



Short Note

Seropositivity of *Chlamydia trachomatis* & *Toxoplasma gondii* among male partners of infertile couples in Odisha, India: A facility-based exploratory study

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Chlamydia trachomatis (CT) is the most common curable sexually transmitted non-viral infection, which can cause urethritis, prostatitis, orchitis and epididymitis among males. *Toxoplasma gondii*, a protozoan causes toxoplasmosis among humans. These infections may lead to decreased fertility. Hence, this study was aimed to estimate the seropositivity of CT and *T. gondii* infection among the male partners of infertile couples in Odisha, India. In this facility-based cross-sectional study, first void urine, seminal fluid and blood sample were collected from 153 males attending infertility clinics including 74 healthy controls. Urine and seminal fluid were tested through polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay was used to detect immunoglobulin (Ig) G, IgA and IgM anti-*Chlamydia* and IgM anti-*Toxoplasma* antibodies through serum. The prevalence of CT was 1.26 per cent (95% CI: 0.03-6.85) as estimated from semen samples using PCR. We detected anti-*Chlamydia* antibodies IgM-four per cent [95% confidence interval (CI): 0.83-11.24]; IgA-28.16 per cent (95% CI: 18.13-40.09) and IgG-12.5 per cent (95% CI: 5.87-22.4) among participants. Anti-*Toxoplasma* antibodies IgM were observed in 27.63 per cent (95% CI: 17.98-39.08) of participants. None of the control samples were found positive. Overall seropositivity of CT and *Toxoplasma* infections is comparable, which suggests that greater attention is required for screening these infections at clinics, especially among infertile couples.

Key words *Chlamydia trachomatis* - India - Infertility - male - sero-prevalence - sexually transmitted infection - *Toxoplasma gondii*

Chlamydia trachomatis (CT) is the most common curable sexually transmitted non-viral infection caused by a Gram-negative obligate intracellular bacterium¹. A recent meta-analysis reported a 2.9 per cent [95% confidence interval [CI], 2.4-3.5%) global prevalence of genital CT among general population². In males, it can cause non-specific urethritis, prostatitis, orchitis

and epididymitis, leading to mechanical hindrance for the sperm to reach the female reproductive tract³. About 50 per cent of the cases in males are asymptomatic and hence under-diagnosed⁴. A previous study reported association of decreased pregnancy rates with the presence of anti-*Chlamydia* IgG antibodies among infertile males⁵.

Toxoplasma gondii is a protozoan which causes toxoplasmosis among humans. A study in Northern India reported 36.8 per cent seroprevalence of *Toxoplasma*⁶. Although toxoplasmosis is generally asymptomatic, *in vitro* studies suggest that toxoplasmosis can decrease male fertility by altering sperm parameters⁷.

The burden of these infections negatively impact upon the quality of life of people in sexually active reproductive age group. A asymptomatic nature of these infections along with milder clinical presentation and associated stigma contribute to their under-reporting. Although CT commonly affects both partners yet, the World Health Organization (WHO) guidelines recommend that screening strategies be aimed at females only, considering it a primary problem among the latter⁸.

Serum antibodies such as immunoglobulin (Ig) help in the detection of past (through IgG) and present (through IgA/IgM) infection state^{9,10}. In addition, a considerable variation in the prevalence across various regions has been reported for these infections^{2,11}. Only a few studies on CT and *T. gondii* infection among males are available from within India with sparse evidence from eastern India. Hence, this study was planned to estimate the seropositivity of CT and *T. gondii* infection among male partners of infertile couples in Odisha.

Material & Methods

This facility based exploratory cross-sectional study was conducted in patients consulting the department of Obstetrics & Gynecology (O&G), Shri Ram Chandra Bhanj (SCB) Medical College and Hospital, Cuttack, Odisha, India, during June 2018 to September 2019. The samples of suspected cases were referred to the Virology Research and Diagnostic Laboratory, Indian Council of Medical Research (ICMR)-Regional Medical Research Centre (RMRC), Bhubaneswar, Odisha. The study was approved by the Institutional Ethics Committee of the ICMR-RMRC, Bhubaneswar, and SCB Medical College and Hospital, Cuttack. Informed written consent was obtained from the participants before enrolment and screening.

Adult male partners of infertile couples belonging to the reproductive age group and attending the O&G Clinic for treatment of infertility were enrolled in this study; those providing informed written consent were included. Patients with already diagnosed cases of sperm dysfunction or primary and secondary testicular failure were excluded. Patients diagnosed with infections such as *Mycoplasma* and *Gonococci*

along with those not willing to respond/give samples in the midst of the interview were excluded. The control group comprised males in reproductive age bracket (who had at least one biological child) accompanying their partners at the O&G clinic for routine check-ups during pregnancy and had no medical issues.

Since this was an exploratory study, the average outpatient attendance and number of infertile males visiting the clinic was noted. Based on this observation, every tenth male reporting infertility was enrolled for the specified duration of the study. Hence, a total of 153 males were enrolled which included 74 fertile males as a comparator group.

The detailed clinical history of each participant was recorded; first void urine and 2-3 ml blood samples were collected from participants with the help of an experienced phlebotomist. In addition, seminal fluid was also obtained. Urine and seminal fluid samples were examined to detect CT with the help of PCR. Serum samples were examined to detect IgG/IgM/IgA antibodies to CT to evaluate the history of infection.

Bacterial DNA was extracted using Qiagen DNA Mini Kit (Cat. 51304, Qiagen GmbH, Qiagen Strasse 1, 40724 Hiden, Germany) and specific CT primer was used for PCR detection. A detailed description of DNA extraction and PCR is published elsewhere¹². CT-IgA antibody assay was done using enzyme-linked immunosorbent assay (ELISA) kit (Cat, E5-350; Immunospec Corporation, 14155 Framington Rd. D, Livonia, MI, USA), with a sensitivity and specificity of 95 per cent. CT-IgG (Cat. E190313AE; Euroimmun, D-23560 Lubeck, Seekamp, Germany) and IgM (Cat. E190329AS; Euroimmun) as well as IgM antibodies against *T. gondii* were detected using ELISA Kit (E190402AB; Euroimmun), with a sensitivity of 100 per cent and specificity of 97.2 per cent.

Statistical analysis: Data were analyzed using SPSS software version 25.0 (IBM SPSS Inc, Chicago, IL, USA). Continuous data were presented as median with interquartile range (IQR), mean \pm standard deviation (SD). Descriptive measures were provided in the form of percentage and frequencies; 95 per cent confidence interval (CI) estimates were provided for all proportions as measures of uncertainty.

Results & Discussion

The median (IQR) age of the males was 35 (29-59) yr. Participants were married for a period ranging from two to 18 yr. The most commonly reported

symptoms were burning sensation during micturition (4%) and painful erection (3%). PCR was conducted on 153 urine samples to detect CT infection; none was positive. Of the 139 seminal fluid samples tested, only one male (1.26%; 95% CI: 0.03-6.85), tested positive for CT infection (Table).

IgG antibodies to CT in patient's serum were detected in nine out of 72 samples (12.5%; 95% CI: 5.87-22.4) reflecting past infection. Ninety one participants were evaluated for presence of IgM antibodies, out of which only three (4%; 95% CI: 0.83-11.24) tested positive. IgA antibodies to CT were tested in samples to detect present infection by ELISA; 20 (28.16%; 95% CI: 18.13-40.09) out of 84 samples tested positive. In IgM antibody assay for *T. gondii* infection 27.63 per cent (21/88; 95% CI: 17.98-39.08) of the participants tested positive. Antibodies were not detected in any of the control samples.

Infertility affects 8-12 per cent of the couples worldwide¹³, and male infertility factors account for 40-50 per cent of all infertility causes¹⁴. In the present investigation, 12.5 per cent of males had IgG anti-*Chlamydia* antibodies suggestive of past infection, whereas four per cent had IgM anti-*Chlamydia* antibodies and 28.16 per cent had IgA anti-*Chlamydia* antibodies indicating present infection. The proportion of positivity for CT in this study was lower compared to a Mumbai-based study, which reported 60 per cent of male partners to be infected with CT¹⁵. A probable reason for lower detection of CT infection in our study could be due to mass use of over-the-counter antibiotics in the community.

Further, the presence of CT infection indicated that males might have either harboured the infection from their spouse or could potentially infect them as stated by a previous study reporting a frequency of 40 per cent of males transmitting CT infection to their spouses¹⁶.

Noticeably, the chances of transmission to spouse could further lead to infertility in females. Hence, we recommend screening for CT infection among both males and females of reproductive age group who are vulnerable to exposure due to young age, multiple or new sex partners, symptomatic partners or irregular use of condoms. Effective vaccine development is a long-term desired goal in achieving complete protection.

Chlamydial antibodies providing long-term natural immunity are questionable due to lack of evidence. Hence, early diagnosis and prompt treatment of CT infection with effective use of antibiotics is the key. Being asymptomatic, there is a greater chance of CT infection remaining untreated, which can lead to severe urogenital complications and associated sequelae. This can further potentially decrease quality of life and increase psychosocial impact such as stigma, shame and loss of self-worth.

This study reports 27.63 per cent positivity of *T. gondii* through estimating IgM antibodies reflecting their present infectious state, which is in contrast with the two per cent prevalence estimated in a national sero-prevalence study¹¹. Such high prevalence of *Toxoplasma* infection in this region further alerts clinicians to look for clinical presentation and advice for screening accordingly.

This is a hospital-based study and hence does not represent community-based prevalence. However, it provides preliminary evidence on CT and *T. gondii* infection among male partners of infertile couples in eastern India. Another limitation of this study is that few of the samples could not be processed due to insufficiency in amount or technical errors. In addition, being an exploratory study, we could not analyze other commonly associated pathogens such as *Mycoplasma*, *Ureaplasma* and *Gonococci*.

Table. Prevalence of *Chlamydia trachomatis* and *Toxoplasma gondii* infections among male partners of infertile couples

Type of samples	Male partners of infertile couples		Healthy control	
	Sample tested (n)	Samples positive, n, % (95% CI)	Sample tested (n)	Samples positive (n)
Urine (<i>C. trachomatis</i>)	79	Nil	74	Nil
Seminal fluid (<i>C. trachomatis</i>)	79	1, 1.26 (0.03-6.85)	60	Nil
Anti- <i>Chlamydia</i> IgG	72	9, 12.5 (5.87-22.4)	24	Nil
Anti- <i>Chlamydia</i> IgM	75	3, 4 (0.83-11.24)	16	Nil
Anti- <i>Chlamydia</i> IgA	71	20, 28.16 (18.13-40.09)	13	Nil
Anti- <i>Toxoplasma</i> IgM	76	21, 27.63 (17.98-39.08)	12	Nil

C. trachomatis, *Chlamydia trachomatis*; CI, confidence interval

Overall seropositivity of CT and *Toxoplasma* infections are comparable, which suggest that greater attention is required for screening these infections at clinics, especially amongst infertile males. In addition, differential diagnoses of these conditions should be considered in clinical practice.

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Conflicts of Interest: None.

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