). Culture positivity was seen in 11 of the 63 (17.5%) males and in 16 of 80 (20%) females. DFA detected \textit{C. trachomatis} specific antigen in 35 patients (24.5%); 15 (23.8%) males and 20 (25%) females. The rate of \textit{C. trachomatis} diagnosis increased to 25.2 per cent by adopting both the methods as against 18.9 per cent by culture only and 24.5 per cent by DFA only. No association of \textit{C. trachomatis} infection with any predictable genitourinary symptom (s), was seen.

Interpretation & conclusion: The findings show a high infection rate for \textit{C. trachomatis} in symptomatic patients with STD. Clinical symptoms alone can be unreliable in specifically predicting infections with \textit{C. trachomatis}. Specific diagnostic tests need to be recommended for routine inclusion in the STD diagnosis to facilitate risk reduction of HIV infection in STD patients.

Key words Antigen detection - \textit{Chlamydia trachomatis} - direct fluorescent antibody test - \textit{Neisseria gonorrhoeae} - sexually transmitted diseases

Genital infection due to \textit{Chlamydia trachomatis} is one of the most prevalent sexually transmitted infections worldwide\(^1\). \textit{C. trachomatis} infection in the genital tract is a common cause of urethritis, epididymitis, mucopurulent cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility \textit{etc}\(^2\). The infection if left undiagnosed and untreated may lead to serious clinical consequences. Untreated individuals serve as reservoirs for the transmission of this infection to their sexual partners. These infections have gained great importance due to their implications in the transmission of human immunodeficiency virus (HIV)\(^3,4\)
and hence documentation of *C. trachomatis* infections in high-risk populations can assist in designing HIV-risk reduction strategies as well.

Various methods to diagnose chlamydial infections include cell culture, immunoassays (EIA), nucleic acid amplification tests like polymerase chain reaction (PCR) and ligase chain reaction (LCR). Though genital infections caused by *C. trachomatis* and its important sequelae are well documented in the developed countries, these infections are not well studied in developing countries like India. This is probably due to the lack of sophisticated diagnostic facilities which are required for *C. trachomatis* diagnosis. While few reports from north India suggest high prevalence rates of infection in high-risk patients with STDs, there is relatively little information available from the southern part of the country. The present study was conducted in symptomatic patients attending STD clinic to analyse the rate of genital chlamydial infections by culture and antigen detection by direct fluorescent antibody (DFA) testing, and to assess whether clinical signs and symptoms could be helpful in specifically predicting the infection with *C. trachomatis*. The feasibility of these tests for routine inclusion in STD diagnosis in Indian setting was also assessed.

**Material & Methods**

A total of 143 symptomatic patients (80 women, 63 men) attending the out patient clinic at the Institute of Sexually Transmitted Diseases (STDs), Government General hospital, Chennai were enrolled in this study during September 1998 to August 2000. Detailed history, demographic data and clinical features were recorded. Urethral and endocervical swabs were collected from males and females respectively. First, a swab was collected and used to make a smear for Gram staining and then cultured for *Neisseria gonorrhoeae*. Two swabs were further collected for chlamydial culture and DFA test respectively. The order doing the above tests with these swabs was randomized throughout the study. The genital swab specimens for culture were placed in 0.2M sucrose phosphate (2SP) buffer (pH7.2) with 5 per cent foetal bovine serum and antibiotics and transported on ice to the Microbiology department of Dr ALM Post Graduate Institute of Basic Medical Sciences, Chennai. The specimens were either cultured on the same day or stored at -85°C for 12 to 24 h until processed for culture. For DFA test, a smear was prepared by rolling the sampled swab over 1 cm circular area on an immunofluorescence slide, air dried and fixed with methanol.

The isolation of *C. trachomatis* was done using McCoy cell line as per the method of Iwen et al with modifications. Briefly, the specimens in 2SP culture transport medium (0.2ml) after vortexing with 2-3 sterile glass beads were inoculated in quadruplicates in 80 per cent confluent McCoy cell monolayers in 24 well tissue culture plates containing coverslips. Positive control (*C. trachomatis* serovar E; provided by Dr W.H.F. Goessens, University of Rotterdam, The Netherlands) and negative controls (2SP transport medium) were included in every batch of the test. The plates after centrifugation at 1000 × g for 1 h was subsequently incubated for 2 h at 37°C in 5 per cent CO₂. The inoculum was replaced with 1 ml of chlamydial growth medium containing 1µg/ml cycloheximide (Hi-Media, Mumbai, India). The plates were further incubated for 48-72 h. After removal of the medium, the monolayers were fixed with 95 per cent methanol for 10 min and subsequently stained with Lugol's iodine (Hi-Media, Mumbai, India) to detect intracytoplasmic inclusions (primary passage). The coverslips from the respective wells for each specimen were removed and parallely stained with fluorescein-labeled species-specific monoclonal antibodies against the major outer membrane protein (MOMP) of *C. trachomatis* (Chlamyset Antigen FA, Orion Diagnostika, Finland) and examined for chlamydial elementary bodies (EBs) in a fluorescent microscope (Optiphot-2; Nikon Corp, Tokyo, Japan). The cells in the two unstained wells of each specimen were processed for a second blind passage of culture on fresh McCoy cell monolayers.

The DFA test was performed for urethral/endocervical smears from the patients using the commercial kit (Chlamyset Antigen FA, Orion Diagnostika, Finland) as per manufacturer's instructions. The results obtained from direct smears were counterchecked by an additional test performed with specimens on culture transport medium. The aliquots of swab specimens in 2SP transport medium were centrifuged at 13,000 x g for 15 min, the supernatant was decanted, and 10µl of the pellet was placed on a Chlamyset immunofluorescence slide, air dried, fixed with absolute methanol for 5 min, and then stained using Chlamyset DFA reagent. Culture was used as the gold standard for analysing the performance characteristics of DFA.
Ethical clearance: The study was approved by the Ethics committee of the Government General Hospital, Chennai. A written informed consent was obtained from each patient. The patients found positive for *C. trachomatis* were provided a course of doxycycline at a dose of 100mg given twice daily for 7 days, if they had not received syndromic treatment soon after specimen collection.

Statistical methods: The data were analysed using statistical software SPSS for windows version 8.0. Chi-square and Mc Nemar’s tests for significance were performed by using Epi Info version 6.04.

Results

The mean age of the 143 patients (63 males and 80 females) enrolled in this study was 29.7±6.1 yr, with a range of 19 to 50 yr (mean age of males was 30.1±6.7 yr with a range of 19 to 50 yr and that of females was 29.3±5.7 yr; with a range of 20-45 yr). *C. trachomatis* was isolated in 27(18.9%) patients. Culture positivity was seen in 11 of the 63 (17.5%) males and in 16 of 80 (20%) females with no significant difference in the isolation rates. While culture isolation of *C. trachomatis* gave an overall positivity of 18.9 per cent (27/143), DFA detected *C. trachomatis* specific antigen in 24.5 per cent patients (35/143); 15 (23.8%) in males and 20 (25%) in females, the detection rate was not significantly different between males and females. Though detection rate of *C. trachomatis* by both the tests was higher in patients in the age group of 26-30 yr, the difference between the positivity rates was not statistically significant in different age groups. The number of polymorphonuclear cells (PMNs) per high power field (hpf) in the Gram stained smear was associated with *C. trachomatis* positivity by culture and DFA. As the number of PMNs/hpf increased on microscopy, the rate of positivity for *C. trachomatis* also increased. For the cell counts of 5-10 PMNs, >10-20 PMNs, >20-30 PMNs and >30 PMNs, the rate of culture positivity was 2 per cent (1/50), 14.6 per cent (7/48), 35.3 per cent (12/34) and 63.6 per cent (7/11) respectively. Correspondingly, the positivity rate for DFA was 4 per cent (2/50), 22.9 per cent (11/48), 44.1 per cent (15/34) and 63.6 per cent (7/11) respectively.

Both culture and antigen positivity was seen in 26 patients, DFA alone was positive in 9. The increased detection rate by DFA compared to culture was statistically significant (Mc Nemar’s test, *P*<0.05). However, DFA test did not pick up one culture positive female patient with endocervicitis. Compared to culture, antigen detection by DFA technique showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 96.3, 92.2, 74.3 and 99.1 per cent respectively.

*C. trachomatis* was detected in 36 patients by either of the two methods thereby giving an overall prevalence

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>N</th>
<th><em>C. trachomatis</em> positivity</th>
<th><em>C. trachomatis</em> and <em>N. gonorrhoeae</em> positivity pattern</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>By culture</td>
<td>By DFA</td>
</tr>
<tr>
<td>Urethritis</td>
<td>58</td>
<td>1 (19.0)</td>
<td>14 (24.1)</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>53</td>
<td>11 (20.8)</td>
<td>14 (26.4)</td>
</tr>
<tr>
<td>Epididymitis</td>
<td>5</td>
<td>1 (20.0)</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>PID</td>
<td>22</td>
<td>3 (13.6)</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>Infertility</td>
<td>5</td>
<td>1 (20.0)</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>27 (18.9)</td>
<td>35 (24.5)</td>
</tr>
</tbody>
</table>

Values given in parentheses are percentages. PID, pelvic inflammatory disease
of 25.2 per cent in the study population (23.8% and 26.6% in men and women respectively). Of the 63 male patients, 58 had urethritis and 5 epididymitis. Of the 80 female patients, 53 had cervicitis, 22 had PID and 5 with infertility (Table I). *N. gonorrhoeae* was detected in 21 (14.7%) patients. Coinfection with *C. trachomatis* and *N. gonorrhoeae* was seen in 6.9 per cent (4/58) patients with urethritis, 3.8 per cent (2/53) with cervicitis and 4.5 per cent (1/22) with PID. The overall coinfection rate for *C. trachomatis* and *N. gonorrhoeae* was 4.9 per cent (7/143) in the study population.

Discharge in 66.4 per cent (95/143) and dysuria in 49.7 per cent (71/143) were the predominant symptoms observed among the study subjects. Other symptoms observed were inflammation, pain, itching and rashes in 34 (23.8%), 24 (16.8%), 23 (16.1%) and 10 (7.0%) cases respectively. In the PID cases, lower abdominal pain was seen in 86.4 per cent (19/22) and chronic pelvic pain was observed in 63.6 per cent (14/22). Among the confirmed patients of *C. trachomatis* infection, discharge was observed in 69.4 per cent (25/36) while dysuria was seen in 55.5 per cent (20/36). Both of these symptoms were observed in 11 (30.6%) positive cases (Table II). The overall analysis revealed that *C. trachomatis* infection was not significantly associated to any particular symptom.

**Discussion**

The occurrence of *C. trachomatis* infection in the STD patients of the present study is relatively higher in comparison to the positivity rates of 5-15 per cent reported in STD clinic populations of other countries like the United States, Australia and Netherlands.10-12 Earlier Indian studies from Mumbai and Delhi have also reported high prevalence rates of genital chlamydial infections varying from 27.3-50 per cent in STD patients. Most of the studies conducted in India were focused on female patients. Very little information is available about the prevalence of genital chlamydial infections in men attending STD clinics in India. A recent study from Mumbai reported an infection rate of 33.3 per cent in male partners of the infected women who attended gynaecology and infertility clinics. Our results revealed similar infection rates in men (23.8%) and women (26.6%), which emphasize the need for screening both men as well as women attending STD clinics for *C. trachomatis* infection and screening of spouse should be an important component of STD control.

Culture positivity in 18.9 per cent patients suggest active chlamydial replication in these cases. Increased number of viable organisms being shed and isolated in culture may indicate increased potential for transmission in persons with clinical signs of urogenital infection. Patients with acute urethritis and cervicitis are more often seen in a STD clinic compared to those with upper genital tract syndromes such as PID, epididymitis and infertility. In the present study, higher infection rates were seen in patients with urethritis and cervicitis. From the prevention perspective, it is of considerable importance to identify and treat men and women with acute urethritis and cervicitis, as they may most likely transmit infection as well as potentially develop upper genital tract disease complications. The rate of *C. trachomatis* positivity was higher in patients in younger age groups though statistically significant association could not be demonstrated in the present study. Our observations are consistent with that of an Indian study by Joshi et al, which showed no difference in the age distribution of

<table>
<thead>
<tr>
<th>C. trachomatis positive group (n=36)</th>
<th>C. trachomatis negative group (n=107)</th>
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<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>Men (n=15)</strong></td>
</tr>
<tr>
<td>Discharge only</td>
<td>-</td>
</tr>
<tr>
<td>Dysuria only</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>Both discharge and dysuria</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>Both the symptoms negative</td>
<td>1 (6.7)</td>
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study subjects with and without \(C. \text{trachomatis}\) infection. However, the association of \(C. \text{trachomatis}\) genital infection with young age has been documented in many western studies\(^1\)\(^5\)\(^1\)\(^7\).

The present study reports the use of both culture and DFA in the diagnosis of \(C. \text{trachomatis}\) infection in STD patients. The DFA technique has detected nine additional patients who were culture negative. Possible failure of immunochromatographic reaction by the presence of inhibitory substances (such as antibodies in the secretions) could have led to the negative result by DFA in one of the culture positive female patient with endocervicitis in our study. The DFA test has shown good sensitivity, specificity and predictive values when compared to culture. Culture methods are time consuming, technically demanding and labour intensive. Moreover, factors like transport, storage, viability and specimen adequacy may affect the detection rate by culture. Though commercial nucleic acid amplification tests are widely available and have been shown to be highly sensitive and specific in the diagnosis of \(C. \text{trachomatis}\) in different study populations\(^1\)\(^6\)\(^8\)\(^1\)\(^9\), these tests are not cost-effective for routine use in the developing countries like India. Taking in to account the rapidity, technical simplicity and relative sensitivity, the DFA test may be a useful routine diagnostic test for genital chlamydial infections in the STD referral centres in India. However, there is a subjective element in the microscopic evaluation and interpretation of results and therefore, care should be taken to maintain the diagnostic accuracy by proper training.

The microscopy of genital swabs for polymorphs has shown that higher cell counts per high power field are directly proportional to the positivity rate for \(C. \text{trachomatis}\). This might be due to a higher chlamydial load in those samples with more number of cells.

It has been suggested that clinical signs of genital chlamydial infection are often mild and non-specific in most cases\(^2\)\(^0\). There is also sufficient overlap between the symptoms of chlamydial infections and gonococcal infections. Consequently, chlamydial infections cannot be predicted on the basis of clinical grounds alone. In the present study, though discharge and dysuria were observed to be the predominant symptoms among the patients, there was no significant difference in the presentation of these symptoms in patients with and without \(C. \text{trachomatis}\) infection. We observed a poor association between \(C. \text{trachomatis}\) positivity and other genitourinary symptoms in this group of symptomatic patients. The findings of the present study are in concordance with that of a recent study conducted at a STD clinic setting by Tchoudomirova \textit{et al.} in Bulgaria\(^2\)\(^1\).

In view of the epidemiological studies showing an association between genital chlamydial and HIV infection\(^3\)\(^4\)\(^2\)\(^2\)\(^-\)\(^2\)\(^4\), it needs to be emphasized that controlling genital chlamydial infection may have positive implications in the control of HIV transmission and spread in India.

In conclusion, the finding of the present study suggest that clinical symptoms alone can be unreliable in specifically predicting infections with \(C. \text{trachomatis}\) and hence there is a need for adopting a specific strategy for the screening of STD patients in India to reduce the overall rate of sexually transmitted infections, which would in turn, reduce the risk of HIV infection in them.

**Acknowledgement**

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**References**


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