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Study of *Chlamydia trachomatis* Infection in Infertile Females at a Tertiary Care Hospital in Mumbai, India

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A B S T R A C T

C.trachomatis is one of the most frequently detected sexually transmitted bacterial pathogen as per CDC. Chlamydial infection has major clinical and epidemiological significance in women. This study aims to determine the magnitude of *C. trachomatis* infection in infertile women and study associated sociodemographic factors. Prospective cross-sectional hospital based study. The study was approved by Institutional Ethics Committee. Two endocervical specimen were collected aseptically by per-speculum examination from 120 women aged 18-45 years. Of these, 90 patients presenting with infertility (primary or secondary) were taken as study group and 30 healthy term pregnant women of similar age group as control. Rapid antigen detection test and Grams stain were done using one swab for each test. *C. trachomatis* infection was seen in 2.2% (2/90) patients in primary infertility cases having infertility greater than 10 years. The mean age of all patients was 27 ± 3.6 years. The mean duration of infertility was 3.9 ± 1.8 years. They significantly belonged to lower socioeconomic group and had lower level of education. They presented predominantly with lower abdominal pain, vaginal discharge, polymenorrhea and dyspareunia. Tubal occlusion was significantly observed in the patients tested positive. A low prevalence of *C. trachomatis* infection was found in women presenting with primary infertility. Early screening by Chlamydia rapid antigen detection test and health education would help in early diagnosis and hence prevent complications. Further studies with a larger sample size are suggested.

Introduction

Chlamydia trachomatis infection is known to be one of the most sexually transmitted infection worldwide.[1] According to World Health Organization (WHO) report in 2008, per year 105.7 million new cases of *Chlamydia trachomatis* infection occur worldwide.[2]As per CDC (Centre for Disease Control and Prevention, Atlanta), with increased testing and more sensitive tests, the incidence of reported *Chlamydia trachomatis* infection has been increasing steadily since reporting began in 1984 reaching an all-time peak of 457.6 cases per 100,000 in 2011.[3] Due to their obligate intracellular nature, the detection and manipulation of *Chlamydia* have proved challenging.[4]Diagnosis of

Chlamydial infection is even more difficult in asymptomatic and in chronic or persistent infections where the pathogen load would be low.[5]Prevention and early detection of the infection is essential as both the health and economic consequences of infection are serious. Chlamydial infection has major clinical and epidemiological significance in women.

The large pools of infected women area symptomatic and are at the risk of developing serious long-term sequel when left untreated, like pelvic inflammatory disease (PID), ectopic pregnancy, and infertility and would also transmit the infection. To prevent such sequelae, the Center for Disease Control and Prevention (CDCP) recommends screening for Chlamydiae in women under the age of 24 years, once in a year, for early diagnosis and treatment.[6] However, in India, such a practice has not been adopted, probably due to lack of information on the magnitude of this problem as only few studies with respect to prevalence of *Chlamydia trachomatis* have been done in India. Hence, the present study was undertaken to determine the magnitude of *C. trachomatis* infection in infertile women and to study sociodemographic factors associated with the *C. trachomatis* infection.

Materials and Methods

The study was conducted in Department of Microbiology at a tertiary care hospital in Mumbai as a prospective cross-sectional study to determine the seroprevalence of the *C. trachomatis* in infertile women by using a rapid test for *C. trachomatis* based on the immunochromatogenic method of antigen detection and to study sociodemographic factors associated with the *C. trachomatis* infection. The study was approved by Institutional Ethics Committee. It was carried out for a period of one year (May 2014 to April 2015).

Data and sample collection

Two endocervical specimen were collected aseptically by per-speculum examination from 120 women aged 18-45 years, attending obstetrics and gynaecology (OBGY) Out-Patient department(OPD) at a tertiary care hospital in Mumbai. Of these, 90 patients presenting with infertility (primary or secondary) were taken as study group and 30 healthy term pregnant women of similar age group attending antenatal clinic as control group. Infertility was defined as one year of unprotected intercourse without pregnancy and it was termed as primary if conception had never occurred and as secondary when patient failed to conceive after having achieved a previous conception. [7]Women taking antibiotic therapy within a month of reporting to OBGY OPD were excluded. Sociodemographic pattern of the study group was also noted. The socioeconomic status of the study subjects was calculated as per Modified Kuppuswamy classification 2014. Rapid antigen detection test and Grams stain were done using one swab for each test.

Rapid antigen detection test

C. trachomatis infection was detected using SD BIOLINE Chlamydia Rapid Test (Standard Diagnostic Inc., Korea, Lot No. 094014002, Expiry date 12.11.2015) which worked on principle of a solid phase immunochromatographic assay. Extraction and neutralization solution provided with the kit were used to the samples and the test results were interpreted at 15 minutes. The presence of only one purple band within the result window indicated a negative result. The presence of two color bands (“Test Line” and “Control Line”) within the result window, indicated a positive result. If the purple color band was not visible within the result window after performing the test, the test was considered invalid.

Microscopy

One endocervical swab was used to perform Grams staining. Staining was done as per standard protocol.[8] The number of pus cells per high power field and organisms if any in Grams stain were observed on microscopic examination.

Statistical analysis

The data was statistically analyzed using SPSS version 22. Fisher's exact test and unpaired t-test was applied to test the significance wherever necessary. A p value of < 0.05 was considered significant for the study.

Results and Discussion

Of 120 women aged 18-45 years, attending OBGY OPD at a tertiary care hospital 90 patients presenting with infertility were taken as study group and 30 healthy term pregnant women of similar age group as control. The mean age of the cases presenting with infertility was26.5 ± 3.9 years (mean ± S.D) and of control group was 27 ± 2.5years, no statistical difference was found (p=0.89). 37.78% of the cases and 66.67% of the controls were in the age group of 25 to 29 years (Figure 1). 68.9% cases and 50% controls were Hindu by religion (Figure 2). 50% cases and 43.3% were educated up to secondary school, 20% cases and 23.3% controls were having higher secondary education (Figure 3). Socioeconomic status of the subjects was analyzed according to Modified Kuppuswamy Classification 2014. Most of the subjects belonged to upper middle class i.e. 66.7% cases and 73.3% controls, 15.5% cases and 13.3%controls belonged to upper socioeconomic class (Figure 4). The prevalence of *Chlamydia trachomatis* in the infertile group was 2.2% (n=90) and was not found in control group, however no statistical significance was seen (Figure 5). A statistically significant association was found between *C. trachomatis* infection and lower socioeconomic status as well as lower education level (Table 1). Majority of the infertile women i.e. 85 (94.4 %) had primary infertility whereas 5 (5.6%) women had secondary infertility. *Chlamydia trachomatis* infection rate of 2.4% was found in patients with primary infertility and there was no statistical significance in the infectivity between the primary and secondary infertility groups (Figure 6). The mean duration of infertility was 3.9 ± 1.8 years. 72.2 % (n=90) infertile women had history of infertility of less than 5 years, 22.2% (n=90) had 5 to 9 years history of infertility and 5.5% (n=90) cases had more than 10 years of infertility. Infection rate of *Chlamydia trachomatis* was found to be 40% (n=5) in more than 10 years infertility cases and a statistically significant association was observed in patients with 10 years or more than 10 years of infertility and *C. trachomatis* infection (Table 2). The study cases presented predominantly with lower abdominal pain (52.2%), vaginal discharge (19.3%), polymenorrhea and dyspareunia (17.8%). Both the patients tested positive *C. trachomatis* infection reported of lower abdominal pain, vaginal discharge and only one of the two patients complained of scanty menses. Both the infertile study group and control group did not have history of tuberculosis in the past (Figure 7). The healthy term pregnant women take as control group were free of all signs and symptoms. A statistically significant association was found between a symptom of vaginal discharge and *C. trachomatis* infection (p value=0.04 by Fisher's Exact test). All the haematological investigations of infertile study group and control group were within normal range except for haemoglobin as anaemia was seen in 26.7% of cases and 23.3% of controls (Table 3). 72.2% (n=90) infertile women were symptomatic whereas 27.8% (n=90) women

were asymptomatic. In symptomatic patients 96.9% (n=65) had primary infertility and 3.1% (n=65) had secondary infertility. In asymptomatic cases 88% (n=25) had primary infertility and 12% (n=25) had secondary infertility. 3.1% (2/65) symptomatic women presenting with infertility were tested positive by Chlamydia Rapid Test were and it was found to be statistical significant (Table 4). Human semen analysis of male partners of all the patients in infertile study group was also performed and reported to be within normal limits. Both the cases tested positive for *C. trachomatis* infection showed tubal occlusion on hysterosal pingography and a significant association between occurrence of tubal occlusion and *C. trachomatis* infection was seen (Table 5). Gram stain was considered suggestive of cervicitis when ≥ 30 pus cells/ 1000X field were present.[2] Thus, on interpretation of the Gram stain smears made from the cervical secretions, 29 cases out of 120 (24.2%) were found to have ≥ 30 pus cellsfield and hence probably cervicitis. The 2 cases positive by rapid detection test for *C. trachomatis* also showed presence of ≥ 30 pus cells on Gram staining.

Table.1 Association of sociodemographic factors and *C. trachomatis* Infection

Risk factors	*Chlamydia trachomatis* infection		Total	Fisher's exact test p value
	Present (n=2) (%)	Absent (n=118) (%)		
Age				
<30 years	0	91(100%)	91	0.06 (NS)
≥30years	2(6.9%)	27(93.1%)	29	
Socioeconomic status				
Upper class	0	100(100%)	100	*0.03
Lower class	2(10%)	18(90%)	20	
Religion				
Hindu	2(2.6%)	75(97.4%)	77	1.0 (NS)
Muslim	0	31(100%)	31	
Other	0	12(100%)	12	
Education				
Illiterate	1(20%)	4(80%)	5	*0.01
Primary	1 (10%)	9 (90%)	10	
Secondary	0	58(100%)	58	
Post-secondary	0	47(100%)	47	
Occupation				
Housewife	1 (0.9%)	100(99.1%)	101	0.1 (NS)
Unskilled	1(16.7%)	5 (83.3%)	6	
Skilled	0	13(100%)	13	
Total	**2**	**120**	**120**	

*Significant by Fisher's exact test; NS: Not Significant

Table.2 Association of *C. trachomatis* infection among infertile women according to duration of infertility

Infertility	Chlamydia Rapid Test (n=90)		Total	Fisher's exact test
	Positive	Negative		
<5 years	0	65 (100%)	65	p value: 0.002 Significant
5-9 years	0	20 (100%)	20	
≥ 10 years	2 (40%)	3 (60%)	5	
Total	**2**	**88**	90	

Table.3 Result of hematology investigations

Hematological investigation	Levels	Cases (n=90)	Controls (n=30)
Hemoglobin level(mg/dl) (12-15.8 mg/dl)	**Anemia**	24(26.7%)	7(23.3%)
	Normal	66(73.3%)	23(76.7%)
***TLC/cmm (4000-11000/cmm)**	**Leucopenia**	00	00
	Leukocytosis	00	00
	Normal	90(100%)	30(100%)
Polymorphs (%) (40-80%)	**Neutrophilia**	00	00
	Neutropenia	00	00
	Normal	90(100%)	30(100%)
Lymphocytes (%) (20-40%)	**Lymphocytosis**	00	00
	Lymphopenia	00	00
	Normal	90(100%)	30(100%)
Platelets/cmm ($165*10^3$-$415*10^3$/cmm)	**Thrombocytosis**	00	00
	Thrombocytopenia	00	00
	Normal	90(100%)	30(100%)
***ESR(mm/hr) (0-20 mm/hr)**	**Increased**	00	00
	Decreased	00	00
	Normal	90(100%)	30(100%)

Table.4 Association of *C. trachomatis* infection among infertile women (Symptomatic versus asymptomatic)

Evidence of symptoms	Chlamydia Rapid Test		Total	Fisher's exact test
	Positive	Negative		
Symptomatic	2 (3.1%)	63 (96.9%)	65	p value :1.00 NS
Asymptomatic	0 (0%)	25 (100%)	25	
Total	**2**	**88**	**90**	

NS: Not significant

Table.5 Association of *C. trachomatis* infection among infertile women And tubal occlusion on hysterosalpingography

Tubal occlusion on *HSG	Chlamydia Rapid Test		Total	Fisher's exact test
	Positive	Negative		
Present	2 (100%)	0 (0%)	2	p value :0.0002 Significant
Absent	0 (0%)	88 (100%)	88	
Total	**2**	**88**	90	

Table.6 Clinical profile of symptomatic infertile women in relation to *C. trachomatis* infection

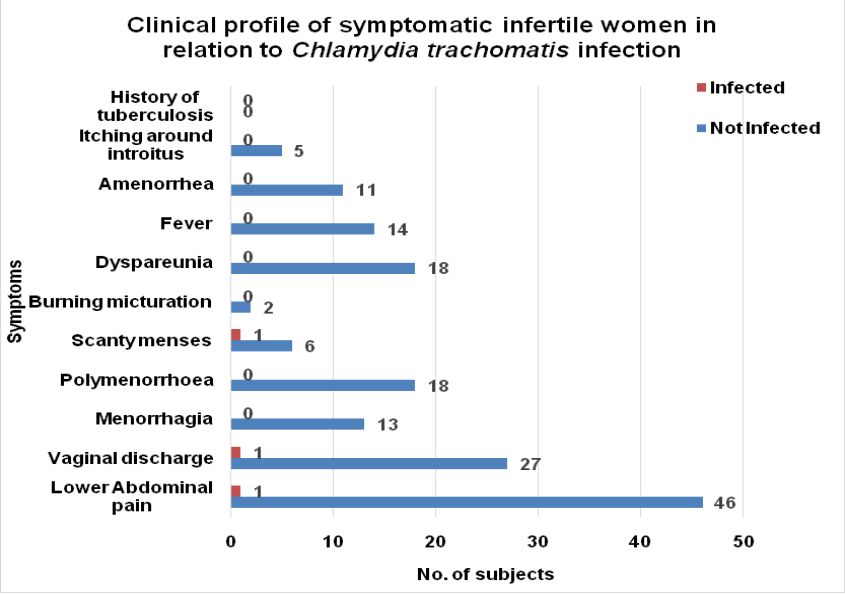


Table.7 Comparison of *C. trachomatis* prevalence in India and worldwide

Authors/ Area/ Year	Age group	Study population	Sample size	Sample used	Test	Prevalence
Brabin L*et al.*, [11]Mumbai, inner city 1998	≤35 years	Suspected PID, infertility	446	Endocervical swab	ELISA	0.5%
		Only infertility	295	Endocervical swab	ELISA	0.3%
Malik A*et al.*,[9] Aligarh, India 2006	Mean age 26.5 ± 4.34 yr	Infertility	110	Endocervical swab	Culture, ELISA	28.1%
Ghosh M*et al.*,[14]Kolkata, India 2015	overall mean age -24.85 ± 4.51 years	Infertility and control group(pregn-ant women attending antenatal clinic)	80	Urine, serum	PCR, ELISA	8.75%
Mania-pramanik J*et al.*,[15]Mumbai, India 2012	16-45 yrs Median age-29 yrs	Gynecological clinic, Complications associated with reproductive health	896	Endocervical swab / Vaginal swab / serum	PCR, ELISA	12.2%
		Only infertility	264	Endocervical swab/Vaginal swab/serum	PCR, ELISA	18.6%
Kamel RM*et al.*,[10]Saudi Arabia 2013	Mean age-26.4 ± 4.8 years	Infertility	640	Endocervical swab, urethral swab	Culture	15%
Morhason-Bello I*et al.*,[17]South West Nigeria 2014	Mean age-31.6 yrs	Infertility	130	Serum	ELISA	20.5%
Adesiji YO*et al.*,[16]Southern Nigeria 2015	20-45 and above years	attending Family Planning Clinics and Gynecology clinics	140	Endocervical swab	Chlamydia Rapid Test (ICS)	0.1%
Present study 2015	18-45 years	Infertility	90	Endocervical swab	Chlamydia Rapid Test(ICS)	2.2%

Figure.1 Age wise distribution of subjects

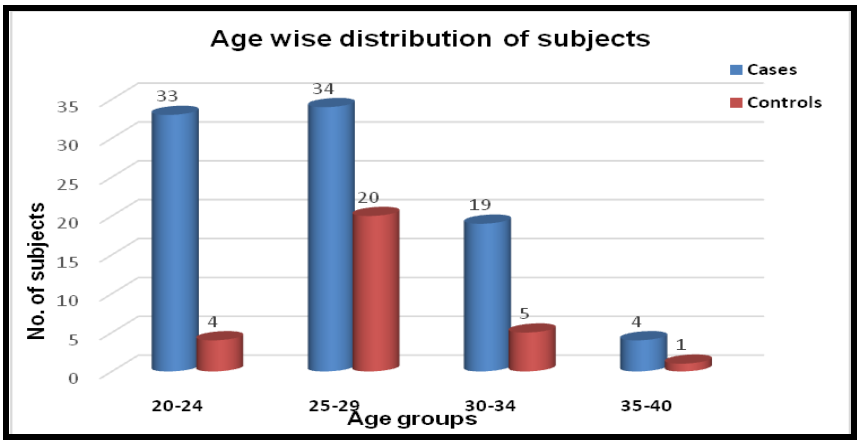


Figure.2 Religion wise distribution of subjects

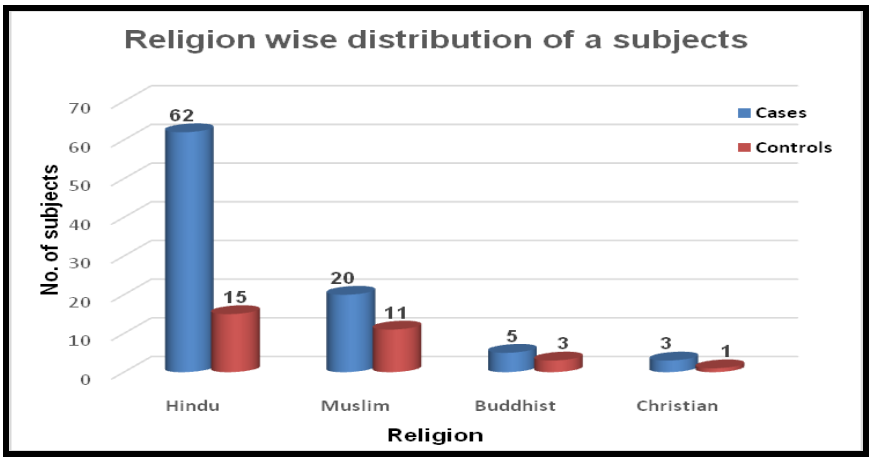


Figure.3 Distribution of subjects according to education

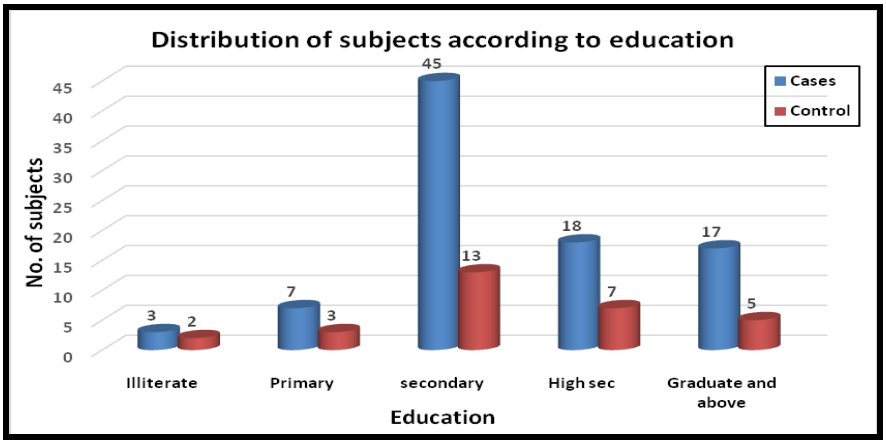


Figure.4 Socioeconomic status of the subjects

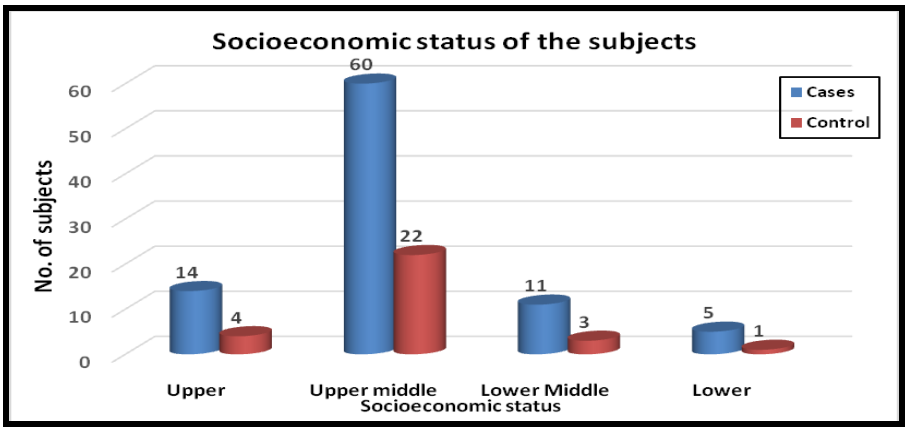


Figure.5 Prevalence of *Chlamydia trachomatis* infection (casesvscontrol)

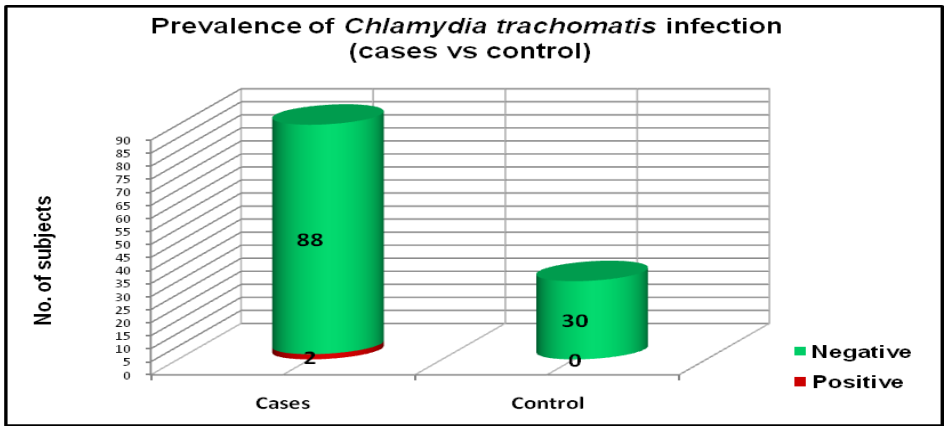
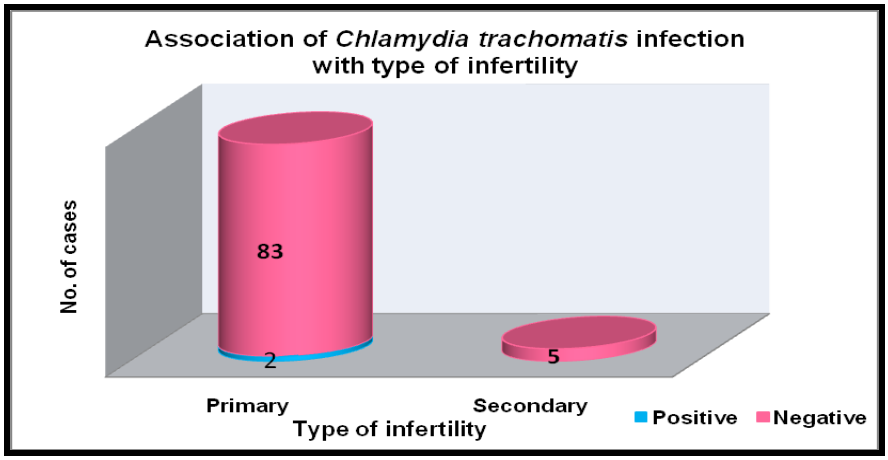


Figure.6 Association of *C. trachomatis* infection among infertile women with type of infertility



NS: Not significant

Demographic profile of the subjects

In this study, all cases were married women aged 20 to 40 years. The youngest case was 20 year old and the case with the maximum age was 36 year old.37.78%cases and 66.67% controls were in the age group of 25 to 29 years. In the age group of 20-24 years, 36.67% were infertile cases and 13.33% were controls. Age group of 30 to 34 years included 21.11% infertile cases and 16.67% controls. Age group 35 to 40 years included 4.44% infertile cases and 3.33%controls.The mean age of 120 patients was 27±3.6 years, of infertile group was 26.5±3.9 years and of control group was 27±2.5 years. There was no statistical difference in the mean age between the study and the control group. On comparing the age group of the study cases with *C. trachomatis* infection, there was no statistical significance. In the study conducted by Malik *et al.*[9], the mean age of the 110 women enrolled was 26.5 ± 4.34 year while the mean age of the control group was 25.4 ± 2.31year, which was in concordance with the present study. In the same study, among the infertile cases 52(47.29%) were in the 21-25 year age group followed by 40 (36.36%) in the26-30 year age group. Of the remaining 18 women,2 were in the 18-20 year bracket, 10 in the 31-35yrage group and 6 were more than 36 year 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. Age group distribution of the subjects was not with the present study however status of control group was comparable with the current study. In Kamel *et al.* study[10], the mean age of infertile women enrolled in this study as the study group was 26.4±4.8 years. This parameter was comparable with the present study.

In the present study, maximum subjects i.e. 68.9% of cases and 50% of controls were Hindu by religion while 22.2% of cases and 36.7% of controls were Muslims, only 5.6% cases and 10% controls belonged Buddhism and 3.3% cases and 3.3% controls were Christian by religion. So the predominant religion of the subjects, including both infertile cases and controls, was Hinduism which is a reflection of the population composition in India. This was not of statistical significance. In study by Brabin *et al.*,[11] conducted in Mumbai, India, the three main religions of the cases and controls were Hinduism (73.8%), Islam (12.3%), and Buddhism (10.9%), which is in concurrence with the present study, however there is no mention about the statistical significance. No data was found about religion distribution in the Indian studies conducted with infertile women as the study population, specifically for *Chlamydia trachomatis* infection. However, as per 2011 census of India, Hindus are the predominant religion group followed by Islam and the same is reflected in the current study.

In the present study, maximum number of subjects i.e. 50 % cases and 43.3% were educated up to secondary school, 20% cases and 23.3% controls were having higher secondary education, 18.9% cases and 16.7% controls were having education of graduation and above, 7.8% cases and 10% controls were having primary education while very few subjects i.e. 3.3% cases and 6.7% controls were illiterate. *Chlamydia trachomatis* infection was found significantly higher in illiterates (20%) followed by women educated up to primary school (10%) than in higher educated group. In El Qouqa *et al*., (2009)[12], 33.3% subjects had elementary education level, 21.1% had preparatory level, 20% had secondary education and least number of patients i.e. 8.3% had higher education. It was not found statistically significant. In the study conducted by Adamson *et al.*, (2011) [13], 45.1% cases had secondary level education, 30.1% cases had primary level education, and 11.5% cases had post-secondary level

education whereas 19.5% cases were illiterate. Compared to the other studies, maximum number of the present study cases had an education level of secondary and above. A significant correlation was seen between a lower education level and *C. trachomatis* infection as of the two cases tested positive, one was illiterate and the other had a primary level education. A good education would definitely help in acquiring proper knowledge of health, hygiene and prevention infection.

In the present study, most of the subjects belonged to upper middle class i.e. 66.7% cases and 73.3% controls, 15.5% cases and 13.4% controls belonged to upper socioeconomic class. 12.2% cases and 10% controls belonged lower middle class and very few subjects belonged to lower socioeconomic class i.e. 5.6% cases and 3.3% controls. The occurrence of *Chlamydia trachomatis* infection was significantly higher in women with lower socio economic class (10%) than in upper socioeconomic class (0%).

In the study reported from Kolkata, India by Mallika Ghosh *et al*[14]., 45.5% cases were from upper middle class, 25% cases belonged to lower middle class, 22.5% were from middle class and 7.5% belonged to upper class. In a study conducted in 2012 by Jayanti Mania-Pramanik *et al.*[15], amajor cause of infertility, all cases belonged to middle socioeconomic status. Observations of the present study were correlating well with Mallika Ghosh *et al.* as the subjects in both studies fall in upper middle class, though the scale used in Mallika Ghosh *et al.*, study was B.G Prasad Classification where as in the present study Kuppuswamy classification was used. In comparison with Jayanti Mania-Pramanik *et al.*, the majority of the subjects included in the present study were from higher socioeconomic class.

Prevalence of *Chlamydia trachomatis* infection infertile women

In the present study, *Chlamydia trachomatis* infection was seen only in the infertile study group and not in the control group and this was not found statistically significant. Thus, the prevalence of *Chlamydia trachomatis* infection in infertile women was 2.2% and there was no evidence of the infection in the control group.

In study conducted by L. Brabin *et al.*[11],the prevalence of *Chlamydia trachomatis* was found to be 0.3% in infertile cases and 0.2% in their control group. In the study by Adesiji *et al.*[16], a low prevalence of 0.1% in the population sampled across three Western States of Nigeria. These studies show a lower prevalence than the present study. On the contrary, In Ghosh *et al.*, study[14], a total of 7 out of 80 (8.75%) infertile patients were diagnosed as infected. In study conducted by Morhason-Bello *et al.*, [17], the prevalence of *C. trachomatis* was 20.5% (27/132). In the study by Malik *et al.*[9], the overall Chlamydial positivity in the infertile women was found in 28.1% cases who were positive for one or both Chlamydial markers while 3.3% healthy at term control women were found positive for *C. trachomatis*. Thus, based on the comparison of *C. trachomatis* prevalence in India and worldwide, it is observed that the prevalence of *C. trachomatis* infection causing infertility remained low in Mumbai even in 1998 although their control group showed a positivity of 0.2% unlike the present study where infectivity was not observed in the control group. The prevalence of *C. trachomatis* infection causing infertility in various studies ranged from 0.3% to 28% and in the control group it was 0.2% to 3.3% (Table 7).

On studying the association of demographic factors and *C. trachomatis* infection in the

present study, a statistically significant association was observed between *C. trachomatis* infection and lower level of education and also lower socioeconomic status among the *C. trachomatis* positive patients as formerly discussed. Maximum numbers of patients in the study were housewives i.e. 84.2%, unskilled workers were 5% and skilled workers were 10.8% by occupation. In a study reported from Zaria, Nigeria by Koledade *et al.*[18], there were no significant statistical association between the sociodemographic characteristics of the patients considered and seropositivity for *Chlamydia trachomatis*, except for the statistically significant association between educational status and patients who were seropositive for *Chlamydia trachomatis* IgG, with primary educational status having the most and tertiary having the least association. In study conducted by Morhason-Bello *et al.*[17], the proportion of women with primary education or less 44.1% (15/34) with positive results was significantly higher than in those with higher education. This correlated well with the present study stating prevalence of *Chlamydia trachomatis* infection is less educated group. In Joanna Crichton *et al.* study[19], which was a metanalysis for socioeconomic factors and other sources of variation in the prevalence of genital chlamydia infections, it was found that there is an association between lower educational opportunities/attainment and increased risk of *Chlamydia* infection and it also provides evidence of a consistent association between socioeconomic disadvantage and higher risk of *Chlamydia* infection. This was in concordance with the present study. The statistically significant association between *C. trachomatis* infection and lower level of education and lower socioeconomic status may be due to low levels of hygiene, ignorance about various health problems, less awareness about the infections and preventive modalities of these infections.

In the present study, majority of the infertile women i.e.85 (94.4 %) had primary infertility whereas 5(5.6%) women had secondary infertility. The mean duration of infertility was 3.9 $\pm$ 1.8 years. *Chlamydia trachomatis* infection rate of 2.4% was found in patients with primary infertility whereas it is zero percent in patients of secondary infertility. There was no statistical significance in the infectivity between the primary and secondary infertility groups. In Mallika Ghosh *et al.* (2015)[14], 75% of cases were suffering from primary infertility and rest 25% from secondary infertility. In Kamel *et al.*[10] study conducted in 2013, 425 out of 640 (66.4%) of the Saudi infertile women had primary infertility whereas 215 out of 640 (33.6%) women had secondary infertility. The mean infertility duration for the study group was 3.28 ±1.73 years. The results of both the studies were comparable with the present study. In all the above studies, the majority of patients presented with primary infertility. The probable reason as to the majority of the cases belonging to the group of primary infertility could be the desire of women unable to conceive to pursue in their quest to find the cause and treatment for infertility.

In a study by Heloisa Lopes Lavorato *et al.*[20], infertility, the prevalence of chlamydial infection was 8% with similar prevalence between primary (8.1%) and secondary (8.0%) infertility but having no statistical significance between the two groups. In a study reported from Aligarh by Malik A *et al.*in 2006[9], the overall chlamydial positivity in the infertile women was found in 31 (28.1%) cases who were positive for one or both chlamydial markers while 1(3.3%) healthy at term control women was found positive for *C. trachomatis*. These results were in discordance with the present study. All these various studies including the present study shows the association of infertility and *C. trachomatis* infection predominantly in primary infertility.

In the present study, 72.2% of the infertile group had a history of infertility of less than 5 years, 22.2% gave a history of 5 to 9 years of infertility and 5.6% presented with more than 10 years of infertility. The mean duration of infertility was 3.9 $\pm$ 1.8 years. Infection rate of *C. trachomatis* was found 40 % (2 were positive from 5 cases) amongst the women with infertility of more than 10 years, while there was no evidence of infection in other groups. Hence, a statistical significance was proved in patients with 10 years or more than 10 years of infertility and *C. trachomatis* infection. In a study by Morhason-Bello *et al.* (2014)[17], 72.0% had been infertile for 5 years or less, another 20% for 6-10 years while the remaining 6.8% had been infertile for more than 10 years. The findings of the present study were comparable with this study. In a study conducted by Remah M Kamel *et al.* [10] in 2013, the mean duration of infertility was 3.9 ± 1.8 years which is in concordance with present study. In Malik A *et al.*[9], the duration of infertility seen was less i.e. 2-4 year in 74.2% of cases. In the remaining 25.8%, the infertility was observed to be greater than 4 years of duration. In Remah M Kamel *et al.*[10], 498 out of 640 women with infertility of a duration of less than5 years had an infection rate of 12.5%, whereas 142 out of 640 women with infertility duration longer than 5 years had an infection rate of 2.5%. This finding was not of statistical significance. In the various studies including the present one, the period of infertility ranged from 1 year to 12 years. *C. trachomatis* can cause infection at any period in the reproductive life of a woman giving rise to primary or secondary infertility as observed in the various studies. Hence patient should be screened for causal microorganisms at the earliest by appropriate screening test to prevent further morbidities.

In the present study, on taking a history of symptoms presented by the infertile group of women, majority reported lower abdominal pain as seen in 47 (52.2%) patients followed by vaginal discharge in 19 (21.1%), polymenorrhea and dyspareunia in 16(17.8%), fever in 14(15.6%), menorrhagia in 13(14.4%). Amenorrhea was reported in 11(12.2%), scanty menses in 7(7.8%), itching around vaginal introitus in 5(5.6%) and burning micturition in 2(2.2%).The two cases tested positive for *C. trachomatis* infection reported lower abdominal pain, vaginal discharge and scanty menses. A statistically significant association was found between a symptom of vaginal discharge and *C. trachomatis* infection (Table 6). In a study conducted by Kamel *et al.*, [10], painful micturition was observed in 115 women (17.97%), vaginal discharges in 98 women (15.31%), pelvic pains in 96 women (15.0%), irregular uterine bleeding in 47 women (7.34%), postcoital bleeding in 27 women (4.22%), and urethral discharge in three women (0.47%). In Brabin *et al.*[11]study conducted at Mumbai, India, patients complained of dysmenorrhea (7.8%), lower abdominal pain (1.7%), vaginal discharge (9.2%), irritation/itching (1%) and pain during intercourse (21.8%). All these were significantly associated with *C. trachomatis* infection in this study. Comparing various studies, symptoms like persisting vaginal discharge, lower abdominal pain and burning micturition should draw attention to causative microorganisms and investigated accordingly.

In the present study, 72.2% infertile women were symptomatic whereas 27.8% women were asymptomatic. Most women of the study group were symptomatic (65 women) with an infection rate of 3.1%. This finding was not of statistical significance. Of the symptomatic cases 96.9% had primary infertility and 3.1% had secondary infertility. Of the asymptomatic cases 88% had primary infertility and 12% had secondary infertility. In a study reported from Aligarh by Malik A

et al.[9], asymptomatic cases (52.7%) slightly predominated in the study. Majority of the asymptomatic cases i.e. 79.3% had primary infertility while 20.7% had secondary infertility. Among the 47.3% symptomatic cases there were 54% cases of primary infertility and 24(46%) cases of secondary infertility.

In the present study, occurrence of tubal occlusion (finding in hysterosalpingography) was seen in both the cases tested positive by rapid antigen detection test. A statistical significance was observed in association of tubal occlusion with *C. trachomatis* infection. None of the other cases in the infertile study group had tubal occlusion on hysterosalpingography. In the study conducted by Malik *et al.*[9], 12 out of 31 (38%) Chlamydia positive cases had tubal occlusion, 5 of these had primary infertility and 7 had secondary infertility. However, both the studies have shown the evidence of tubal occlusion in the infected cases. In a study conducted by Spandorfer *et al.*[21] in USA on previously undetected *Chlamydia trachomatis* infection, immunity to heat shock proteins and tubal occlusion in women undergoing in-vitro fertilization, out of 58 tubal occlusion cases 15 had *C. trachomatis* infection (25.9%). It was found that *C. trachomatis* infection was significantly related to tubal occlusion. Both this study and the present study have found statistically significant relation between *C. trachomatis* infection and tubal factor infertility. Besides, in both symptomatic and asymptomatic cases more than 88% had primary infertility in the present study. This highlights the relation between Chlamydial pelvic inflammatory disease (PID) and tubal infertility. The results emphasize the need for *C. trachomatis* screening of symptomatic and asymptomatic sexually active women at risk of this infection as well as couples seeking an infertility evaluation. Identification and prompt treatment of *C. trachomatis* genital infection in women and their sexual partners will decrease the prevalence of tubal factor infertility.

In the present study, Gram staining was also done. Gram stain was considered suggestive of cervicitis when more than 30 pus cells/1000X field were present.[2] On interpretation of Gram stained smears in the present study, 29 cases were found suggestive of cervicitis as they had more than 30 pus cells/1000X field. This included the 2 cases detected positive for *Chlamydia trachomatis* infection by rapid antigen detection test. A study by Linda Myziuk *et al.*[22] in 2001 at Canada, states that the presence of any signs of cervicitis - which includes mucopus, friability, erythema, and ectropion together with greater than 10 polymorphonuclear cells(PMN)/HPF was statistically significant for the presence of *C. trachomatis*. It further concludes that use of endocervical Gram smear results together with clinical information can be used to identify high risk women for *C. trachomatis* infection.

In conclusion, a low prevalence of 2.2% of *C. trachomatis* infection was found in the infertile women in the present study. *C. trachomatis* infection can lead to long term complications like tubal factor infertility which can be successfully prevented by screening of the infertile patients in the initial stages of infertility and also an early therapeutic intervention can be done. Increased awareness of STIs through health education amongst all socioeconomic classes especially low and middle socioeconomic class will greatly help in women seeking medical advice, thus resulting in early accurate diagnosis and treatment. Association of *Chlamydia trachomatis* infection and infertility, its prevalence and risk factors would be determined more accurately by conducting further studies with a larger sample size.

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