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Prevalence of Chlamydia Trachomatis in Patients with Sexually Transmitted Disease

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ABSTRACT

Genital chlamydial infections are the leading cause of sexually transmitted infections with annual detection of 92 million new cases worldwide, including 43 million from South-east Asia (WHO, 2001). C. trachomatis infection, if undiagnosed and untreated, can result in pelvic inflammatory disease, epididymitis etc., finally damaging the human reproductive tract irreversibly. Although the gold standard for detection of C. trachomatis is culture, tissue culture setup is not available in most hospitals. Therefore, serological assays have been recommended as an alternative. This study was undertaken to find the prevalence of Chlamydia trachomatis in sexually transmitted disease patients using Giemsa stain and ELISA for presence of IgG and IgA antibodies. Overall prevalence of C. trachomatis was found to be 17.5%. Prevalence of C. trachomatis by ELISA for IgG and IgA was 13.75% and 5% respectively and 2.5% by Giemsa stain. Serologic tests like ELISA are simple, rapid and a valuable tool in sero-epidemiological studies. C. trachomatis serology has been used for both diagnostic purposes and large epidemiological studies.

INTRODUCTION:

Chlamydiae are obligate intracellular parasites with a biphasic life cycle.[1] The type species of the genus is *Chlamydia trachomatis.*[2] *C. trachomatis* has been the most extensively studied species because of its association with ocular trachoma and its importance as a sexually transmitted pathogen.[3]

Genital tract infections with *C. trachomatis* are even more prevalent and have as major complications acute pelvic inflammatory disease (PID), ectopic pregnancy, infertility, and infant pneumonia.[4] In sexually active men, *C. trachomatis* causes nongonococcal urethritis and, occasionally, epididymitis.[5] The World Health Organization (WHO) has estimated that the number of new genital infections by *Chlamydia* has almost reached 100 million annually, which makes these infections one of the most prevalent sexually transmitted diseases (STD).[6] However, the epidemiological situation varies by country. Young age is identified as a risk factor to contact genital chlamydial infections and also for reinfection.[7] Untreated individuals serve as reservoirs for the transmission of this infection to their sexual partners.[17] The biggest challenge to the control of Chlamydial disease is that as many as 70 to 80% of women and up to 50% of men who are infected do not experience any symptoms.[8,9,10] This results in a large reservoir of unrecognized, infected individuals who are capable of transmitting the infection to sexual partners.[11]

There are several methods for detection of Chlamydial infection such as: cell culture, ELISA, micro immunofluorescence (MIF), direct fluorescent antibody (DFA) and molecular methods such as PCR.[12] The organisms may be demonstrated in cells from the site of the lesion by light microscopy with the material stained by Giemsa, Macchiavello or Gimenez stains, or by fluorescence microscopy with acridine orange stain, or by immunofluorescence with antibody against the group antigen.[13]

This study was carried out to find the prevalence of *Chlamydia trachomatis* among sexually transmitted disease patients attending STD Clinic.

METHODS:

A total of 80 (60 females and 20 males) clinically diagnosed STD cases attending the STD clinic of a tertiary care hospital in North East India were taken for this study and screened for *Chlamydia trachomatis.* Diagnosis was based on history and clinical examination of patients. Patients presenting with symptoms suggestive of STI (i.e., vaginal discharge, urethral discharge, dysuria, urethral itching etc.) and giving consent to participate were included in the study. The approval of the Institutional Ethics committee was obtained before conducting the study. Urethral swabs in male patients were obtained at least 1 hour after the patient had passed urine. A sterile swab was inserted 2–4 cm into the urethra and rotated gently two to three times.[14] Smears were immediately prepared by rolling the swab on a pre-cleaned slide.

In female patients at first, inflammatory exudates and other secretions were removed. A sterile swab was inserted at least 1 cm into the endocervical canal under direct vision (using a speculum) and rotated for 15-20 seconds.[14] Then smears were immediately prepared by rolling the swab on a pre-cleaned slide.

Smears were stained by Gram stain and Giemsa stain. Only those smears yielding more than 4 polymorphonuclear leukocytes per oil immersion field in Gram stain were included in the study.[15]

About 5 ml of venous blood was collected from each patient. Serum was separated and then transferred to a sterile vial, labelled and stored at -20^{0}C till the assay was done (as per ELISA kit). All the sera were examined by *Chlamydia trachomatis* IgG and IgA ELISA kits manufactured by Nova Tec Immunodiagnostica GmbH, Germany. Cut off values were calculated according to the manufacturers' instructions.

RESULTS:

Seropositivity for IgG was found in 11 (13.75%) cases and IgA was found in 4 (5%) cases but only 2 (2.5%) cases were positive by Giemsa stain.

Table 1: Findings in the different tests

Total No. of Samples	Giemsa stain		Seropositivity			
		IgG		IgA		
80	No. +ve	%	No. +ve	%	No. +ve	%
	- 2	- 2.5	- 11	- 13.75	- 4	- 5

IgG was also found to be positive in the 2 cases showing elementary bodies in Giemsa stain and in one of the IgA positive case. Therefore, the total number of positive cases was 14 (17.5%).

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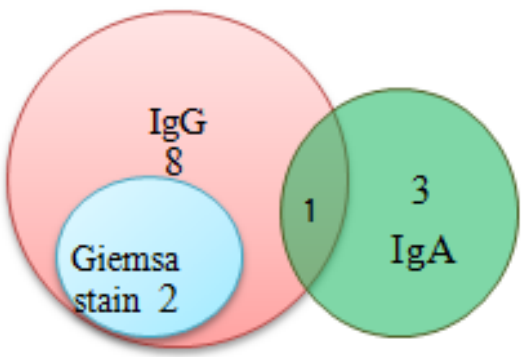
Table 2: Showing the overall prevalence of positive cases

Tests	Giemsa stain & IgG	IgG	IgG & IgA	IgA	Total
No. Positive	2	8	1	3	14
Percentage (%)	2.5	10	1.25	3.75	17.5

Fig 1: Showing positivity of Giemsa stain, IgG and IgA



Highest number of 48 (60%) cases was in the age group of 21-30 years. Vaginal discharge (45%) was the most common presenting feature in females and urethral discharge was the most common complaint in males (60%).

Among the positive cases 71.4% (10 cases) belonged to the age group of 21-30 years.

Table 3: Showing the age distribution:

Age in years	No. of cases	Positive cases			
		IgG	IgG & Giemsa	IgG & IgA	IgA
15-20	4	1	-	-	-
21-30	48	7	-	-	3
31-40	25	-	2	1	-
41-50	3	-	-	-	-
>50	0	-	-	-	-

71.4% (10 cases) of the positive cases did not have history of contraceptive use. 21.4% (3 cases) of the female patients taking OCPs were also positive for IgG. However, none of the cases using barrier method of contraception was found to be positive.

DISCUSSION:

In the present study, the prevalence of *Chlamydia trachomatis* was found in 17.5% patients attending STD clinic. This finding correlate well with a study of Hashemi *et al*.[16] who reported the prevalence of *C. trachomatis* infection to be 17%. Slightly higher prevalence of 25.2% was reported by Joyee AG *et al*.[17] Lower prevalence of 11.5% and 10.7% were reported by Jensen IP *et al*.[18] and Köksal F *et al.*[19] respectively.

Of the total 80 cases, elementary bodies in Giemsa stain was found in two (2.5%) cases. In contrast, Mohammadzadeh M *et al.*[20] found no positive case in Giemsa staining. But Mittal A *et al*.[21] found a higher prevalence of 41% and 36% by Giemsa staining of endocervical smears in women with vaginal discharge and infertility respectively.

Among the seropositive cases, IgG was found in 13.75% cases. Thawani G *et al.* found *C. trachomatis* IgG antibody in 15.97% of patients attending STD clinics.[22] Lower prevalence of 6.2% was reported by Jenab A *et al.*[12]

IgA seropositivity was found in 5% cases in the present study. This correlates well with the finding of Jenab A *et al.* who reported prevalence of 5.1% in symptomatic patients.[12] Lower prevalence of 3% was reported by Morré SA *et al.*[23] But Joyee AG *et al.* reported a higher prevalence of 28.7%.[24]

In the present study, 71.4% of positive cases belonged to the age group of 21-30 years. This correlates with finding of Agrawal SK *et al.* who reported peak incidence of 70% in the age group of 21-25 years.[25] Also Moss TR *et al.* reported highest prevalence of 56.4% between ages of 19-29 years.[26]

In this study, Giemsa staining was found to be less sensitive technique for detection of *C. trachomatis* infection as elementary bodies were found in only 2 cases though serologic tests shows a higher prevalence. Lack of use of barrier contraceptive was also associated with increased prevalence. Highest number of positive cases was found in the younger age group (< 30 years).

The value of serodiagnosis compared to direct tests for *C. trachomatis* is limited in uncomplicated Chlamydial infections. However, in cases of deep-seated upper genital tract Chlamydial infections in whom the bacteria are no longer detectable locally, a positive serological test may be the only indication of Chlamydial involvement.[24] Anti-*Chlamydial* IgM is uncommon in adults with genital tract infection. The prevalence of anti-*Chlamydial* IgG is high in sexually active adults, even in those who do not have an active infection, and is likely due to past infection. A test for IgG antibodies to *C. trachomatis* has also been argued to be a screening test for tubal occlusion due to a previous *Chlamydia* infection and to support the diagnosis of ectopic pregnancy.[6] There is a statistically significant association between *Chlamydia* specific serum IgA and active disease.[4] The presence of IgA anti-*Chlamydia* antibodies have been believed to predict a persistent Chlamydial infection, as IgA antibodies in general disappear quicker than IgG antibodies.[6]

CONCLUSION:

Prevention of STD in general and Chlamydial infection in particular requires education at all levels, training, information and counselling. Examination of sexual partners is important to prevent the spread of *C. trachomatis* infection. Efforts should be made to promote condoms as use of barrier contraceptives will reduce transmission. Non-invasive serological tests may be employed to identify Chlamydial etiology and thereby initiate treatment at an early stage to reduce associated morbidity, particularly in settings where costly molecular methods of diagnosis such as PCR may not be feasible.

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