

Detection of Chlamydia Trachomatis among Infected Women

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Abstract: *Chlamydia trachomatis* (*C. trachomatis*) is the most common bacterial sexually transmitted infection (STI). *C. trachomatis* has a high rate of asymptomatic infection; approximately 80% of cases in females and 45% in males are estimated to be asymptomatic. The aim of this study was to estimate the prevalence of *C. trachomatis* among women with chronic cervicitis, abortion, full term pregnancy and infertile women and compare between different methods of diagnosis for detection of *C. trachomatis*. Eighty women were subjected to our study, they were classified as 20 women with chronic mucopurulent cervicitis, 20 women with spontaneous abortion, 20 infertile and 20 full term pregnancy, also 40 controls normal women were examined. Endocervical specimens and blood samples were taken from all previous groups and subjected to examination ELISA for detecting *Chlamydia trachomatis* (*C. trachomatis*) antigen and immunoglobulin A (IgA). IgA and *C. trachomatis* ELISA gave positive chlamydial infection of 25% and 20%, respectively. IgA proved good sensitivity and specificity of 93.8% and 92.8%, respectively. Chlamydial infection was detected among 30% of abortion cases, 25% among infertile women, 15% among chronic mucopurulent cervicitis, 10% in full term pregnancy and 5% of the control women. *C. trachomatis* infection was significantly prevalent among examined cases in comparison to controls ($P < 0.05$). Our results revealed increased incidence of chlamydial infection among nulliparous women but non statistical significant differences were recorded. Also, chlamydial infection was inversely related to young and marital duration less than 5 years, significant statistical differences were recorded ($P < 0.004$ and < 0.001) respectively. *Chlamydial trachomatis* has an important role especially in infertile women and those with spontaneous abortion. Cases of abnormal vaginal discharge particularly in young sexually active women belonging to low socioeconomic classes should be considered as at high risk of chlamydial infections. So, strategies for the treatment of females early in pregnancy must be carried. IgA and ELISA testes are sensitive methods of diagnosis of *C. trachomatis*.

Key words: *Chlamydia trachomatis* • *Chronic cervicitis*

INTRODUCTION

The majority of *Chlamydia trachomatis* infections in women is asymptomatic, but may give rise to pelvic inflammatory disease (PID) and tubal infertility. Screening programmes aim at reducing morbidity in individuals by early detection and treatment and at decreasing the overall prevalence of infection in the population. A number of modeling studies have tried to calculate the threshold prevalence of Chlamydia lower genital tract infection above which screening becomes cost-effective [1].

In developing countries, the prevalence of lower genital tract chlamydial infection in sexually active women may be of the order of 26% [2]. The challenge is to develop cheap and reliable diagnostic tests for chlamydial infection. Of women being evaluated for infertility, 40% are infected with Chlamydia, Mycoplasma or Ureaplasma, as are 36% of those with a previous history of uterine infection and 50% of those with tubal blockage. More than 60% had evidence of a past infection [3].

Prematurely is one of the leading causes of perinatal mortality. Uterine contractions may be induced by cytokines, proteolytic enzymes or prostaglandins released

or induced by microorganisms. Asymptomatic bacteriuria, gonococcal cervicitis and bacterial vaginosis are strongly associated with preterm delivery, but the role of *C. trachomatis*, *Trichomonas vaginalis* and *Ureaplasma urealyticum* is less clear. However, a substantial number of studies suggest that maternal *C. trachomatis* infection in pregnancy is associated with premature delivery [4,5].

Andrews *et al.* [6] looked at the prevalence of genitourinary *C. trachomatis* infection in 190 women who spontaneously delivered after less than 37 weeks of gestation versus 190 control, women with *C. trachomatis* infection at 24 weeks' gestation were twice as likely as uninfected women to have a spontaneous preterm birth and three times as likely to have a spontaneous preterm birth at <35 weeks' gestation. *C. trachomatis* has also been associated with intrauterine growth retardation and has been experimentally shown to induce pre-term birth [6-8].

A large number of studies have shown that there is a high prevalence of *C. trachomatis* genital tract infection among women seeking termination of pregnancy. Moreover, post-abortion pelvic inflammatory disease is a well recognized complication of termination of pregnancy, with its attendant risks of tubal dysfunction and either infertility or subsequent ectopic pregnancy [9].

This is particularly the case for sexually active women under the age of 24 years who are likely in many countries to have rates of chlamydial carriage in excess of 5%. Of course the costs of screening for genital infection have to be balanced against the overall risk factors for premature birth [10].

Prenatal implications of chlamydial infection for the mother and newborn include associations with ectopic pregnancy, spontaneous abortions, preterm labour, amnionitis, premature rupture of membranes, low birth weight, prematurity, still birth and neonatal deaths [11,12].

Chlamydia infection is both treatable and easily cured when detected. The most sensitive method for diagnosis of genital *C. trachomatis* infection is based on culture of microorganisms on Hela 299 or MacCoy cells, which requires extensive laboratory facilities [14]; also its disadvantage is that it takes several days before the test result. Several serological methods have been developed to detect *C. trachomatis* as complement fixation test, immunofluorescent test, ELISA and recently PCR technique [15].

The aim of the present study was to detect the prevalence of *C. trachomatis* among women with chronic cervicitis, abortion, full term pregnancy and infertile women and compare between different methods of diagnosis as IgA and ELISA.

MATERIALS AND METHODS

Patients: This study included 80 women attending the Gynecology and Obstetrics Clinic at King Abdulaziz University Hospital. Also, 40 gynecologically free women attending to the family planning clinic were included as a control group.

The investigated women were classified as the following; twenty women presented with abnormal vaginal discharge and diagnosed as chronic mucopurulent cervicitis, twenty full term pregnant women, twenty infertile cases and twenty women with spontaneous abortions.

Then diagnosis was based on the following five basic criteria; fertile husband (having at least three times normal semen analysis), ovulatory cycles, satisfactory postcoital test, normal pelvic organs and tubal patency and regular sexual actions). The duration of infertility was at least two years and the age of wife was less than 35 years.

All the previous cases were subjected to the following:

- History; taking, age, marital status, residence, parity.
- Menstrual history, obstetric history, history of abortions and types of contraceptive used.
- Complete general examination.
- Pelvic examination, the cervix was inspected for signs of trauma, chronic cervicitis and purulent discharge.

Sampling:

- Sterile plastic swabs were used to collect endocervical specimens and were immediately immersed into 1ml of chlamydial antigen detection transport medium (IDEIA, Novobio labs, Cambridge, UK).
- Peripheral venous blood samples (3-5 cm) were also obtained from patients. The serum was separated by centrifugation and stored at (-20°C) until assay.

Elisa Assay: The chlamydia antigen was assayed using commercial ELISA kit (IDEIA, Novobio labs, Cambridge, UK). Briefly, samples were put into 2ml of transport medium and boiled for 15 minutes to extract Chlamydia antigen. The assay was carried out in duplicate with 200 μ l extract added to each well. The principle of the assay is that, monoclonal antibody bound to the prepared EIA tray captures chlamydial antigen from the extract, the antigen is detected with an alkaline phosphatase.

Labeled monoclonal antibody with the formation of red formazan dye could be detected spectrophotometrically on wave length 450.

Detection of Immunoglobulin a (IgA): Detection of antichlamydial IgA antibodies using VIROTECH System Diagnostika GmbH (West Germany) was carried out according to instruction of manufacturers. The test principle is ELISA intended for qualitative detection of IgA serum antibody in the human serum which formed an immune complex with the chlamydia antigen coated on the test strips which attracted the enzyme conjugate. After adding the substrate solution, an orange yellow dye was produced by the bound enzyme (peroxidase). Unbound immunoglobulins were removed by washing.

Statistical Analysis: The chi-square and t-test were used for statistical contrasts. Sensitivity, specificity, positive and negative predictive values for each test were estimated ($P < 0.05$).

RESULTS

Table (1) shows that, IgA and ELISA methods were used for diagnosis *C. trachomatis* infections. IgA antibodies and ELISA technique gave giving prevalences of 25% and 20%, respectively.

Table (2) shows that, on detecting the sensitivity and specificity of IgA a confirmed by ELISA test, IgA showed high sensitivity and specificity of 93.8 and 92.8%, respectively.

Table (3) shows that among 80 examined women; *C. trachomatis* was found at highest percentage among women with abortion (30%) followed by infertile women, women with mucopurulent cervicitis and full term pregnancy (25, 15 and 10%) respectively.

Table (4) shows that *C. trachomatis* was more prevalent among rural women, than urban ones and *C. trachomatis* infection was inversely related to age. Prevalent associations were recorded between *C. trachomatis* infection and age less than 25 years ($P < 0.05$).

Table 1: Different techniques used for diagnosis of *C. trachomatis* infections.

Different techniques	Positive cases detected (Total=80)	
	No.	%
ELISA	16	20
IgA	20	25

Table 2: Validity test of ELISA (IgA) in comparasion to antigen detection by ELISA

	ELISA		Total
	Positive	Negative	
IgA			
Positive	15	5	20
Negative	1	59	60
Total	16	64	80
Sensitivity = 93.8% Predictive - ve=75%			
Specificity =92.8% Predictive +v =98.3%			

Table 3: Chlamydia infection among the different examined groups.

Examined groups	No. of examined cases	Chlamydia infection	
		No.	%
Chronic mucopurulent cervicitis	20	3	15
Abortion cases	20	6	30
Full term pregnancy	20	2	10
Infertile women	20	5	25

Table 4: Distribution of women infected with Chlamydia according to their residence and age.

	Chlamydial cervicitis (3)				Abortion (6)		Full term pregnancy (2)		Infertility (5)	
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Data	No.	%	No.	%	No.	%	No.	%	No.	%
Residence:										
Urban (38)		0	3	50	1	50	2		40	
Rural (42)	3	100	3	50	1	50	3		60	
	X2=0.8		P=0.3		N.S					
Age:										
<25(35)	2	6.77	4	66.7	2	100	2		40	
>25(45)	1	33.3	2	33.3	0	0.0	3		60	
	X2= 7.94		P= 0.05		NS					

Table 5: Distribution of women infected with Chlamydia according to parity and marital duration.

	Chlamydial cervicitis (3)		Abortion (6)		Full term pregnancy (2)		Infertility (5)	
Data	No.	%	No.	%	No.	%	No.	%
Parity:								
Nullipara(43)	2	66.7	2	33.3	2	100	2	40
Multipara(37)	1	33.3	4	66.6	0	0.0	3	60
	X2= 0.0		P= 1.0		NS			
Marital duration:								
<5 years(32)	3	100	2	66.7	2	100	4	80
>5 years(48)	0	.	2	33.3	0	0	1	20
	X2= 14.18		P= <0.001					

Table 6: Distribution of positive *C. trachomatis* cases with different methods of contraception

Methods of contraceptive	Positive cases detected (Total=16)	
	No.	%
IUD	6	37.5
Oral contraceptive	8	50
Other methods	2	12.5

Table (5) shows that *C. trachomatis* infection was inversely related to marital duration. Significant association was recorded between *C. trachomatis* infection and marital duration <5 years ($P<0.001$).

Table (6) shows that infection was high among women who used oral contraceptive (50%), compared to others who used intra-uterine device IUD (37.5%).

DISCUSSION

The true incidence of Chlamydia infection in developing countries is difficult to establish because of several factors. There is a socio cultural inhibition that

prevents women from reporting sexual symptoms, non availability of facility to detect the organism in many health units and the largely asymptomatic nature of the disease [16]. In spite of these limitations, it is still reported that there is a high prevalence of chlamydia infection in most parts of Africa [17].

Chlamydia trachomatis are now widely recognized as the most common cause of sexually transmitted diseases both in man and women. In almost all populations of women studied in developed countries, the prevalence of *C. trachomatis* exceeds that of *N. gonorrhoeae* [18]. Recent studies have shown that chlamydial genital infections and its complication are

common in industrialized countries and some countries of Africa. The endocervix is the most common site for *C. trachomatis* infection in women. Infection and destruction of the cervical endometrial and fallopian tube lining cells may impair fertility and increase the risk of ectopic pregnancy or damage of a developing pregnancy [19].

Our results revealed that IgA and ELISA techniques gave positivity results of 25% and 20%, respectively. IgA proved high sensitivity specificity, predictive negative and predictive positive values with 93.8, 92.8, 75 and 98.3%, respectively. These results are in agreement with others [20] who found that IgA is detected in the cervix of 28% and 33.5% of women with *C. trachomatis* infection. Also, IgA sensitivity, specificity and positive and negative predictive values were calculated as 84.7, 98.6, 98.4 and 86.3%, respectively. We detected that 5 women were positive by IgA and negative with ELISA test, this might be explained by that, there was a recently cleared chlamydial infection in these women and the IgA immune response had not get subsided. Also, chlamydial antibodies ELISA are genus specific, not species specific and women infected with *C. pneumoniae* or *C. psittaci* and who have antibodies to these organisms circulating may be scored as false positive in *C. trachomatis* antibody testing [21].

The results of this work revealed that 15% of infected women had chronic cervicitis, this result is confirmed with Manhart *et al.* and Taylor-Robinson, [13, 22] reported that the two most regularly identified causes of cervicitis are gonococci and *Chlamydia trachomatis*. Gonococci and Chlamydia are of particular importance as being likely to give rise for chronic cervicitis [13, 22]. It was suggested that the high rate of asymptomatic infection by serovar E conferred a transmission advantage in this high risk population [18].

Also, the results detected that 25% of infertile women had *C. trachomatis* infection. Our results are in agreement with Siemer *et al.* [23] reported that 33% of women with unexplained infertility had chlamydial infection. IT was found that among women evaluated for infertility; 40% are infected with Chlamydia, Mycoplasma or Ureaplasma, 36% with a previous history of uterine infection and 50% of with tubal blockage [23]. More than 60% had evidence of a past infection [24]. An infection can prevent pregnancy by blocking the uterine tubes, damaging the sperms (14A), so they can't swim toward the egg and it can cause abortions, premature birth and low birth weight babies [25]. Infection with chlamydia is the most common cause of blocked Fallopian tubes that cause infertility [28-29].

The results of this study revealed the prevalence of *C. trachomatis* infection in rural area, but non significant difference was recorded. Belongia *et al.* [30] detected a geographic variation in the rate of Chlamydia infection, where they detected *C. trachomatis* infection is increased in rural areas and explained that by the different in sexual habits and socioeconomic status between rural and urban areas. Also, our results found significant difference between chlamydial infection and different age groups, high prevalence of infection was recorded in age group <25 years [30].

Our results are in agreement with Rassjo *et al.* [31] declared that younger age group was associated with active sexual practice and higher rates of chlamydial infection. In addition, the presence of chlamydial infection was correlated with nulliparous women, but non significant association was recorded between chlamydial infection and parity [32]. Also, our results revealed significant increase of *C. trachomatis* infection in the early years of marriage (< 5 years) during which the sexual relation is usually active. It was reported that *C. trachomatis* is a symptomatic infection in both women and men and transfer of infection can occur easily between husband and wife [33]. In this study, the frequency of *C. trachomatis* recovered from control cervix was (5%), while other authors [34] found that 6% and 8% of their healthy controls had *C. trachomatis* in their endocervical specimens. This may be explained by the *C. trachomatis* infection is an asymptomatic sexually transmitted disease and *C. trachomatis* is a pathogen commonly found in genital tract of normal women and men.

The prevalence of *C. trachomatis* in our study groups were 30% in women with abortion, 25% in infertile women, 15% in women with chronic mucopurulent cervicitis and 10% in full term pregnancy. Insignificant difference was recorded between investigated groups ($P>0.05$). *C. trachomatis* infection was recorded with (20%) among all investigated groups. Other studies showed variable percentages of chlamydial infection. This variability of results between different studies compared to ours may be explained by the variation in sexual activity between our population and other populations, also, our Islamic religion which may restrict sexual activity to one partner, the husband, while other countries don't apply this restriction and there are several partners [25].

As regard to *C. trachomatis* infection in abortion, our results revealed that 30% of women with spontaneous abortion had *C. trachomatis* infection; the prevalence is comparable to that of the previous studies which revealed

that *C. trachomatis* infection occurs with 17.6% and 21.7% in spontaneous abortion cases. Chronic silent chlamydial infection may results in pregnancy loss. The results of this study revealed the prevalence of oral contraceptive tablets among infected women; this finding was confirmed with others [34].

Chlamydial trachomatis has an important role especially in infertile women and those with spontaneous abortion. IgA and ELISA testes are sensitive methods for diagnosis of *C. trachomatis* infection. There was a high prevalence rate of maternal *C. trachomatis* which necessitate the need for routine testing for *C. trachomatis* in pregnancy-to reduce the significant, yet preventable morbidity associated with chlamydial infection in both the mother and the neonate.

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