TO STUDY INCIDENCE OF CHLAMYDIAL GENITAL TRACT INFECTIONS USING ENZYME IMMUNO ASSAY-ANTIGEN DETECTION AND CELL CULTURE METHODS

Lt Col YOGESH CHANDER^{*}, Dr ASHIMA TALWAR⁺,
Col A NAGENDRA[#], Lt Col AK PRAHARAJ^{**},
Lt Col RK SHARMA⁺⁺, Surg Rear Adm VC OHRI^{##}

Abstract

Endocervical swabs from 315 patients were screened for chlamydial infection by using Enzyme Immuno Assay technique for antigen detection. Of these, 190 patients were of infertility and 125 patients were with history suggestive of pelvic inflammatory disease (PID). 100 age matched controls were also screened for the detection of chlamydial antigen by using EIA. The overall incidence of chlamydial infection in this study group was 15.2%. 21 (11.05%) of the infertility patients and 27 (21.6%) of the pelvic inflammatory disease cases were found to be positive for chlamydial antigen. The prevalence rate was found to be high in the age group of 31-40 years in both study groups i.e. infertility group (14.7%) and PID group (50%). All the ELISA positive cases (48) and randomly selected (10) age matched controls were screened by tissue culture using McCoy cell line. In the tissue culture, 44 of the 48 samples were found to be positive and none of the controls groups were found positive. 4 samples showed discordant results possibly due to the presence of non-viable organism or inhibitory material present at the sample site. The sensitivity and specificity of ELISA with respect to tissue culture are 100% and 71% respectively. The positive predictive value and the negative predictive value of the ELISA are 91.6% and 100% respectively. The efficiency of the test was found to be 93.1%.

MJAFI 2001; 57 : 197-202

KEY WORDS :*Chlamydia trachomatis*; Infertility; PID

Introduction

Chlamydial diseases have been known from the earliest records as one of the major scourges of man. Trachoma, a Chlamydial disease affecting eyes is one of the oldest recognised human diseases as described in the ancient Chinese writings and Egyptian Ebers Papyrus (1500 BC) [1]. Chlamydia are obligate intracellular parasites and have been classified taxonomically as a unique class of bacteria on the basis of their phenotypic properties and sequence data of their rRNA [2].

Chlamydiae are classified under the order Chlamydiales that contain one family Chlamydiaceae and one genus *Chlamydia*. Within this genus three species are recognised which have been further classified into biovars and serovars, which are responsible for different clinical syndromes. *C. trachomatis* is divided into 15 serovars. The D through K serovars are commonly associated with sexually transmitted infections. The target cells of the trachoma biovars of *C. trachomatis* are the squamo-columnar epithelial cells of the endocervix and upper genital tract in women and the conjunctiva, urethra and rectum in men and women.

C. trachomatis infections impose a significant burden on humans globally. Besides there being 500 million people affected with ocular trachoma with 7-9 million blind as a result, the genital tract infections are even more prevalence and have major complications [3]. Because of lack of universal testing and reporting, reliable prevalence data is not available. The Centre for Disease Control and Prevention (CDC) has estimated that there are approximately 4 million new *C. trachomatis* infections every year in USA [4]. The proportion of infected sexually active women ranges from 8%-40% with median of about 15% and approximately 10% of sexually active men are infected. In Indian scenario, being a developing country the prevalence data of this infection is inadequate in view of limited Indian studies having been carried out. The available data suggest a prevalence rate of 10%-40% in various target populations. The present study was carried out to know the prevalence rate of Chlamydial genital infections in the local target population. This study has used ELISA for antigen detection to know the prevalence of chlamydial genital tract infection in women and to compare this with the 'gold standard' - tissue culture using McCoy cell line. The present

^{*,**}Reader, ⁺Post Graduate Student, [#]Professor and Head, Department of Microbiology, ⁺⁺Reader, Department of Obstetrics and Gynaecology, Armed Forces Medical College, Pune 411 040. ^{##}CMO, HQ Western Naval Command, Fort, Mumbai 400 001.

study was under-taken to determine the incidence of *Chlamydia trachomatis* infection in female patients attending gynaecology OPD with history suggestive of infertility and pelvic inflammatory disease (PID) and to compare Enzyme Immunoassay for chlamydial antigen detection with cell culture methods.

Material and Methods

315 female patients attending gynaecology OPD of a large hospital between the period of January 98 to June 99 were included in this study. Patients with history of infertility and history suggestive of pelvic inflammatory disease such as vaginal discharge, backache, abdominal pain and menstrual disorders were included in the study. 100 females were also selected as controls after being carefully matched for age from the patients attending antenatal clinic. These patients did not give history suggestive of PID and / or discharge per vaginum. A detailed clinical protocol was maintained in each case.

Specimen Collection

2 endocervical swabs were taken from each patient and control after wiping the endocervix to remove excess mucous and inserting the swab in the endocervical canal and giving rotation for 5-10 sec to ensure that the endocervical cells were sampled. The swabs were numbered as Swab 1 and Swab 2. Swab 1 was put into the transport tube without transport medium which was used for EIA for chlamydial antigen detection, Swab 2 was put into 5ml of the transport medium Sucrose Phosphate Glutamate and this was used for tissue culture of *Chlamydia*.

Swab 1 : was processed by ELISA Microtrak-II Chlamydia EIA KIT (Antigen Detection).

Storage : The swabs were stored at 4-8°C and were tested within 7 days. However, if not tested within 7 days they were stored at -20°C and were brought to room temperature before testing. The test was carried out with commercially available diagnostic kit and universal reagent kit marketed as MICROTRAK - II CHLAMYDIA EIA KIT for antigen detection. The manufacturer's instructions as regards procedure and interpretation of results were adhered to. Biosafety level 2 precautions as described by Centre of Disease Control were followed.

SWAB - 2 was processed by Tissue culture technique.

Storage : Swab - 2 was collected in the transport medium (Sucrose-Phosphate-Glutamate medium or SPG) and was processed for tissue culture. It was stored at -20°C and processed within 7 days.

Preparation of Cell Lines

Mc-Coy cell line was used for this study, stock culture of which was obtained from National Cell Culture Sciences (NCCS) Pune. 200 ml McCartney bottles which were seeded with 2.5-5 million cells suspended in 15 ml of Eagles Minimum Essential Medium were obtained from NCCS.

Maintenance of Mono Layer

Splitting of the cell lines in order to maintain cell line, the monolayer was treated with Trypsin Versene Phosphate Glutamate medium (TPVG) so as to split the monolayer. Complete detachment of the cells was confirmed under the inverted microscope. Cells were dispensed in another bottle for maintenance and on coverslip kept in petridishes for the processing of the samples. After 1/2 hour of incubation the bottles and petridishes were flooded with MEM with 10% fetal calf serum.

Processing of Samples

Stored samples were thawed and vortexed. The media was decanted from the cover slips in the petridish and 100µL of processed sample was layered on the coverslip and then it was kept for incubation at 37°C for 2 hours. After 2 hours MEM medium and 10% FCS were flooded in the petridish containing monolayer. Petridishes were kept at 37°C in CO₂ incubator undisturbed for 48-72 hours after which they were examined for inclusion by Giemsa staining.

Results

315 female patients attending gynaecology OPD of a large hospital during the period January 98 to June 99 were included in the study. The selection of patients was based on those having history of infertility and pelvic inflammatory disease. 100 females were also selected as controls after being carefully matched for age from the antenatal clinic. All the patients were subjected to Chlamydial antigen detection by ELISA. Positive samples by ELISA and randomly selected controls were subjected to tissue culture techniques for isolation of *Chlamydia*.

Amongst the study group the majority of the cases were from the age group of 21-30 years both in cases of infertility and pelvic inflammatory disease. Same trend was present in the age matched control group of 100 cases. The mean age $\pm$ SD in infertility case and PID case was 25.5 $\pm$ 3.99 and 24.5 $\pm$ 6.16 respectively. The control group had mean age $\pm$ SD of 26.0 $\pm$ 3.38. Majority of the cases were of primary infertility and maximum number fell in the age group 21-30 years in both primary and secondary infertility cases. The mean age $\pm$ SD of the patient in primary infertility group was 25.0 $\pm$ 4.1 and in secondary infertility group was 27.0 $\pm$ 4.29. The predominant presenting symptom of PID at the time of reporting of the case in gynaecology OPD is depicted in Table-1. Whereas all the cases presented with more than one symptomatology listed above, the commonest complaint of the patients coming to OPD was vaginal discharge followed by backache and menstrual disorders.

TABLE 1
Presenting symptoms of PID (n = 125)

Symptoms	Present (%)*	Absent (%)*
Vaginal discharge	53 (42.4)	72 (57.6)
Backache	30 (24.0)	95 (76.0)
Pain abdomen	09 (7.2)	116 (92.8)
Dysuria	07 (5.6)	118 (94.4)
Menstrual disorder	24 (19.2)	101 (80.8)
Fever	01 (0.8)	124 (99.2)
Vomiting	01 (0.8)	124 (99.2)

* Figures in parenthesis are percentages

The overall incidence of chlamydial genital tract infection was 15.2% as compared to 0% in age matched control group, the positivity of infection being 11.05% and 21.6% in cases of infertility and pelvic inflammatory diseases respectively. When the values in these 2 groups were compared, the prevalence rate was statistically significant in PID group ($p < 0.01$). χ^2 Yates corrected = 5.70 (Table-2). The incidence of chlamydial infection is more in the age group of 31-40 years and 21-30 years, sexually most active age groups. When these two age groups were compared statistically, no significant difference in the incidence of chlamydial infection was found by ELISA (Table-3).

TABLE 2

Incidence of chlamydial infections in study and control group by ELISA

Types of cases	No. of cases	No. of cases		Positive cases (%)
		Positive	Negative	
Control	100	0	100	0
Infertility	190	21	169	11.05
PID	125	27	98	21.6

 χ^2 Yates corrected = 5.70, $p < 0.01$

TABLE 3

Incidence of chlamydial infection in different age groups of infertility cases (n=190) and PID cases (n=125) by ELISA

Age in years	Infertility cases	No. of positive* cases	PID cases	No. of positive cases
< 20	24	02	38	0
21-30	132	14	45	10
31-40	34	05	32	16
> 41	0	0	10	01
Total	190	21	125	27

* χ^2 Yates corrected = 0.14, $p > 0.05$ * χ^2 Yates corrected = 5.27, $p > 0.05$

The incidence of chlamydial infection in PID cases is more in the age group of 31-40 years and 21-30 years, the sexually most active age groups. When these two age groups were compared statistically the incidence was significantly higher in the age group of 31-40 years ($p < 0.05$) than age group 21-30 years (Table-3). The commonest presenting complaints of the patients of PID were vaginal discharge, backache and menstrual disorder. 14 cases (26.45%) of the vaginal discharge, 6(20%) patients of backache and 3(12.5%) cases of menstrual disorder were found to be positive by ELISA (Table-4).

TABLE 4

Incidence of chlamydial infection in relation to different presenting symptoms of PID

Symptoms	ELISA		Total	+ve cases (%)
	Positive	Negative		
Vaginal discharge	14	39	53	26.4%
Backache	06	24	30	20.0%
Pain abdomen	02	07	09	22.2%
Dysuria	02	05	07	28.5%
Menstrual disorder	03	21	24	12.5%
Fever	0	01	01	0.0%
Vomiting	0	01	01	0.0%

Table-5 shows the result of tissue culture in both the study group and the control group. In the study group 44 out of 48 ELISA positive cases were found positive by culture. 4 discordant results by culture can be explained as the result of presence of non-viable organism or the presence of inhibitory material at the sample site. 10 randomly selected control groups were found to be negative by culture.

TABLE 5

Results of tissue culture in ELISA positive cases study group (n=48) and control (n=10)

Type of cases	No. of cases	No. of cases	
		Positive	Negative
Controls	10	0	10
Infertility	21	20	01
PID	27	24	03

The sensitivity and the specificity of ELISA with respect to tissue culture was found to be 100% and 71% respectively. 44 cases were true positive and 4 cases were false positive. None of the cases were false negative while 10 cases were true negative. The Positive Predictive Value and the Negative Predictive Value of ELISA were 91.6% and 100% respectively. The efficiency of the test was 93.10% (Table-6).

TABLE 6

Comparison of ELISA and tissue culture in test group (n=48)

		Cell culture	
		Positive	Negative
ELISA	Positive	44	04
	Negative	0	10

Sensitivity = 100%

Specificity = 71%

Positive predictive value = 91.6%

Negative predictive value = 100.0%

Efficiency of the test = 93.1%

Discussion

Since the discovery of *C. trachomatis* in 1907 by Helberstaedter and Von Prowazek [1], as inclusion bodies in ocular scraping this organism has received an active consideration from different workers, Lindner in 1911 [5] recognised its relation to genital tract infection. Since then wide variation in the prevalence rate of this infection in the general population group has been found out by different workers, the variation is to the extent from 4 to 40% [6]. This wide variation is partly due to different types of tests used for screening by different workers like antigen detection by tissue culture and antibodies detection by different types of antigen antibody reactions. (Gordon and Quan 1965) [7]. Therefore it is important to know the prevalence rate in local target population in order to establish its cause and effect in genital tract infection.

315 female patients attending gynaecology OPD were investigated, of these 190 patients were those of infertility, while 125 patients had history suggestive of pelvic inflammatory disease. Majority of women in both infertility group (69.4%) and PID group (36%) were in the age group of 21-30 years (Table-3) and this is the group which is sexually most active. JD

Fig. 1 : McCoy cell line uninoculated methanol fixed Giemsa stain (x 200)

Fig. 2 : McCoy cell line uninoculated methanol fixed Giemsa stain (x 400)

Fig. 3 : Cytoplasmic inclusions in McCoy cells stained with Giemsa stain after methanol fixation (x 400)

Fig. 4 : Cytoplasmic inclusions in McCoy cells stained with Giemsa stain after methanol fixation (x 400)

Oriel et al [8], in Medical School London, Maxwell and Diana et al [9] from Royal Womens' Hospital, Melbourne, Victoria, Australia and J. Mahoney et al [10] from Canada had 58.2%, 84% and 90% women respectively belonging to this age group in their test population. Majority of patients (72%) amongst 314 were in this age group in the study conducted by Ray and Yadav in 1997 [11] at Safdarjung Hospital, New Delhi. This is the most sexually active age group. Thus majority of cases in most of the studies belong to this age group.

Surveys carried out all over the world indicate the variable prevalence rate of *Chlamydia trachomatis* genital tract infection in females. The present study was conducted at a large hospital in Pune, and the target populations was selected from the gynaecology OPD. In this study the prevalence rate was 15.2% among the target patients using EIA for antigen detec-

tion. This result is comparable with the work done by Gilanfer et al [12] and Chernesky et al [13]. The other workers like Mohaney et al [10], have shown higher prevalence rate. The rate is high in their studies because they selected the patients from clinics for Sexually Transmitted Diseases whereas in the present study cases attending gynaecology OPD with history of infertility and PID were included. Ray and Yadav in New Delhi at Safdarjung Hospital studied 314 female patients attending gynaecology OPD. They subgrouped the patients in six groups and used EIA for diagnosis and found an average incidence of 28.6% in symptomatic cases [11].

Amongst the 315 cases, 190 belonged to infertility group (Primary infertility n=136, Secondary infertility n=54). The overall incidence of chlamydial infection was found to be 11.05% in this group (Table-2). In primary infertility it was 11.2% and in secondary in-

fertility group it was 11.11%. Sharma M et al in 1989, found overall incidence of 50% among infertile patients screened. The patients with primary infertility in her group of cases studied showed a positivity of 11.7% [14]. Our study is in accordance with this study. Ray and Yadav in 1997 found a detection rate of 27.2% in the infertility group of patients. Their incidence is high possibly because they have included less number of cases in this group [11].

Incidence of chlamydial infection associated with PID in this study was 21.6% (Table-2). Incidence of *C. trachomatis* detected by Oriel et al amongst PID cases in 1978 at Hospital Medical School, London, England was 41.2% [8]. Sharma M et al in 1989 have shown a prevalence rate of 25.2% in PID cases [14] which is almost similar to our results. Lorraine et al in 1993 have shown similar finding and have found incidence of 22% in PID group [15]. In year 1997, Ray and Yadav while doing studies at Sufdarjung Hospital in New Delhi have shown a prevalence rate of 42.8% chlamydial infections in patients attending STD clinics [11]. This is quite high as compared to this study and can be explained by the fact that Ray and Yadav divided the total number of patients into 6 study groups and the number included in PID group were less (49) as compared to present study.

Overall higher incidence was detected in age group 31-40 years and 21-30 years, the sexually most active age group. On statistical comparison of the incidence between these two age groups, it was found that incidence is significantly high statistically in 31-40 years age group in PID cases whereas no statistically significant difference was noticed in the infertility cases belonging to these two age groups (Table-3).

In recent years the overall incidence of sexually transmitted diseases is on the rise the world over, including *C. trachomatis* genital tract infection. Therefore diagnostic techniques are required to detect these infections by screening of the sexually active population and providing effective treatment for prevention of transmission of disease to the sexual partner and also to the new born. Various diagnostic methods include techniques for antigen detection by complement fixation test, radioisotope precipitation test, and enzyme immunoassays. Recently new technique of nucleic acid hybridisation and PCR has been developed. This study has determined the incidence of *C. trachomatis* infection by using Enzyme Linked Immunoabsorbant Assay and compared it with cell culture methods considered as gold standard.

The sensitivity and specificity of ELISA in this

study was 100% and 71% respectively (Table-6). Chlamydia antigen detection by ELISA has been reported to have sensitivity of greater than 70% and specificity of 97.99% in population of men and women with prevalence of infection of 55 or more and it has been accepted as an alternative, to tissue culture for diagnosis of chlamydial genital tract infection in women [16]. Various other workers have also done work with ELISA and found different sensitivities and specificity respectively [12]. Pugh and Slack et al by using Enzyme Immunoassay have shown sensitivity and specificity of 92% and 99% respectively. Grillner et al in 1986 have shown sensitivity of ELISA for detecting *C. trachomatis* infection as 100% which is comparable to our studies [17]. Mahoney et al in their study at Regional Virology and Chlamydiology Laboratories at Canada in year 1989 have shown sensitivity and specificity of 85.2% and 99.3% respectively by using Chlamydiazyme (Abbott Laboratories, North Chicago Hill) in which they have measured antigenic components of *C. trachomatis* [10]. Ray and Yadav have shown sensitivity of 70%-90% by EIA in their study [11]. The high specificity of 71% of the test is attributable to the use of a monoclonal antibody directed against genus specific lipopolysaccharide antigen. This good co-relation was due in part to the fact that the monoclonal antibody detects not only elementary bodies but also free lipopolysaccharide antigen.

The "gold standard" for detection of *C. trachomatis* has been cell culture as culture has always fascinated man in the field of research. In this study out of 48 ELISA positive cases 44 were found to be culture positive (Table-5). The 4 cases, which were culture negative, can be explained as a result of presence of non-viable organism or the presence of inhibitory material at the sample site. Galanfer et al in 1985 have shown 100% concordant results between immuno assay and culture methods [12].

Because of the cumbersome procedure involved with cell culture techniques, we recommend Enzyme-Immunoassay for the detection of chlamydial infection, as there are many advantages associated with it such as speed of diagnosis, potential for automation and ease of transport of specimen. The EIA can be performed in four hours while the cell culture takes minimum of three days, before the result can be reported and is also technically demanding. Also the assays are not dependent on the viability of the organism, and transport of the specimen is not a critical factor [12]. Considering all the advantages this study recommends Enzyme-Immunoassay for the diagnostic evaluation of chlamydial infections.

It is concluded from the study that the prevalence rate of *Chlamydia trachomatis* infection is high in infertility and pelvic inflammatory diseases in the female population. Enzyme immuno assay for the detection of *Chlamydia trachomatis* is simple, convenient, less time consuming, sensitive, specific and reliable test for the diagnosis and screening of large population group in comparison to tissue culture.

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