Original Research Article

The socio-demographic profile and clinical correlation of *Chlamydia trachomatis* among infertile women at a tertiary care center in North India

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ABSTRACT

Background: The frequency of *Chlamydia trachomatis* infection in infertile Indian women and potential risk factors associated with the infection is not well understood. To improve the primordial prevention *C. trachomatis* infection in developing countries, there is an urgent need to understand the prevalence of the infection among women with infertility and establish the common risk factors associated with this. This study was conducted to determine prevalence of *Chlamydia trachomatis* infection in infertile women attending an infertility clinic in north India and the associated socio-demographic and clinical features associated with it.
Methods: Endocervical swabs, collected from 105 infertile women were tested for *C. trachomatis* by real time-PCR and direct gram's stain. A detailed clinical history and examination was done on each subject during sample collection. The study group was then divided into two comparison groups and p factor was determined and factors with significant correlation were established.
Results: Total 9 out of 105 infertile women visiting infertility clinic were RT-PCR positive for *Chlamydia trachomatis*. The socio-demographic factors that significantly correlated with *Chlamydia trachomatis* infection were lower age group, rural locality and illiteracy. The clinical history and examination findings that significantly correlated with the infection were past history of RTI/STI in the subject, history of RTI/STI in husband, cervical/ vaginal discharge, lower abdominal pain, burning micturition, erythema of genitalia, backache, dyspareunia and dysmenorrhea. The gram's stain finding confirmed the active infection by presence of pus cells.
Conclusions: The study concluded that the socio-demographic risk factor for *Chlamydia trachomatis* infection among infertile women is lower age group, rural locality and illiteracy while several clinical features that are red flags for the presence of such infection are past history of reproductive tract infection along with partner, cervical/vagina discharge, lower abdominal pain, burning micturition, erythema of genitalia, backache, dyspareunia and dysmenorrhea that should never be overseen.

Keywords: *Chlamydia trachomatis*, Clinical features, Infertility, Real-time PCR, Reproductive tract infection, Sexually transmitted infection, Socio-demography

INTRODUCTION

Around 15% of couples are affected with infertility, worldwide. In 2010, around 1.9% women of reproductive age group were affected by primary infertility and ~10.5% of women were affected with secondary infertility.[1] Among several causes of infertility, urogenital infections

serve as an important cause that when approached correctly and treated timely may prevent the morbidity.[2]

The genital infections that are reported to have been associated as cause of infertility are *Candida, Mycoplasma hominis, Ureaplasma infections, Bacterial vaginosis, Chlamydia trachomatis, Neisseria gonorrhoeae, Genital tuberculosis,* human papilloma virus (HPV). Furthermore, Chlamydia trachomatis infection serves as cause of pelvic inflammatory disease, that may end up into complication causing chronic pelvic pain, ectopic pregnancy and infertility.[1,3] According to WHO 2019, approximately 1 million sexually transmitted infections are acquired each day worldwide and estimated 374 million newly acquired reproductive tract infections with 1 out of 4 being caused by, *Chlamydia trachomatis* ~129 million, *Neisseria gonorrhoeae* ~82 million, syphilis ~7.1 million and *Trichomonas vaginalis* (156 million). Chlamydia are microscopic, ovoid or spherical obligate intracellular bacteria classified in the order chlamydiales and family chlamydieaceae containing single genus and three species as *C. trachomatis, C. psittaci* and *C. pneumoniae*. They are non-motile organism possessing both DNA and RNA as the genetic material, carry their own ribosome, multiply by binary fission and have a peptidoglycan free cell wall with inner and outer cytoplasmic membranes and lipopolysaccharide layers.

Chlamydia clinical diagnosis

Since up to 70% to 80% of infected women and 50% of infected males remain asymptomatic, the clinical picture of individuals with chlamydial infection may be deceiving. The typical vaginal discharge of a female with an uncomplicated chlamydial infection is mucoid, odourless, and free of pruritis. If the urethra is affected, dysuria without frequency or urgency will be reported. Additionally, PID can reveal a history of intermenstrual bleeding, dyspareunia, delayed menstrual cycles, and severe abdominal pain with high fever. Cervicitis with a yellow, hazy, mucoid discharge is visible from the os upon examination. When the cervix is scraped with a spatula or brush, it frequently bleeds freely. Clinically, chlamydial infections cannot be separated from other urethral infections.[4]

Laboratory diagnosis

The asymptomatic character of the condition and the widening range of infections brought on by *C. trachomatis* highlight the demand for accurate and sensitive laboratory techniques. The likelihood of finding the pathogen can be affected by the sampling locations used. The typical samples collected from female patients include endocervical swabs, vaginal/introital swabs, vulval swabs, urethral and rectal swabs, and first catch urine. If possible, samples should be collected and processed in less than 48 hours; however, if that is not practicable, samples can be stored at -70 °C until processing.[5]

METHODS

This is a hospital based prospective observational study. Duration of Study is from September 2021 to August 2022.

Study population

Married women of age group 18-45 years visiting infertility clinic, were included in the study while patients who refused to participate in the study and patients with well-established diagnosis for infertility other than infectious cause were excluded from the study.

In the present study 105 infertile women fulfilling the inclusion criteria were enrolled in the study after obtaining informed consent. Patients were clinically examined and were subjected to necessary laboratory/microscopic examinations. Samples were processed in the Laboratory of Post Graduate Department of Microbiology, K.G.M.U., Lucknow. Two Cervical Swabs were collected as per guidelines by National AIDS Control Organization, 2013. Using first cervical swab gram staining was the commercially available IVD Nylon Flocked swab was used for collection and steps are as follows - following aseptic precautions patient was asked to come in lithotomy position. After wearing sterile gloves, a sterile vaginal speculum was inserted in the vagina and exo-cervix was inspected. Type of secretion was observed and then wiped aseptically with cotton swab. The nylon swab was inserted 1-3 cm into endocervix and rotated several times for 10 seconds to 30 seconds. Then, swab was recovered without touching the vaginal surface. The collected cervical swab was put into in commercially available BD Universal Transport Media for storage at -80°C after immediate transport to laboratory.

Nucleic acid extraction and RT-PCR for *Chlamydia trachomatis* was done by using a commercial kit: 1) TRUPCR total nucleic acid extraction kit version 1.0, 2) Real-time PCR for *Chlamydia trachomatis* was done by using Thermo fisher- superscript 3 kit, 3) DNA extraction for *Chlamydia trachomatis* - using the TRUPCR total nucleic acid extraction kit version 1.0. DNA was extracted from 500µl thawed sample suspended in the transport medium. The DNA was then eluted in a final volume of 100 µl of the elution buffer provided in the kit. One negative extraction control was tested in each extraction run (96-well plate). Multiplex Real time PCR for *Chlamydia trachomatis* was carried out on ABI Quant studio 5 (96x 0.2 ml) instrument and for amplification of Nucleic acid TaqMan Probe assay was used. Primer and probe sequences used for Real Time PCR were selected from a previous study done by Jaton et al (2006) and following are sequences for primer probe[7]

Chlamydia trachomatis

Fp - 5'-CATGAAAACTCGTTCCGAAATAGAA-3',
Rp- 5'-TCAGAGCTTTAC CTAACAACGCATA-3'
Probe -5'-FAM-5'-TCGCATGCAAGATATCGA-3'.

Internal control

Fp-5'-AGA TTT GGA CCT GCG AGC G
RP-5'-GAG CGG CTG TCT CCA CAA GT
Probe-5'-VIC-TTC TGA CCT GAA GGC TCT GCG CG-MGBNFQ.

Primers and probe were used in a 25 µl reaction carrying 12.5 µl of (Thermo fisher- superscript 3) PCR master mix,1 µl of each primer of 10 pmol concentrations, enzyme 0.5 µl, 5.0 µl of DNase free water, and 5 µl of DNA template. Cyclic conditions were 2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 sec at 95°C and 1 min at 60°C. Amplification and PCR product detection were performed with ABI Quant studio 5 (Applied Biosystems, USA). Interpretation of result was done. For interpretation, samples with Ct value <35 were considered as positive and samples with Ct value >35 were considered as negative (Figure 1).
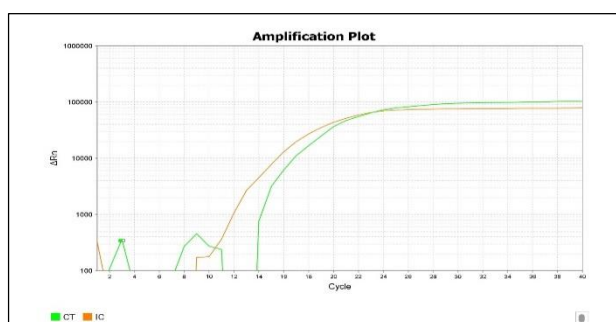
Figure 1: Cervical swab gram's staining under oil immersion (100x) magnification of light microscope.

A second cervical swab was collected immediately and smear was made on glass slide. On smear made form second cervical swab gram's staining was done and observed under oil immersion (100x) magnification of light microscope.

Sample size calculation

Sample size is calculated based on previous study that reported prevalence of *Chlamydia trachomatis* infection in infertile women to be 12.4% by Hossein et al (2008).[6]

RESULTS

Total 9 out of 105 infertile women visiting infertility clinic were RT-PCR positive for *Chlamydia trachomatis*. The study population was divided into two comparison groups to correlate the overall *Chlamydia trachomatis* infection with different associated risk factors and Clinical features. 9 (8.6%) patients with RT-PCR positive for *Chlamydia trachomatis* were classified as Group I, 96 (91.4%) women who were RT-PCR negative were classified as Group II (Table 1).

Table 1: Prevalence of genital *Chlamydia trachomatis* infections among women attending infertility clinic.

Group	Description	No. of women	Percentage
Group I	RT-PCR positive for *Chlamydia trachomatis*	9	8.6
Group II	RT-PCR negative for *Chlamydia trachomatis*	96	91.4

On comparing the two groups, the socio-demographic factors that significantly correlated with *Chlamydia trachomatis* infection were lower age group (44.4%), rural locality (100%), illiteracy (77.8%) with p value less than 0.05 (Table 2).

Table 2: Association of *Chlamydia trachomatis* infection with socio-demographic variables.

Variables	Total (N=105)	Group I (n=9)		Group II (n=96)		Statistics	
		No.	%	No.	%	χ²	'p'
Age group							
≤25 yrs	13	4	44.4	9	9.4		
26-35 yrs	76	4	44.4	72	75.0	9.345	0.009
>35 yrs	16	1	11.1	15	15.6		
Mean age±SD (Range)	30.21±4.71 (19-42)	27.44±5.94 (19-38)		30.47±4.53 (22-42)		't'=1.864; p=0.065	
Religion							
Hindu	94	7	77.8	87	90.6		
Muslim	8	2	22.2	06	6.25	3.188	0.364
Sikh	2	0		2	2.1		
Christian	1	0		1	1.04		
Locality							
Rural	41	9	100.0	32	33.3		
Slum	10	0	0.0	10	10.4	15.366	<0.001
Urban	54	0	0.0	54	56.2		

Variables	Total (N=105)	Group I (n=9)		Group II (n=96)		Statistics	
Education							
Illiterate	31	7	77.8	24	25.0		
Primary	23	2	22.2	21	21.9	12.545	0.005
Secondary	24	0	0.0	24	25.0		
Above Sec.	27	0	0.0	27	28.1		
Occupation							
Housewife	77	7	77.8	70	72.9		
Salaried	6	0	0.0	6	6.25	1.679	0.642
Daily wager	15	2	22.2	13	13.5		
Small business	7	0	0.0	7	7.3		

Table 3: Association of *Chlamydia trachomatis* infection in infertile women with marriage duration and obstetric history.

Variables	Total (N=18)	Group I (n=9)		Group II (n=96)		Statistics	
		No.	%	No.	%	χ^2	'p'
Duration of marriage							
≤5 yrs	44	4	44.4	40	41.7		
>5-10 yrs	36	3	33.3	33	34.3	6.501	0.011
>10 yrs	25	2	22.2	23	23.9		
Mean age±SD (Range)	8.08±5.42 (2-29)	7.44±4.28 (2-15)		8.14±5.53 (2-29)		't'=0.367; p=0.714	
Parity							
G0P0	87	7	77.8	80	83.3		
G0P1	1	0	0.0	1	1		
G1P0	10	1	11.1	9	9.4	1.528	0.822
G2P0	2	0	0.0	2	2.1		
G1P1	5	1	11.1	4	4.2		
Type of infertility							
Primary	87	7	77.8	80	83.3	0.179	0.672
Secondary	18	2	22.2	16	16.7		
History of contraception use							
None	94	9	100.0	85	88.5		
IUD	1	0	0.0	1	1.04	1.152	0.765
Condom	4	0	0.0	4	4.2		
OCP	6	0	0.0	6	6.2		
H/o abortion							
Present	14	1	11.1	13	13.5	0.042	0.837
Absent	91	8	88.9	83	86.5		

Patients with *Chlamydia trachomatis* infection (Group I) had higher prevalence among those married <5 years (44.4%) followed by those married for >5 years. In group II, prevalence of women married <5 years was higher (41.7%) followed by those marriage duration was between 5-10 years (34.3%) followed by those married for >10 years (23.9%). The difference between two groups on the basis of duration of marriage was found to be statistically significant. (p=0.011). The difference of parity between two groups was not statistically significant.

Between the two groups, difference in the percentage of females with primary infertility with PCR positive for *Chlamydia* infection (77.8%) and PCR negative Group

(83.3%) as well as secondary infertility 22.2% vs 16.7% was not statistically significant.

No use of any method of contraception was dominant in both the groups i.e. 100% in Group I and 88.5% in Group II and the difference was not statistically significant.

Positive history of abortion was higher in Group II (13.1%) than in Group I (11.1%) and this difference was not statistically significant. (p=0.765) (Table 3).

Between the two groups, history of RTI/STI was higher in Group I (44.4%) than Group II (25%) but this correlation with history of RTI/STI was not statistically significant.

The history of RTI/STI in husband was higher in Group I (44.4%) than Group II (2.1%) and this difference was statistically significant thereby significantly correlating history RTI/STI in husband with *Chlamydia trachomatis* infection.

Cervical/vaginal discharge was higher in Group I (66.6%) than Group II (27.1%) and this difference was statistically significant. (p=0.014).

The clinical finding of cervix bleeding on touch was higher in Group I (22.2%) than in Group II (12.5%) but this difference was not statistically significant. (p=0.412).

History of burning micturition was present in 55.6% of Group I and 22.9% of Group II and this difference was statistically significant. (p=0.032). History of backache was present in 33.3% of Group I and 4.7% of Group II and this difference was statistically significant. (p=0.031) History of dyspareunia was present in 55.6% of Group I and 8.5% of Group II and this difference was statistically significant. (p=0.004).

Erythema of genitalia was present in 44.4% of Group I and only 15.6% of Group II subjects and this difference was statistically significant. (p=0.032) thereby significantly correlating this finding with genital *Chlamydia trachomatis* infection.

History of dysmenorrhoea was present in 44.4% of women of Group I which was significantly higher than in Group II in whom it was present only in 12.5% of women. This difference was statistically significant (p=0.011) (Table 4).

Table 4: Association of *Chlamydia trachomatis* infection with clinical history and examination findings.

Variables	Total (N=105)	Group I (n=9)		Group II (n=96)		Statistics	
		No.	%	No.	%	χ^2	'p'
H/o RTI/STI	28	4	44.4	24	25.0	1.591	0.207
H/o RTI/STI in husband	6	4	44.4	2	2.08	27.406	<0.001
Cervical/ vaginal discharge	32	6	66.6	26	27.1	6.085	0.014
Cervical hypertrophy	10	1	11.1	9	9.4	0.029	0.865
Genital ulcer	0	0	0.0	0	0.0	-	
Cervix bleed on touch	14	2	22.2	12	12.5	0.673	0.412
Lower abdominal pain	12	5	55.6	7	7.3	18.936	<0.001
Genitalia itching	31	4	44.4	27	28.1	1.053	0.305
Burning micturition	27	5	55.6	22	22.9	4.589	0.032
Erythema of genitalia	27	4	44.4	15	15.6	4.611	0.032
Backache	12	3	33.3	9	9.4	4.666	0.031
Dyspareunia	20	5	55.6	15	15.6	8.509	0.004
Post coital bleed	11	2	22.2	9	9.4	1.448	0.229
Dysmenorrhea	16	4	44.4	12	12.5	6.501	0.011

Table 5: Comparison of Group I and Group II on the basis of pus cells on gram stain.

Total no. of women	Total (N=105)	Group I (n=9)		Group II (n=96)		Statistical significance	
		No.	%	No.	%	χ^2	'p'
Absent	82	0	0.0	82	85.4	35.095	<0.001
Present	23	9	100	14	14.6		

Pus cells on gram's stain was present of all patient of Group I (100%) and only 14 patients of Group II. The distribution was significantly higher in Group I (100%) than in Group II (14.6%). This difference was statistically significant (p<0.001) (Table 5).

DISCUSSION

In present study, prevalence of genital *Chlamydia trachomatis* infection as detected by Real-Time PCR was 8.6% which was higher than DFA confirmed *Chlamydia trachomatis* (4.1%) in study done by Chaudhary et al.[8] This difference in prevalence could be contributed by difference in type of study population and different method of diagnosis for the same organism. These findings parallels the finding in study done by Parpillewar et al (2021), at Maharashtra in which they found prevalence of *Chlamydia trachomatis* to be higher in endocervical swab of infertile females (18.6%) than among controls (5.6%).[9] Another study performed by Kokkayil et al (2013) have found prevalence of *Chlamydia trachomatis* among infertile females to be 13.5% by Real-Time PCR. The prevalence is slightly higher than that found in our study that could be due to difference in regions of study.[10]

In a study, done by Malik et al (2009) they found that 55% of the women with infertility had past infection with *Chlamydia trachomatis* and 45.4% were found to have current infection of *Chlamydia trachomatis*.[11] Out of this 72.7% patients were found to have tubal factor of infertility and a significant correlation was found between genital *Chlamydia trachomatis* infection with secondary infertility. The prevalence was very much higher in this study as compared to our study (8.6%) as their study subjects included only women with secondary infertility where prevalence has been proven to be higher than the primary infertility and also due to exclusion of women with secondary infertility who were diagnosed with genital tuberculosis, ovarian dysfunction, and/or abnormal semenogram in husband. In their study they found that 35 women out of 40 (87.5%) had one or more clinical feature of genital tract infection and 17 of these had were positive for *Chlamydia trachomatis* while in our study only 62.8% of study population showed positive clinical feature and all the subjects with RT-PCR positive for *Chlamydia trachomatis* had at least one positive history or examination finding for genital tract infection. In their study, the most common feature associated with *Chlamydia* infection was bad obstetrics history, then bleeding per vaginum and then abnormal vaginal discharge, chronic cervicitis and menorrhagia which was different than finding in our study where most common clinical feature was vaginal/cervical discharge.

In another similar study done by Al-Farraj et al (2016), found that the *Chlamydia trachomatis* infection was significantly associated with lower age group (<30 years), irregular menstruation which is similar to finding in our study and history of previous abortion which is in contrast with finding in our study as other features that were not significantly correlated in their study were burning sensation during urination, genital bleeding, history of abnormal discharges, low-seated abdominal pain, history of pelvic inflammatory disease, signs of vaginosis and signs of cervicitis but were found to be significant among *Chlamydia* positive patients in present study as there was co-infection in 6 out of 8 patients.[12]

Similarly, in the study of Somayaji et al (2017) also found significant correlation of *Chlamydia trachomatis* infection with lower age group which parallels the finding in our study.[13]

In a similar study done by Ogbu et al (2017) they found the prevalence of *Chlamydia trachomatis* among infertile females (cases) to be 75% as compared to 23.5% among pregnant women (control) as tested by anti-chlamydial antibody test.[14] Among socio-demographic risk factors associated with *Chlamydia trachomatis* infection among cases they found three factors that were significantly correlated with tubal factor infertility and exposure to *Chlamydia trachomatis* were lower age of sex debut (<30 years), higher number of multiple sexual partner and nulliparity which was similar to finding in our study where the infection was significantly prevalent among lower age group (<30 years) and most of the cases were among nulliparous (77.8%). Clinical features associated with genital tract infection in their study that showed higher prevalence among infertile women with tubal factor of infertility and risk of previous *Chlamydia* infection were vaginal discharge (24.5%), dysmenorrhea (24.5%), lower abdominal (23.1%), asymptomatic (16.1%), and intermenstrual bleeding (3.5%) while in our study we found the prevalence of cervical/vaginal discharge to be the most prevalent feature (66.6), 50% of which were vaginal discharge and 50% were cervical discharge followed by lower abdominal pain and burning micturition 55.6%, followed by erythema of genitalia and dysmenorrhea among 44.4% cases. The slight difference in the prevalence of different clinical features might be contributed due selection of all patients suggestive of tubal factor of infertility and at risk of *Chlamydia* infection in their study while in our study the prevalence of different clinical features were studied specifically in RT-PCR positive and negative for *Chlamydia trachomatis* group and also due to presence of co-infection in *Chlamydia trachomatis* positive group.

CONCLUSION

The study concluded that the socio-demographic risk factor for *Chlamydia trachomatis* infection among infertile women is lower age group, rural locality and illiteracy while several clinical features that are red flags for the presence of such infection are past history of reproductive tract infection along with partner, cervical/vagina discharge, lower abdominal pain, burning micturition, erythema of genitalia, backache, dyspareunia and dysmenorrhea that should never be overseen.

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