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Seroprevalence of *Chlamydia trachomatis* Genital Infection among Women of Reproductive Age Group-A Pilot Study Conducted in South India

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A B S T R A C T

Chlamydia trachomatis is an obligate intracellular bacterium associated with sexually transmitted infection in humans and cause genital infection in both men and women. It is also associated with negative impacts on women of reproductive age group. The current study was conducted as a pilot study to assess the prevalence of *C. trachomatis* specific IgG antibodies among the women of reproductive age group. The study population included 100 women of all child-bearing age groups attended the antenatal clinic with and without genital infections. Micro immunofluorescence (MIF) assay was done to detect the presence of IgG antibodies. Among the 100 samples tested, 20 patients were positive for *C. trachomatis* specific IgG antibodies. The data strongly indicating the need for mandatory screening for *C. trachomatis* infection among women of fertile age group to prevent the later complications.

Introduction

Chlamydia trachomatis infection is the most prevalent bacterial sexually transmitted infection with over 130 million new cases reported annually (Newman *et al.*, 2015). It 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 neighbouring 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 due to the under reporting of symptoms, associated stigma and misuse of antibiotics (Kant *et al.*, 2015). Past research information 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 a reservoir and source for spreading the infection (Ma Guadalupe Aguilera-Arreola *et al.,* 2014). 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).

The majority of infected women have uncomplicated lower genital tract infections, but some women develop a persistent or ascending infection. Women who have ascending infection, it can lead to severe reproductive morbidity, including tubal factor infertility (Paavonen and Eggert-Kruse, 1999). Tissue damage resulting from chlamydial infection has been attributed to inflammatory processes in the upper genital tract, leading to pelvic adhesions and scarring of the tubal epithelium (Mardh, 2004). Infections with *C. trachomatis* can severely impact the reproductive health of women, causing severe conditions such as ectopic pregnancies, repeated and spontaneous abortions and stillbirths (Baud and Greub, 2011).

Materials and Methods

The study was conducted for a period of three months from March 2015 to May 2015 as part of a pilot study prior to PhD thesis work, after obtaining institutional ethical committee clearance. The study population includes women of reproductive age group with and without clinical symptoms of genital infection attended the Obstetrics & Gynaecology department of Azeezia medical college hospital, Kollam. Patients' consents were obtained before collecting the samples. A total of 100 blood samples were collected and Micro immunofluorescence assay was performed.

Serum was separated from the collected blood samples and kept at -20º C till it is tested. It was further subjected for the screening of *C. trachomatis* specific IgG antibodies and the samples were processed at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India. The method used was Micro immunofluorescence (MIF) by using Chlamydia IgG SeroFIA-Fluorescent Immuno Assay (Saadouni *et al.,* 2013). The Savyon Chlamydia Sero FIA kit (Savyon Diagnostics, Israel) was used in our study (FIG 1(a)). 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.

Procedure

As per the kit manufacturer's instructions, the patient's sera should be started with an initial dilution of 1:20 for the primary, preliminary qualitative analysis of *C. trachomatis* specific IgG antibodies. Further in order to find out the end point titre of the positive sample, the patient's sera were serially diluted and screened for *C. trachomatis* specific IgG antibody. The Sero FIA kit provided slides coated with as antigen purified elementary bodies (EB) of *C. trachomatis* (FIG 1b). The diluted patients' sera (1:20) was delivered to the Ag coated slides and incubated for 30 minutes at 37ºC. Unbound serum components were removed by washing. Fluorescein- conjugated anti-human IgG was added and incubated for 30 minutes at 37ºC.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 (FIG 2c&2d). Qualitative determination achieved by a single dilution of sera.

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

Among the 100 samples tested, 20 were positive for *C. trachomatis* specific IgG antibodies with an overall prevalence of 20%. Among the asymptomatic group, majority of them had the history of genital tract infections. In the symptomatic group, mixed symptoms such as mucopurulent vaginal discharge or leucorrhoea, severely eroded cervix with hypertrophic cervical erosions, signs of burning micturition and lower abdominal pain were observed. The positive results were appeared as apple green IgG fluorescence-stained *C. trachomatis* elementary body (FIG 2c & 2d). Tissue damage resulting from *Chlamydial* infection has been attributed to inflammatory processes in the upper genital tract, leading to pelvic adhesions and scarring of the tubal epithelium (Mardh, 2004).

Prevalence of *C. trachomatis* infection of our study group was found to be 20 % in the women of reproductive age group (Table 1& FIG 3). A study conducted in Netherlands revealed a percentage of 23.1% among women of sexually active women (Hoenderboom *et al.,* 2019). Similar studies conducted in New Delhi showed a high prevalence rate of 28% and 23 % respectively by Singh *et al.,* 2003 and Patel *et al.,* 2010. The prevalence of *C. trachomatis* IgG antibodies in the predominant age groups among the asymptomatic and symptomatic individuals are given in Table 2 & FIG 4. Among the asymptomatic and symptomatic groups, the percentage of infection was found to be 60 % and 40 % respectively. The positive results of serological tests strongly indicating the presence of an immune response to the pathogen either as a result of past or chronic exposure (Horner *et al.,* 2013).

Off the 100 samples tested, about 70%, 20% and 10% found to possess IgG antibodies in their serum with age range 18-24, 25-29, and 30-39 respectively. In both symptomatic and asymptomatic group, women belong to 18-24 years age group found to be the predominant group who had shown the highest percentage of IgG antibody in their serum sample. Young age is a consistent risk factor for *C. trachomatis* acquisition, which could suggest an acquired and protective immune response (Rekart *et al.,* 2008). However, younger age at first sexual intercourse also increased the risk of being susceptible to *C. trachomatis* infection (Woodhall *et al.,* 2017 and Horner *et al.,* 2016).

Whereas comparatively decreased percentage (20 & 10%) was recorded with the other age groups included in our study (Table 3 & FIG 5). The overall *C. trachomatis* antibody positivity rate of 20% found in this study was higher than that found in a population-based seroprevalence study in Netherlands among 25–39-year-old women (Van *et al.,* 2014).

Table.1 Details of the total number of individuals under the study and the percentage of positive population

Test Method	Total number of patients under the study group	Total positive patients	Positive percentage
MIF	**100**	**20**	**20**

Table.2 Details of *C. trachomatis* positivity among the asymptomatic and symptomatic study population

Study population	C trachomatis positivity	Percentage
Asymptomatic	12	**60%**
Symptomatic	**8**	**40%**

Table.3 Specific details about the test method used in the screening of *C. trachomatis* IgG antibody with specific age group

Test method	Age group (years)			Total positive
	18-24	25-29	30-39	
MIF	14 (70%)	4 (20%)	2 (10%)	20 (20%)

Fig.1 (a) & 1(b) Savyon Chlamydia Sero FIA Kit (Savyon Diagnostics, Israel)

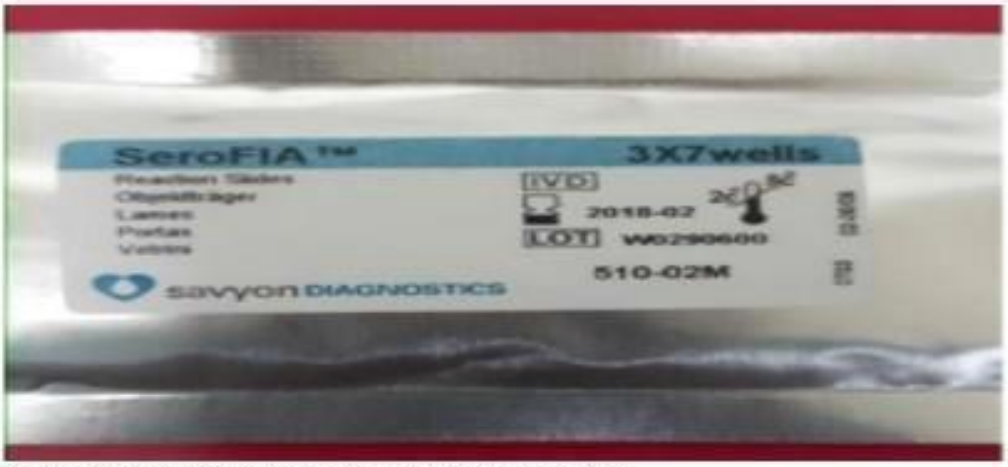


FIG 1(a) Sero FIA Kit pouch with coated slides

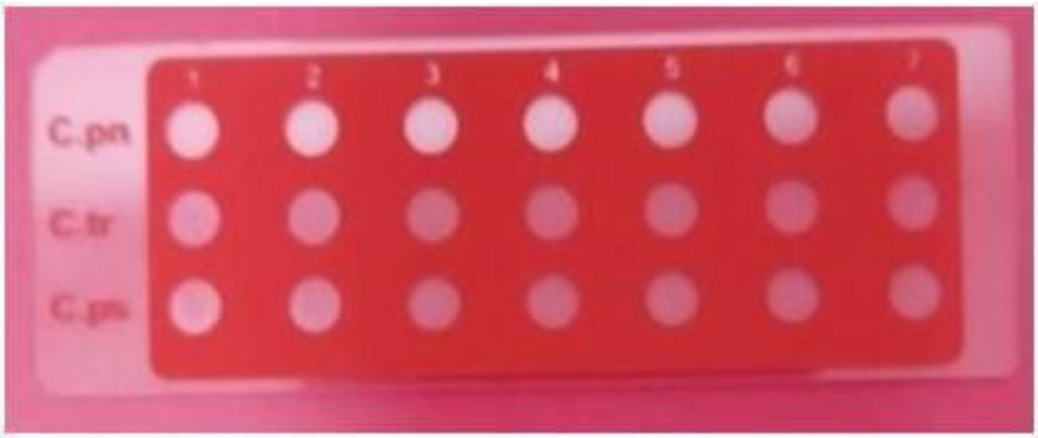


FIG 1(b) Sero FIA Kit provided slides coated with antigen purified elementary bodies (EB)) of *C. trachomatis*

Fig.2 Confocal Microscopic image-Apple green fluorescence-stained C. *trachomatis* elementary body appear against dark background indicating positive for C. *trachomatis* specific IgG antibodies in sera among the study population.

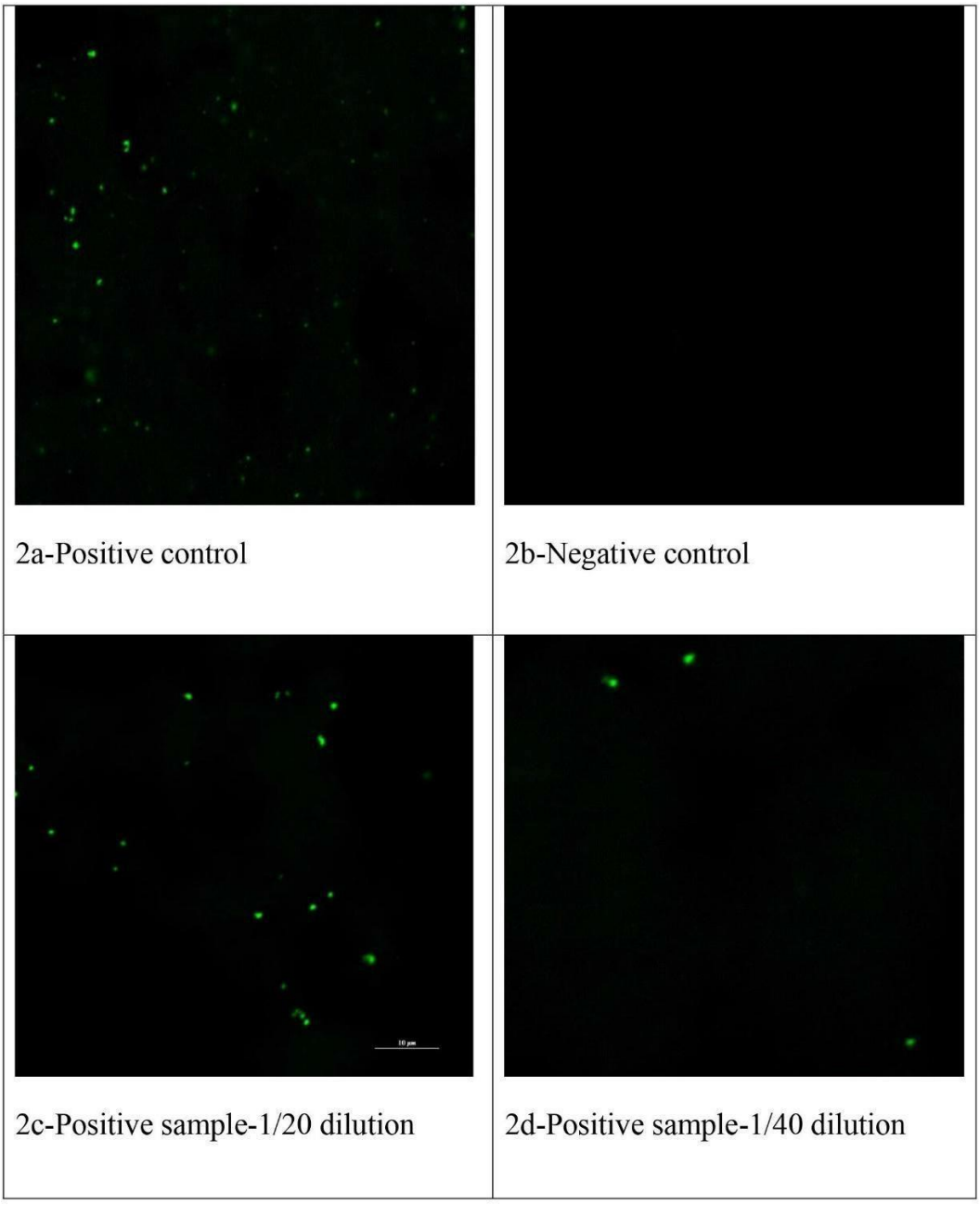


Fig.3 Details of the total number of individuals under the study and the percentage of positive population

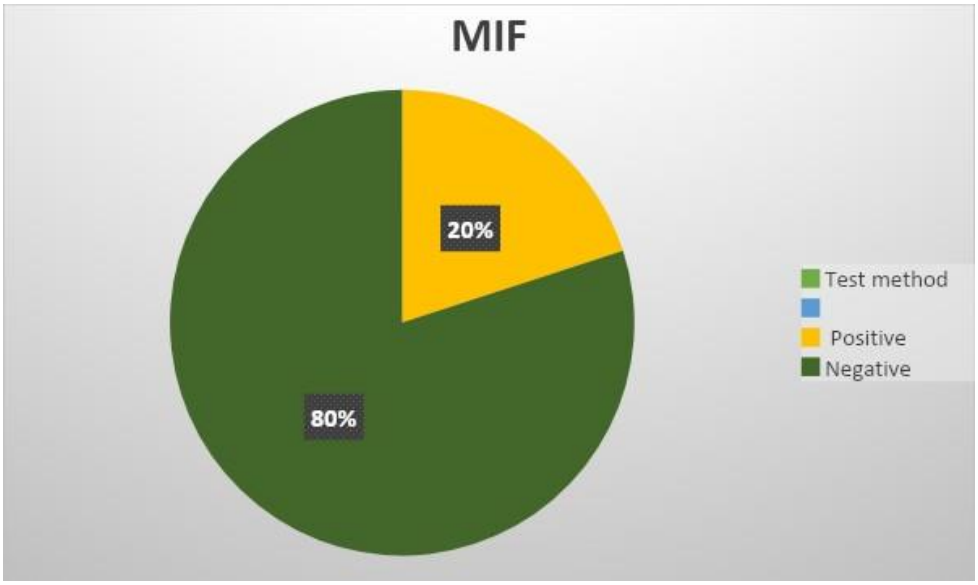


Fig.4 Details of *C. trachomatis* positivity among the asymptomatic and symptomatic study population

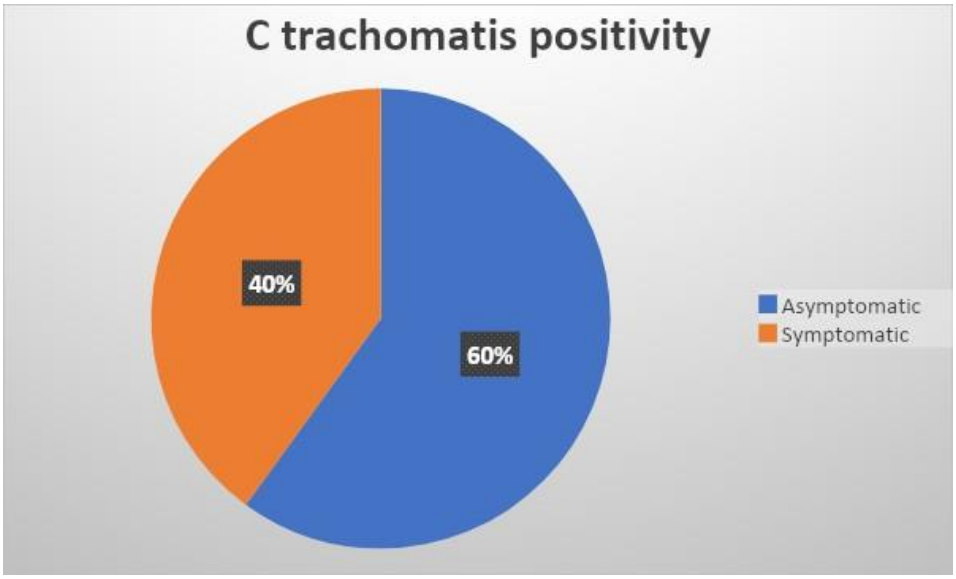
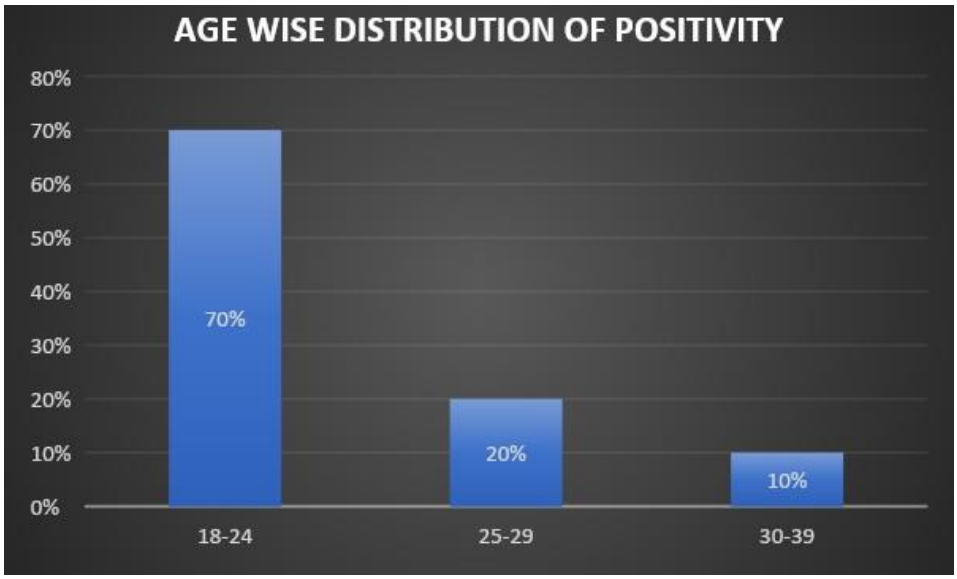


Fig.5 Specific details about the test method used in the screening of *C. trachomatis* IgG antibody with specific age group



The high seroprevalences as compared to PCR prevalence suggest that previous *Chlamydia trachomatis* infection is related to long term consequences. In fact, PCR detects bacterial DNA inside of the patient's genital tract, which suggests a current infection rather than a past one. This finding underscores the role played by *C. trachomatis* in chronic infections leading to adverse health outcomes, and particularly, infertility. Multiple *C. trachomatis* infections were strongly associated with antibody positivity. This corroborating that repeated exposure to *C. trachomatis* infections is an important predictor of specific antibody positivity (Horner *et al.*, 2013 and Ohman *et al.*, 2020).

Conclusion

In conclusion, we demonstrated the prevalence of *C. trachomatis* specific IgG antibody in women with unnoticed Chlamydial genital tract infections, with negative NAAT results. The asymptomatic nature of the disease requires evidence-based guidelines for the implementation of population-wide screening programs. Although not all women tested positive for *C. trachomatis* antibodies despite a previous *Chlamydial* genital infection, the extra infections found with antibody testing significantly increased the lifetime prevalence estimates. Consequently, this improved the estimates on proportions of women who experienced *C. trachomatis* related complications. In order to check the prevalence of *C. trachomatis* genital infections, we recommend for screening of IgG antibodies among women of reproductive age group.

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Conflicts of interest

There are no conflicts of interest

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