

Prevalence of Chlamydia Trachomatis IgM Antibody among the Pregnant Women Attending a Tertiary Care Hospital at Kerala - South India

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ARTICLE INFO

Article history:

Received: 8 February 2019;

Received in revised form:

28 February 2019;

Accepted: 9 March 2019;

Keywords

Chlamydia Trachomatis,
Micro Immune Fluorescence,
IgM Antibodies,
Sexually Transmitted
infections,
Pregnancy.

ABSTRACT

Chlamydia trachomatis is an obligate intracellular bacterium associated with sexually transmitted genital tract infection in humans and cause genital infection in both men and women. It is one among the major causes for complications in pregnancy. In this study, prevalence of the *C. trachomatis* genital infection among the pregnant women of reproductive age group has been studied. A total of 300 pregnant women with symptomatic genital infection were included in this study. Blood samples were collected from them and screened for *C. trachomatis* specific IgM antibodies by *Micro Immuno Fluorescence (MIF)* test. The results showed an overall 16% prevalence of *C. trachomatis* infection among the various age groups of the pregnant women. From this informations, we conclude that the *C.trachomatis* infection is quite prevalent among the pregnant women belongs to our area - south India. Hence, we suggest the need of implementing the screening programme as mandatory for the detection of Chlamydial genital infection among pregnant women, in view of preventing the adverse effects related to pregnancy outcomes.

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Introduction

Sexually transmitted infections (STIs) have major negative impacts not only on the reproductive health of women but in fetus also. Centre for Disease Control and prevention (CDC) notified that the genital Chlamydial infection was found to be one among the five sexually transmitted diseases such as gonorrhea, Human Immunodeficiency virus (HIV) infection, syphilis, and hepatitis B. The World Health Organization (WHO) reports 101 million Chlamydial infections annually (World Health Organization, Reproductive Health and Research, 2012).

C. trachomatis is an important causative agent of genital tract infections such as cervicitis, urethritis, and pelvic inflammatory disease (PID). It is an ascending infection of the reproductive organs and neighboring pelvic structures that can vary with their presentation as asymptomatic endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis, perihepatitis and periappendicitis (Paavonen and Eggert-Kruse, 1999). Sexually transmitted infections are a challenging public health problem in India due to the under reporting of symptoms, associated stigma and misuse of antibiotics (Kant et al., 2015).

Past research informations saying that the Chlamydiae are not routinely screened during pregnancy and can result with adverse pregnancy outcomes, which creates significant public health problems at global level (Michael G Gravett; 1986). The female population with asymptomatic Chlamydial infections serving as reservoir and source for spreading the infection (Ma Guadalupe Aguilera-Arreola et al., 2014).

The predominant biovar responsible for *C. trachomatis* infections are D-K strains of *C. trachomatis* which infects the genital epithelium (Jyoti Rawre et al., 2017). Untreated *C.trachomatis* genital infections can lead to serious reproductive sequelae among pregnant women including pelvic inflammatory disease (PID) (Brunham et al., 1998) tubal factor infertility, ectopic pregnancy (Chow et al., 1990) and an increased risk of acquiring other STIs (Fleming et al., 1999) are the so far recorded complications. The pregnant women with *C.trachomatis* genital infections are at an increased risk of having a preterm birth and giving birth to infants with low birth weight (Silva et al., 2011). Pneumonia and neonatal conjunctivitis are the major complications among the neonates born to infected mothers (Kristina et al., 2016).

In India, the negative impact of *C trachomatis* infection in reproductive health seems to be unnoticed and a neglected area of investigation (Jayanthi et al., 2012). The up-to-date knowledge of the prevalence of the *C. trachomatis* infection is essential to design the appropriate Chlamydial infection control programs. Though countless research informations has been explored about the genital infections by *C. trachomatis* at National and International level, still it seems less research informations are revealed by the Indian authors comparatively. Hence, the present study was undertaken to assess the presence of *C. trachomatis* IgM antibody in the sera of pregnant women especially the age group ranged between 18-39 years. We proud to say that the present study is a bit of a PhD research work and still more information may be explored in future.

Materials and methods

Study population

Total of 300 pregnant women aged between 18-39 years attended the Obstetrics & Gynecology department of Azeezia medical college hospital, Kerala, Kollam, India, were included in the study. Ethical clearance was obtained from the Institutional Ethical committee of Azeezia medical college hospital. Prior to specimen collection, the study plan and its purpose was thoroughly explained to the patients and obtained their consent in the specified form.

Specimens

Specimens were collected from the pregnant women with both symptomatic and asymptomatic genital infections during either of the first, second or third trimester of pregnancy

Serum was separated from the collected blood samples and further subjected to *C. trachomatis* screening and it was carried out at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India.

Test used

Serum samples were processed for the detection of *C. trachomatis* specific IgM antibodies by Micro immunofluorescence (MIF) method by using Chlamydia IgM SeroFIA-Fluorescent Immuno Assay (Saadouni et al., 2013). The Savyon Chlamydia Sero FIA kit (Savyon Diagnostics, Israel) was used in our study. It is a micro-IF assay based on the principles of MIF. Purified elementary bodies (EB) of *C. trachomatis* (L2) were used as the antigen.

As per the kit manufacturer's instructions, the patient's sera should be started with starting dilution 1:20 for the primary, preliminary qualitative analysis of *C. trachomatis* specific IgM antibodies. Further in order to find out the end point titre of the positive sample, the patient's sera was serially diluted and screened for *C. trachomatis* specific IgM antibody. The sero FIA kit provided slides were coated with antigen purified elementary bodies (EB) of *C. trachomatis*. The diluted patients sera (1:20) was delivered to the Ag coated slides and incubated for 90 minutes at 37°C. Unbound serum components were removed by washing. Fluorescein-conjugated anti-human IgM was added and incubated for 30 minutes at 37°C. Unbound conjugate was removed by washing with diluted wash buffer. Slides were dried and mounted by adding 3 drops of mounting fluid. Slides were examined under Confocal microscopy (Model-Nikon A1R and software nis elements). Positive results were appeared as bright apple green fluorescence elementary bodies against a dark background. Qualitative determination achieved by a single dilution of sera and for the end point titre, we have restricted only with three dilutions 1:20, 1:40 and 1:80.

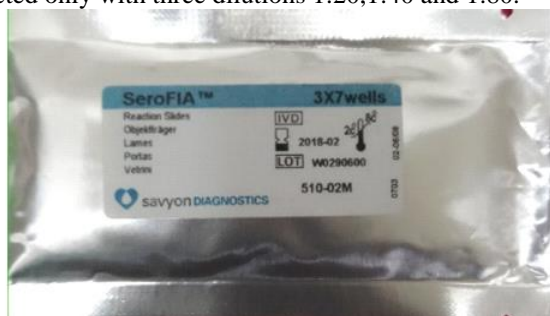


Fig 1. Savyon Chlamydia Sero FIA kit (Savyon Diagnostics, Israel) used in our study.

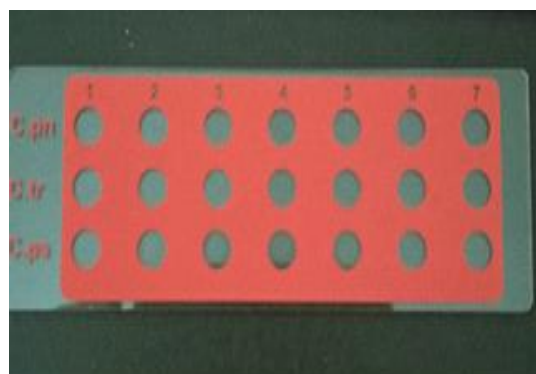


Fig 2. Sero FIA kit provided slides coated with antigen purified elementary bodies (EB) of *C. trachomatis*.

Statistical analysis

The statistical Analysis was performed by using Systems software SPSS version 20.0. Chi-square was used to assess differences in proportions and p values <0.05 were considered statistically significant.

Results and Discussion

The pregnant women with symptomatic genital infection were included in this study. All these women had shown either or mixed symptoms such as mucopurulent vaginal discharge or leucorrhoea, severely eroded cervix with hypertrophic cervical erosions, signs of burning micturition and lower abdominal pain. According to the kit manufacturer's procedure, the serum samples were first screened for *C. trachomatis* IgM with 1:20 diluted serum randomly. Among 300, diluted (1:20) serum specimens, only 48 had shown to have IgM antibody against *C. trachomatis*, which appeared as apple green IgM fluorescence stained *C. trachomatis* elementary body (Fig.3)

Negative control



1/ 20 dilution

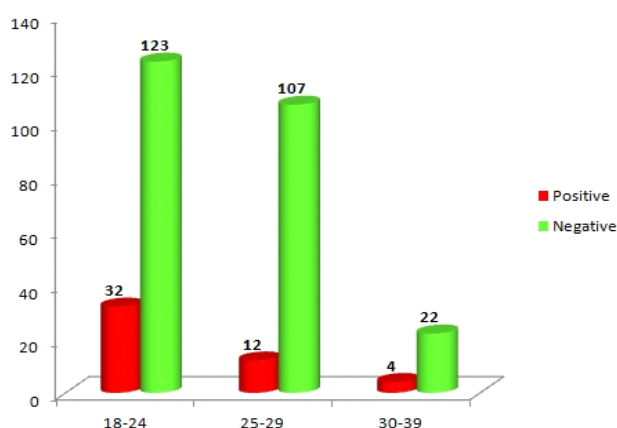
Fig 3. Confocal microscopic image -Apple green IgM fluorescence stained *C. trachomatis* elementary body appear against dark background indicating positive for *C. trachomatis* IgM antibody in sera of study population.

Table 1. Result of *C. trachomatis* IgM antibodies among study population.

Sl. no	Age range (years) (18-39)	Study population-Total N=300	Chlamydia trachomatis IgM positive MIF N = 48	Age wise prevalence MIF positive N =48(16%)
1	18-24	155	32 (21 %)	11%
2	25-29	119	12 (10 %)	3.3%
3	30-39	26	4 (15 %)	1.3%

The value of the Chi-Square Statistic is 6.012 and the p-value is less than 0.05 (<0.041)

Prevalence of *C.trachomatis* infection of our study was found to be 16 % in women of reproductive age group as showed in the table. The prevalence of *C.trachomatis* and the predominant age groups are presented with symptomatic genital infection. Among 300 symptomatic genital infection groups, of pregnant women, about 21%, 10% and 15% found to possess IgM antibodies in their serum with age range 18-24, 25-29, and 30-39 respectively. In both symptomatic and asymptomatic group, the pregnant women belong to 18-24 years age group found to be the predominant age group who had shown the highest percentage of IgM antibody in their serum sample. Whereas comparatively decreased percentage (10 & 15%) was recorded with the other age groups included in our study (table.).However, the results of asymptomatic group are not presented in this paper, and that will be published with other related informations shortly.



The infection is frequently remaining unrecognized due to asymptomatic nature and uncertain clinical presentations in the patient (Singh et al., 2003). The reports of Silverman et al.,2007 and Subramanian et al.,2007 states that the STIs were found to be major health impact on women of reproductive age group and Chlamydial infection contributes a significant portion of female reproductive morbidity.

The patients included in this study had shown the clinical symptoms such as muco purulent vaginal discharge or leucorrhoea, severely eroded cervix with hypertrophic cervical erosions, signs of burning micturition and abdominal pain which was also mentioned by Robert et al.,1984 in his study. Thick mucopurulent discharge was found as the predominant symptom which we have recorded with the individuals among those the *C. trachomatis* infection had been recorded.

Peuchant et al in 2015 had recorded the highest prevalence of genital Chlamydial infection among younger women in France. A similar, but population based study conducted in Netherlands also reported the highest prevalence rate of *C. trachomatis* infection among younger women, which significantly increased with urban population (Van Bergen et al., 2005).

Therefore, correct and early diagnosis of the disease is of critical importance for the timely medical intervention. In India, there are a few studies performed on the prevalence of *C.trachomatis* infection among pregnant women. So, there is a definite need to have a cost effective method for routine diagnosis of genital Chlamydial infections in India.

The overall prevalence of 16% was observed in the study which is lesser than the rate of prevalence which was recorded by Singh et al.,2003 (28%) and Patel et al.,2010 (23%),from New Delhi.

Few Indian authors performed study on *C.trachomatis* genital infection, among the symptomatic women, and documented different rates of prevalence. A hospital based study from India revealed the increased recurrence of *C.trachomatis* infection among women of reproductive age group. The incompetent public health programs conjoined with ongoing socioeconomic and demographic trends have led to an epidemic of *C. trachomatis* infection in many developing countries (Becker et al., 2010, Vishwanath et al., 2000).A study conducted in Orissa shows the prevalence of Chlamydial infection to be 7.04% in symptomatic women (Bhagirathi et al.,2009). All these authors suggested the diagnosis of *C.trachomatis* genital infection which could help the clinician to enhance the effective treatment. Our study report gain the support of Singh et al.,2003 who conducted similar study at a hospital Gynecology clinic in New Delhi, the authors recorded a highest persistence of 28.5% among 18–25-year-old symptomatic patients. Comparatively our study results have been documented with higher percentage (16%) prevalence of *C.trachomatis* genital infection.

Conclusion

Prevalence of genital infection with *C. trachomatis* found to be significant in the pregnant women belongs to the age group 18-24 years of our study population especially, the women living in highly urbanized areas. Both symptomatic and asymptomatic genital infection infected with *C.trachomatis* has been recorded in our study. This information provides evidence for the need to implement active screening of Chlamydial infection in young sexually active women of all socioeconomic groups with special focus on pregnant women. From this we came to understand that the *C.trachomatis* genital infection among the pregnant women belongs to the age group 18-24 years seems to be prevalent with younger age group, compared to other age group (25-39 years). This was occurred with pregnant women of both symptomatic and asymptomatic genital infection. When we think about the reason for the above mention condition, factors such as age, hormonal status, number of sex partners, frequency of intercourse, above all the status of personal hygienic practice of the individual and the sex partners condition (transmitting any STD, infectious agents ?) all should be considered since they are also either directly or indirectly contributing quite significant role in the establishment of these sexually transmitted infectious agents including *C.trachomatis*.

Acknowledgement

I express my heartfelt gratitude to the H.O.D.Department of Obstetrics& Gynecology and other staffs of Azeezia medical college Hospital, Kollam, Kerala for their support in collecting samples from symptomatic pregnant women attending the OBG clinic. I also express my sincere gratitude to the Director and technical staffs of Rajiv Gandhi Centre for Biotechnology (GCB), Thiruvananthapuram for their support for conducting the test at RGCB.

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