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Microbiology Section

Seroprevalence of *Chlamydia trachomatis* among Infertile Female Patients: A Cross-sectional Study

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ABSTRACT

Introduction: Among many sexually transmitted pathogens, *Chlamydia trachomatis (C.trachomatis)* is increasingly being connected with long-term sequelae like infertility, in addition to its ability to cause genital tract infection.

Aim: To determine the seroprevalence of *C.trachomatis* Infection (CTI) among the infertile female patients at a tertiary care centre.

Materials and Methods: This was a cross-sectional study conducted from March 2019 to February 2020 at the Outpatient Department (OPD) of Reproductive Medicine and Surgery, Sri Ramachandra Hospital, SRIHER, Chennai, Tamil Nadu, India. Women with primary and secondary infertility were included in the study and their clinical history was collected in a proforma. Estimation of Immunoglobulin G (IgG) antibody by Enzyme Linked Immunosorbent Assay (ELISA) to *C.trachomatis* was done in the patient's serum.

Results: Out of the 130 female patients with infertility, 10 (7.6%) of them were positive for *C.trachomatis* IgG antibody. Among the 10 patients, five were primary infertility and the rest were secondary infertility. The most common risk factors were lack of awareness of contraceptive usage, young age, use of Intrauterine Device (IUD) and multiple sexual partners were generally associated with genital Chlamydial infection.

Conclusion: The prevalence of *C.trachomatis* was 7.6% in the present study and was found to vary based upon the culture, hygiene and education. Hence, the present study recommends screening of IgG antibody by ELISA for *C.trachomatis*, which plays a significant role in the diagnosis of infertility.

Keywords: Contraceptives, Genital *chlamydia*, IgG antibody, Sexually transmitted disease

INTRODUCTION

Sexually Transmitted Disease (STD) is mainly due to *Neisseria gonorrhoeae*, *Treponema pallidum*, Herpes Simplex Virus (HSV), Human Papilloma Virus (HPV), Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV), *Trichomonas vaginalis*, etc., *C.trachomatis* is one among them but increasingly seen in infertile females worldwide. *Chlamydiae* spp., are obligate intracellular microorganisms. Based on disease produced *C.trachomatis* was subdivided into two strains or biovars, Trachoma Inclusion Conjunctivitis (TRIC) and Lympho Granuloma Venereum (LGV) [1,2]. Genital infections with *C.trachomatis* (serovars D-K) cause cervicitis and urethritis in females and proctitis in both the sexes [2,3]. The untreated CTI in women may develop Pelvic Inflammatory Disease (PID) which can lead to long term sequelae like infertility (Infertility defined as failure to conceive even after a year of frequent unprotected intercourse [2]), ectopic pregnancy and chronic pelvic pain [4-6]. It is the most frequently reported bacterial Sexually Transmitted Infection (STI) in the United States.

The PID is one of the most significant complications of STD and this is primarily caused by *C.trachomatis* and *N.gonorrhoeae* or both [7]. The incidence of Chlamydial infections in women has increased dramatically from 50.8 to 319.6 per 100,000 between 1987 and 2004 [8]. Recent studies from India have revealed the prevalence of *C.trachomatis* in young females to be 43% in the Gynaecology Outpatient Department [9] and 19.9% in the STD patients [10]. Since the prevalence of Chlamydial diseases is rising, development of sensitive, specific and rapid methods to diagnose these infections is highly preferred [11]. CTIs are diagnosed by cultivation and identifying the cytoplasmic inclusion bodies in the cell lines, Direct Immuno Fluorescence Assay (DFA), ELISA, Deoxyribonucleic Acid (DNA) hybridisation techniques and Polymerase Chain Reaction (PCR) are several different methods which are used to diagnose the *C.trachomatis* [2,7,12]. Endocervical swab culture is considered as a gold standard and has several limitations, so non cultural methods using the endocervical swab is preferred for the rapid diagnosis [13]. Apart from this recently Nucleic Acid Amplification Technique (NAAT) was also found to be significantly more sensitive for the diagnosis of *C.trachomatis* [14]. Hence, the present study aimed to determine the seropositivity for *C.trachomatis* among the females with infertility and their risk factors.

MATERIALS AND METHODS

A cross-sectional study was conducted from March 2019 to February 2020 amongst females with infertility issue attending the OPD of Reproductive Medicine and Surgery, Sri Ramachandra Hospital, SRIHER, a tertiary care hospital in Chennai, Tamil Nadu, India. The present study is cleared by Institutional Ethics Committee with the enrollment number IEC-NI/20/FEB/74/11.

Inclusion criteria: All Infertile women in the reproductive age group between 18 and 42 years of age with both primary (when a conception has never been achieved by a couple after a year of regular unprotected sexual intercourse) and secondary (Inability to conceive after a previous successful conception) causes of infertility, having no history of genital tuberculosis, and patients willing to give consent for taking part in the study were included.

Exclusion criteria: Patients with antibiotics treatment in the previous two months those with history of recently treated genital tuberculosis, repetitive specimen from the same patients and patients who were not willing to give consent were excluded from the study.

Study Procedure

Study participants comprised of 130 females with infertility. Of these primary were 86 (66.2%) and the rest 44 (33.8%) were secondary infertility. A detailed clinical history was taken.

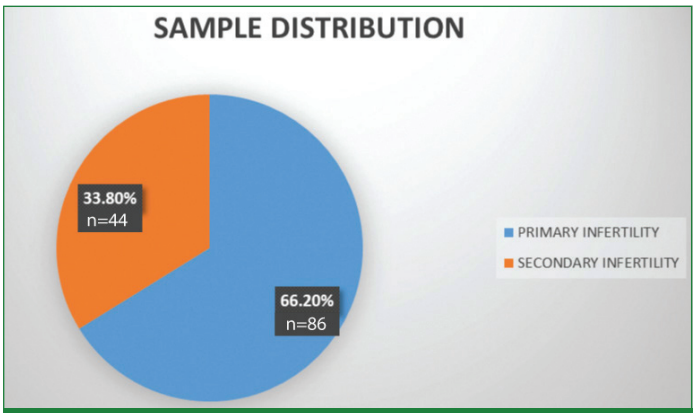
Specimen: Blood sample (n=130) was collected under strict aseptic precautions, serum separated to detect IgG antibodies against *C.trachomatis*. *C.trachomatis* IgG ELISA kit (EI 2191-9601 G) procured from EUROIMMUN (Germany) was used as per the manufacturer's instruction.

STATISTICAL ANALYSIS

The risk factors and their statistical significance in contributing infertility were determined by using the Chi-squared test (Open Epi software). The p-value <0.05 was considered statistically significant.

RESULTS

Of the 130 samples, 44 (33.8%) and 86 (66.2%) had primary and secondary infertility [Table/Fig-1] were subjected to *C.trachomatis* IgG ELISA, 7 (5.3%) were more than cut-off value of 22 RU/mL and reported positive, three samples (2.3%) had borderline titer and failed to reviewed because of lockdown and hence a repeat test of this patients could not be carried out to see increase in titer. Of these positive patients, 5 (50%) were from primary infertility and 5 (50%) were secondary infertility. The risk factors commonly associated with genital Chlamydial infection in the present study were lack of use of a contraceptive device (60%), young age (50%), nulliparity (50%), use of IUD (40%), multiple partners (20%) and symptoms suggestive of PID (20%). Risk factors of more than three were found in 2 (20%) cases of females with infertility. The commonest being lack of contraceptive use, young age, use of IUD and multiple partners [Table/Fig-2].



[Table/Fig-1]: Sample distribution.

S. No.	Risk factors	Total number of patients who had the risk factors (%) n=130	*C.trachomatis* IgG positive	p-value
1	Intrauterine devices (IUD)	12 (9.2%)	4 (33.3%; n=12)	**0.000467**
2	Young age	62 (47.7%)	5 (8.1%; n=62)	0.879129
3	Multiple partner	7 (5.4%)	2 (28.6%; n=7)	**0.033069**
4	Nulliparous	44 (33.8%)	5 (11.4%; n=44)	0.261168
5	Symptoms of Pelvic Inflammatory Disease (PID)	11 (8.5%)	2 (18.2%; n=11)	0.172382

[Table/Fig-2]: Risk factors and its statistical significance for contributing infertility.

DISCUSSION

C.trachomatis genital infections are often asymptomatic [7,13,15]. Usually, the cervix is the initial site of the infection but the urethra and rectum may also be infected [13]. Some individuals experiences burning micturition, vaginal discharge, dysuria, lower abdomen pain, pelvic pain, postcoital bleeding, nausea and fever [13]. Even in the present study, the most frequent complaints were abdominal pain, back pain, foul smelling white discharge among the seropositivity patients and one patient also had endometritis diagnosed earlier. The clinical conditions associated with the genital *C.trachomatis* are urethritis, cervicitis, cervical dysplasia, PID [3,4,9,15]. Svensson LO et al., and Malhotra M et al., stated, the *C.trachomatis* is taken into consideration to be a main purpose of PID and female infertility worldwide [15,16]. Repeated episode of PID doubles the risk of tubal factor infertility and development of ectopic pregnancy. Ray K also emphases *C.trachomatis* as a preventable cause of infertility [14]. PID is seen in 15-40% of women with cervical Chlamydial infection [17,18]. About 20% of women with PID will become infertile as a result of tubal scarring, 18% will experience persistent pelvic pain and 9% will have an ectopic pregnancy [19]. In the present study, 20% of the patients were diagnosed to have PID. Chlamydia DNA seems to persists in human fallopian tube tissues, the persistence of the infection may stimulate immune mediated tissue damage leading to tubal factor infertility [20]. Several studies stated the risk factors of CTIs are young age, single status, oral contraceptive use, IUD, inconsistent condom, multiple sex partner and previous history of STI [21]. In the present study, 50% of them were nulliparous and belonged to age group less than 30 years, 40% had inconsistent IUD usage, 20% had multiple partner and 20% of them had symptoms of PID as shown in [Table/Fig-2].

When comparing the risk factors and their statistical significance for contributing to infertility, in the present study, usage of IUD and multiple partners had a significant p-value of 0.000467 and 0.33069, respectively. *C.trachomatis* causes the most prevalent bacterial STI in the world [22]. Global STI surveillance reported 127 million new cases of *C.trachomatis* in 2016 [23]. In Europe and America, *C.trachomatis* genital infections are the most frequent STI. They primarily affect younger age people. More than 1.3 million CTIs were reported to the CDC in the United States in 2010 [24]. In 2017, the European Centre for Disease Prevention and Control received over 4,09,646 *C.trachomatis* cases from 26 European countries [25]. In 2016, the global prevalence of *C.trachomatis* was estimated to be 3.8% in women and 2.7% in men [26]. According to a systematic review, the prevalence of *C.trachomatis* among reproductive age women in different region of Africa like Eastern Africa, Middle Africa, Southern Africa and Western Africa was 8.9%, 7.2%, 5.9% and 7.4%, respectively [22]. In the present study also, the prevalence of CTI was 7.6%. In Bombay, the prevalence of *Chlamydia* was 23.2% among female sex workers attending an STD clinic [27].

In Assam, the seropositivity of *C.trachomatis* was 25% in primary and secondary infertile women but only 7.5% in the control group [28]. But in the present study the seroprevalence of *C.trachomatis* is only 7.6% probably due to increased awareness and hygienic condition associated. The prevalence of *C.trachomatis* was 5.9% and 1.8% among rural and urban women, respectively [29]. Ghosh M et al., had 8.75% prevalence of *C.trachomatis* among the infertile women by ELISA and PCR [29]. Bajpai T et al., in their study observed 4.5% positive and 6.5% borderline positive for IgG ELISA among infertile women [30]. Malik A et al., and associates did a detailed study on *C.trachomatis* infection in females with secondary infertility specifically [31]. In Aligarh, 55% and 5.5% seropositivity was observed among secondary infertility women and control group by ELISA and cell culture from endocervical and blood sample [32]. The overall positivity rate of *C.trachomatis* was 33% among PID patients from cervical specimen by using EIA [33]. In Karnataka, very low prevalence of 0.88% of *C.trachomatis* was observed using multiplex amplicor Chlamydia trachomatis/Neisseria Gonorrhoeae (CT/NG) kit among men and women [34]. The prevalence of *C.trachomatis* and *Neisseriae gonorrhoeae* was 2.2% and 5.4% on single specimen by Roche amplicor PCR among men attending STI clinic [35].

The prevalence of *C.trachomatis* has been reported in Orissa with 7.04% in patient attending Gynaecology OPD by PCR from cervical swab specimen [36]. In Karnataka, the prevalence of 10.5% *C.trachomatis* was observed among men and very low prevalence

was observed among women [36,37]. The incidence was 3% and 0% among high risk population attending STD clinic and sexually active healthy volunteers [37]. In Mumbai, the prevalence of *C.trachomatis* was found to be 12.1% [4]. In the present study, *C.trachomatis* antibody was found in 7.6% of the infertile female patients. A very low 0.38% prevalence of *C.trachomatis* was observed among pregnant women in Puducherry [38]. So the seroprevalence varying in different parts of the country may be due to the cultural, social behaviour, personal hygiene, literacy, population studied and methodology of the assay. The results of the present study are comparable to seroprevalence in Orissa, India. Most of the studies done by several authors have indicated the presence of Chlamydial antibodies and hence past infection in secondary infertile females. The authors here, in the present study, had antibody prevalence in both primary and secondary infertility. Joyee AG et al., evaluated the different serological markers and recommended that a single serological marker cannot be a diagnostic help for CTI. Concurrent detection of IgA, IgM and IgG is required to identify the infection [10]. However, the authors have done our serological assay by ELISA only. A list of studies conducted at various places and the *C.trachomatis* prevalence percentage are tabulated in the [Table/Fig-3] [4,26-36,38].

S. No.	Name of the study	Place of the study	Year of study	Prevalence (%) of *C. trachomatis*
1.	Divekar AA et al., [26]	Bombay	2000	23.2%
2.	Mohan DG and Borthakur AK [27]	Assam	2015	25% in primary and secondary infertility and 7.5% in control group
3.	Alexander R et al., [28]	South Indian women	1993	5.9% and 1.8% among rural and urban women
4.	Ghosh M et al., [29]	Eastern India	2015	8.75% among infertile women
5.	Bajpai T et al., [30]	Indore	2015	4.5% positive and 6.5% borderline positive
6.	Malik A et al., [31]	Aligarh	2009	55% among secondary infertility and 5.5% among control group
7.	Shrikhande SN et al., [32]	Nagpur	1995	33% among PID patients
8.	Sowmya B et al., [33]	Karnataka	2001	0.88% among men and women
9.	Lindan C et al., [34]	Mumbai	2004	CT-2.2% NG-5.4%
10.	Dwibedi B et al., [35]	Orissa	2009	7.04%
11.	Becker M et al., [36]	Karnataka	2010	10.5% among men and very low prevalence among women
12.	Mania Pramanik J et al., [4]	Mumbai	2012	12.1%
13.	Stephen S et al., [38]	Puducherry	2017	0.38% among pregnant women

[Table/Fig-3]: List of studies conducted at various places and *C.trachomatis* prevalence percentage [4,26-36,38].

CONCLUSION(S)

Of the total number of 130 samples tested in the present study, it showed the prevalence of *C.trachomatis* IgG antibody (7.6%). This study emphasises the importance of screening for *C.trachomatis* in the early stages. So that appropriate therapy can be given, thereby preventing long term complications like tubal scarring and blockage which ultimately result in infertility.

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