

## Seroprevalence of *Chlamydia trachomatis* in Healthy Pregnant Women of Puducherry

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### Abstract

**Introduction:** So far, there has been no report of genital *Chlamydia trachomatis* prevalence in the pregnant women of Puducherry. The aim of our study is to determine the seroprevalence of *C. trachomatis* infection in healthy antenatal women attending Mahatma Gandhi Medical College & Research Institute, a tertiary care Teaching Hospital at Puducherry.

**Materials & Method:** We screened 266 healthy pregnant women in the first and second trimesters for anti-*C. trachomatis* IgG antibody by ELISA test.

**Results:** Only one out of 266 sera screened was positive for *C. trachomatis* IgG antibody (0.38%).

**Conclusion:** Seroprevalence of *C. trachomatis* in this part of southern India is very low. However, additional screening of more number of both asymptomatic and symptomatic pregnant women from different geographical locations in the union territory of Puducherry might give a clear picture.

**Keywords:** *C. trachomatis*, Genital Chlamydia, IgG ELISA, Seroprevalence

### Introduction

*Chlamydia trachomatis* infection is a common sexually transmitted bacterial disease with various reproductive complications.<sup>(1-6)</sup> It is the chief cause of mucopurulent cervicitis and is responsible for about 50% of one million cases of pelvic inflammatory disease (PID) reported in the United States each year.<sup>(5)</sup> Worldwide *C. trachomatis* infection among pregnant women has ranged widely from 2 to 30 percent in various studies.<sup>(4)</sup> World Health Organization has estimated a total global prevalence of 3.53% for *C. trachomatis* in women for 2005. At any point in 2005 there were approximately 98 million adults infected with *C. trachomatis*. Global prevalence of *C. trachomatis* infection varies from continent to continent and differences are seen in different parts of the same country.<sup>(1)</sup> Thus, a low prevalence of 3.78% in Cameroon,<sup>(7)</sup> 4.6% in Australia,<sup>(8)</sup> between 5%<sup>(3)</sup> and 22%<sup>(9)</sup> in USA, 10% in UK,<sup>(10)</sup> 19.4% in Ghana<sup>(11)</sup> and 29.4% in Nigeria.<sup>(12)</sup> In the Middle East countries, Hani *et al*<sup>(13)</sup> reported that 8.7% of the total number of 1600 Saudi Pregnant Women tested were positive for IgG antibodies to *C. trachomatis*. Fatholahzadeh *et al*<sup>(14)</sup> reported a seroprevalence of 9.23% asymptomatic women of Iran. Information regarding prevalence and risk factors of this infection in India are limited.<sup>(6,15-21)</sup> Few document a prevalence of 3-20% and 18-30% among antenatal and symptomatic women respectively.<sup>(15)</sup> Endocervical swab Culture, considered as gold standard, has several limitations. Therefore, non-cultural methods such as direct Immunofluorescence (DFA), EIA and PCR carried out with endocervical swabs. In a recent study which compared nucleic acid amplification test (NAAT) and

cell culture, the NAAT was found to be significantly more sensitive and therefore it was recommended as "new gold standard".<sup>(1-6,22-23)</sup> Analysis of the epidemiology of *C. trachomatis* infection, various risk factors, and usefulness of different non-culture methods will help in arriving at an effective strategy for accurate diagnosis and management of this disease. Due to paucity of data in India, especially Puducherry, present research was undertaken to determine the prevalence of *C. trachomatis* infection in healthy antenatal women attending a tertiary care Teaching Hospital at Puducherry.

### Materials and Method

Our Institutional Human Ethical Committee (IHEC) has approved this research and the work was carried out during July 2015 to December 2016 at Mahatma Gandhi Medical College & Research Institute, Puducherry. Based on the national seroprevalence, 266 healthy pregnant women were included in this study. IgG ELISA serology for *C. trachomatis* was performed using NovaLisa Chlamydia trachomatis IgG ELISA – CHLG0070 kit (NovaTec Immunodiagnostica, GmbH, Dietzenbach, Germany). The procedure outlined in the kit's brochure was strictly adhered to. The microtitre wells are precoated with *C. trachomatis* antigen. About 10µl of participant's serum diluted 1:100 with IgG sample diluent was added to each well, incubated for one hour at 37°C and later washed with washing solution. After the addition of conjugate, further incubation for 30 minutes was done at room temperature. At the end of incubation, plates were again washed, TMB substrate was added and incubated for 15 mins at room temperature in the dark.

Finally, stop solution was added to all the wells and Optical Density (OD) readings were taken with the wavelength of 450/620 nm using iMark Microplate Reader, Bio-Rad, Japan.

Cut-off value calculation:

- Cut-off value is the mean absorbance value of cut-off controls kept in duplicate wells.
- Samples are considered positive if OD readings are 10% higher than the cut-off values and those with 10% less than cut-off are negative. Those in the middle are in Grey zone and were repeated.

Calculation in Nova Tec Units

Antibody index = (Sample O.D./Cut-off O.D) x 10.

The samples with OD values above 11 NTU (NovaTec units) were considered as positive and those below 9 NTU were taken as negative. Borderline samples with 9-11 NTU were repeated in triplicate.

## Results

Only one healthy antenatal mother was positive for IgG antibodies to *C. trachomatis* (0.38%). Three samples in the grey zone were negative on repeat testing.

## Discussion

*C. trachomatis* infections are most often asymptomatic.<sup>(1-6)</sup> In symptomatic women, the symptoms usually appear within 1 to 3 weeks after exposure. The most common symptoms are abnormal vaginal discharge, burning micturition, cervical fragility and hypertrophic cervical erosions. Lower abdominal pain, low back pain, nausea, fever, pain during intercourse and bleeding between menstrual periods are the other symptoms. The clinical syndromes associated with genital *C. trachomatis* infections include acute urethral syndrome, cervicitis, Bartholinitis, cervical dysplasia and pelvic inflammatory disease (PID). Women with untreated *C. trachomatis* infections may develop pelvic inflammatory disease, which can result in long-term sequelae, such as infertility, ectopic pregnancy, and chronic pelvic pain.<sup>(6,15,17-20)</sup> The risk factors for *C. trachomatis* infections observed in several studies are young age, single status, oral contraceptive use, intrauterine devices (IUDs) use, inconsistent condom use, multiple sex partners and a previous history of sexually transmitted infection.<sup>(24)</sup> Age dependency was seen in symptomatic patients, with a high chlamydial prevalence rate (28%) found in younger women.<sup>(25)</sup>

The culture of endocervical swab is generally considered as the diagnostic gold standard for detection of cervical chlamydial infection, but due to the complexity of this technique and the difficulties in cytological staining and microscopy, a variety of non-cultural tests are in use. These include antigen detection by rapid kits/EIA, nucleic acid amplification, direct fluorescent antibody (DFA) and ELISA.<sup>(1-6,22,23)</sup> Three classes of immunoglobulins are produced during genital

*C. trachomatis* infection, viz., IgM, IgG and IgA. Presence of IgG antibody indicates previous exposure to *C. trachomatis* and does not point to present infection. Hence, seroprevalence surveys on genital *C. trachomatis* infections in healthy pregnant women target this immunoglobulin. Detection of *C. trachomatis* antigen in endocervical specimens by IFA/PCR would be a better indicator of infection. Nucleic acid amplification tests (NAATs) have been developed for the diagnosis of genital Chlamydia infection.<sup>(22,26)</sup>

CT prevalence in Puducherry is very low with only 0.38%. Several studies in India from different states like Tamil Nadu, Haryana, Karnataka and Maharashtra, reported prevalence rates of 3-15% in asymptomatic women, 9.7% in commercial sex workers and 15-60% in young women with infertility or PID or STD.<sup>(1,15,17,25,27)</sup> A recent report from Assam highlights *C. trachomatis* infection in infertile women. While 7.5% of the control group has *C. trachomatis* IgG antibodies, 25% of the infertile women had IgG antibodies.<sup>(28)</sup> Chandhok *et al*<sup>(15)</sup> reported a range of 1.2 to 33% seroprevalence. The lowest prevalence of 0.2% was recorded by Brabin *et al* in 1998.<sup>(27)</sup> Similar low prevalence of 0.57%<sup>(17)</sup> and 1.7%<sup>(16)</sup> were reported by Mania-Pramanik *et al* in asymptomatic controls in Mumbai. Other states like Orissa recorded 7.04%, whereas New Delhi recorded 23% as well as a higher 29%.<sup>(25,29)</sup> Our results are comparable to seroprevalence in Mumbai. The detection of specific anti-chlamydial antibodies (IgA, IgM, IgG) by ELISA is a valuable, non-invasive diagnostic procedure.<sup>(2,29-30)</sup> A study from Chennai, which evaluated the various serological markers of *Chlamydia trachomatis* infection, suggested that a single serological marker cannot be of diagnostic help and so simultaneous detection of IgA, IgM and IgG is needed to diagnose this infection. The authors also opined that a single elevated serum IgG titer may be diagnostically more helpful to identify deep-seated upper genital tract infection with *C. trachomatis* than paired serum specimens, which are difficult to collect from patients suspected to have STD as they often receive treatment on syndromic basis.<sup>(30)</sup>

## Conclusion

Notwithstanding a very low seroprevalence of 0.38%, genital chlamydial infection in Puducherry needs to be further explored with more number of samples collected from various geographical locations of Union Territory of Puducherry.

## Acknowledgement

This Faculty Research Project has been funded by Sri Balaji Vidyapeeth University, and the authors are grateful to the Chairman, Vice-Chancellor, Dean-Academic and Dean – Research & Allied Health Sciences of Mahatma Gandhi Medical College and Research Institute, Puducherry.

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**How to cite this article:** Stephen S, Ghose S, Anitharaj V, Pradeep J. Seroprevalence of *Chlamydia trachomatis* in Healthy Pregnant Women of Puducherry. *Indian J Microbiol Res* 2017;4(3):295-297.