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Detection of Chlamydia Trachomatis among Infected Women

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Abstract: Background: 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. **Objective:** The aim of this study was to to detect the prevalence of C. trachomatis among women with chronic cervicitis, abortion, full term pregnancy and infertile women. Also to compare between different methods of diagnosis as detection of C. trachomatis as ELISA and IgA antibody. **Material and Methods:** Eighty women were subjected to our study, they were classified as 20 women with chronic mucopurulent cervicities, 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 by enzyme linked immunosorbent assay (ELISA) for detecting chlamydia trachomatis (C. trachomatis) antigen and immunoglobulin A (IgA). **Results:** IgA, and ELISA gave a positive chlamydial infection of 25% and 20% respectively. IgA proved good sensitivity and specificity 93.8% and 92.2% respectively. Chlamydial infection were detected among 30% of abortion cases, 25% among infertile women, 15% among chronic mucopurulent cervicities, 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**. Conclusion:** chlamydial trachomatis has an important role especially in infertile women and spontaneous abortion. Cases of abnormal vaginal discharge particularly in young sexually active women belonging to low socioeconomic classes should be considered at high risk of chlamydia infections. So that strategies for the treatment of females early in pregnancy must be carried. IgA and ELISA testes are sensitive methods of diagnosis C. trachomatis. Detection of IgA gave a good sensitivity and specificity results.

Key words:

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 modelling studies have tried to calculate the threshold prevalence of chlamydia lower genital tract infection above which screening becomes cost-effective (Whiteside *et al*., 2001).

In developing countries, where the prevalence of lower genital tract chlamydial infection in sexually active women may be of the order of 26% (Tiwara *et al*., 1996), 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 (Geisler *et al*., 2008).

Prematurity 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 (Cram *et al*., 2002;

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Locksmith & Duff, 2001). However, a substantial number of studies suggest that maternal C. trachomatis infection in pregnancy is associated with premature delivery.

Andrews *et al*.,(2000) 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 shown experimentally to induce pre-term birth in intrava (K"http://www.chlamydiae.com/restricted/docs/infections/ gentrac_pregnancy.asp"\l"pregnancy_refs1" Blanco *et al*., 1997; Pal *et al*., 1999).

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-abortal pelvic inflammatory disease is a well recognised complication of termination of pregnancy, with its attendant risks of tubal dysfunction and either infertility or subsequent ectopic pregnancy (Cameron & Sutherland, 2002)

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 (Suchland *et al*; 2003)

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. 7–9 Women with chlamydia during pregnancy are also more likely to develop intrapartum fever and or late onset postpartum endometritis after vaginal delivery (Tiller; 2002 and Bennett *et al*.; 2001).

Million chlamydial cases presenting each year, Chlamydia is more difficult to diagnose for women and men. Untreated infections in women evolve serious reproductive tract sequale. The bride side to this epidemic is that these sequale are preventable (Manhart *et al*.; 2003). Chlamydia is both treatable and easily cured when detected. The most sensitive method for diagnosis is of genital C .tachomatis infection was recently based on culture of microorganism on Hela 299 or MacCoy cells, which require extensive laboratory facilities (Marrazzo *et al* .; 2002), also its disadvantage is that it takes several days before the test result. Several seriological methods had been developed to detect C. tachomatis as complement fixation test, immunoflourescent test. ELISA test, and recently PCR technique (Joyee *et al*.; 2007).

The aim of the present study is to detect the prevalence of C .tachomatis among women with chronic cervicities, abortion, full term pregnancy and infertile women. Also, to compare between different methods of diagnosis as ELISA and IgA detection.

MATERIALS AND METHODS

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 Following:

(1) Twenty women presented with abnormal vaginal discharge and diagnosed as chronic mucopurulent cervicitis.
(2) Twenty full term pregnant women.
(3) Twenty infertile cases, then-diagnosis were based on the following five basic criteria (fertile husband having at least three times normal semen analysis, ovulatory cycles, satisfactory postcostal 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.
(4) Twenty women with spontaneous abortions.

All the Previous Cases Were Subjected to the Following:

(1) History taking, age, marital status, residence, parity.
(2) Menstrual history, obstetric history, history of abortions, and types of contraceptive used:
(3) Complete general examination.
(4) Pelvic examination, the cervix was inspected for signs of trauma, chronic cervicitis, and purulent discharge.

Sampling:

Sterile plastic, swab was used to collect endocervical specimens, the swab was inserted into the endocervical canal about 1-1.5 cm until most of the swab tip inside the external cervical opening and left for 5-10 seconds using pressure to obtain more cells from the endocervical canal.

The swab was immediately expressed into 1ml of chlamydial antigen detection transport medium (IDEIA). Novobio labs Cambrigde, UK).

Peripheral venous blood sample (3-5 cm) was also obtained from patient. The serum was separated by centrifugation and stored at (-20°C) until assay.

Enzyme linked immuno-sorbent assay: Chlamydia antigen was assayed using commerical ELISA kit (IDEIA; Novobio labs, Cambridge, UK).Briefly, samples which put into 2ml of transport medium were boiled for 15 minutes to extract C. antigen. The assay was carried out in duplicate with 200 ul extract added to each well. The principle of the assay is that, monoclonal antibody bound to a 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, which can be detected spectophotometrically on wave length 450.

Detection of immunoglobulin A (IgA): Detection of anticlamydial IgA antibodies using VIROTECH System Diagnostika GmbH (West Germany) according to instruction manufacturers. The test principle ELISA is intended for qualitative detection of IgA serum antibody in the human serum which forms an immune complex with the chlamydia antigen coated on the test strips. The enzyme conjugate attracts to this complex. After adding the substrate solution an orange yellow dye is produced by the bound enzyme (peroxidase). Unbound immunoglobulins are 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: Different techniques used for diagnosis of C. trachomatis infections.

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

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

Table 2: Validity test of IgA confirmed by ELISA method.

	ELISA		
	Positive	Negative	Total
IgA			
Positive	15	5	20
Negative	1	59	60
Total	16	64	80

Sensitivity = 93.8% Predicitive –ve=75%
Specificity =92.8% Predicitive +v =98.3%

Table (2) shows that, on detecting the sensitivity and specificity of IgA a confirmed by ELISA test., IgA showed high sensitivity and specificity 93.2% and 92.2% respectively.

Table 3: Chlamydia infection among different examined groups.

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

Table (3) shows that,on diagnosis of C.trachomatis among 80 women from different classified groups C. trachomatis was found at highest percentage among women with abortion cases (30%) followed by infertile women and women with mucopurulent cervicities and full term pregnancy (25%, 15% and 10%) respectively.

Table 4: Distribution of women infected with chylamydia according to their Residence and age.

Clinical data	C.cervicitis		Abortion		Full term pregnancy		Infertility	
	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	66.7	4	66.7	2	100	2	0
>25(45)	1	33.3	2	33.3	0	0.0	3	60
X2= 7.94	P= 0.05	NS						

Table (5) Shows that on detecting C. trachomatis infection among different investigated cases. C. trachomatis was more prevalent among rural women, than urban ones also, C. tracomatis infection was inversely related to age (<25 years). Prevalent associations were recorded between C. trachomatis infection and age less than 25 years (P<0.05).

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

Clinical data	C.cervicitis		Abortion		Full term pregnancy		Infertility	
	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	4	66.7	2	100	4	80
>5 years(48)	0	0	2	33.3	4	80	1	20
X2= 14.18	P= <0.001							

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

Table 6: Distribution of positive C.trachomatis with different methods of contraceptive.

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

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

Discussion:

The true incidence of Chlamydia infection in developing countries is difficult to establish because of several factors. There is a sociocultural 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 (Fioravante *et al* 2005). In spite of these limitations, it is still reported that there is a high prevalence of the chlamydia infection in most parts of Africa (Sobocinski *et al*., 2001).

Chlamydiae trachomatis are now widely recognized as most common cause of sexual transmitted disease both in man and women. In almost all populations of women studied in developed countries, the prevalence of C. trachomatis exceeds that of N. gonorrhoea (Sturm-Ramirez *et al*., 2000). Recent studies have shown that chlamydial genital infection and its complication are common in industralaized 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 (Silveira *et al*., 2010).

Our results revealed that IgA and ELISA techniques gave postivity results of 25% and 20% respectively. IgA proved high sensitivity specificity, predictive negative and predicitive positive with 93.3%, 92.8%, 75% and 98.3% respectively. These results in agreement with others (Servaas *et al*.; 2002; Bennett *et al*., 2009), they found that IgA was detected in the cervix of 28% and 33.5% of women with C. trachomatis infection. Also, IgA sensitivity, specificity, positive predictive value, and negative predictive value were calculated 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 may be explained by that, there was a recently cleared chalmydial infection in these women and the IgA immune response had not get subsided. Also chalmydia 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 (Wills *et al* ;2009).

The results of this work revealed that 15% of infected women had chronic cervicities, this results confirmed with Manhart *et al*., (2003) and Taylor-Robinson, (2002) they 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 cervicities. It was suggested that the high rate of asymptomatic infection by serovar E conferred a transmission advantage in this high risk population (Sturm-Ramirez *et al*., 2000).

Also the results detected that, 25% of infertile women had C trachomatis infection. Our results in agreement with Siemer *et al* (2008), they reported that 33% of women with unexplained infertility had chlamydia infection. Also, most of women being evaluated for infertility, 40% are infected with chlamydia, mycoplasma or ureaplasma, 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 (Omo-Aghoja,*et al* .; 2007) . An infection can prevent pregnancy by blocking the uterine tubes. It can damage sperm (14A), so they can't swim toward the egg, and it can cause abortions, premature birth and low birth weight (van Valkengoed *et al*., 2004)

An infection can prevent pregnancy by blocking the uterine tubes (Oloyede *et al*., 2003). It can damage sperm, so they can't swim toward the egg, and it can cause abortions, premature birth and low birth weight (Okonofua; 2003). Infection with chlamydia is the most common cause of blocked Fallopian tubes that cause infertility (Land *et al*.; 2003). First, chlamydia paralyzes the cilia so the egg the first study from the Netherlands shows that having antibodies against chlamydia is a potent predictor of blocked tubes. The second study shows that many women infected with chlamydia don't have high antibody titers to chlamydia (Gijsen *et al*.; 2002)

Also, the author suggested that C. trachomatis infection or reaction of an immune response to the C.trachomatis "heat shock protein" may induce an inflammatory reaction in the uterus that impairs embryo implantation and facilitates immune-rejection of the embryo. The result of this study also revealed the prevalence of C. trachomatis infection in rural area, but non significant difference was recorded. Belongia *et al*.; (1996), detected a geographic variation in the rate of chlamydia infection, they detected C. trachomatis infection increased in rural areas and explained that by the different in sexual habitis and socioeconomic status between rural and urban areas. Also on detecting the relation of age with chlamydia infection our result found significant difference was recorded between chlamydial infection and different age groups, high prevalence of infection was recorded in age group <25 years.

Our results in agreement with Rassjo *et al*., (2006), they declared that younger age group was associated with active sexual practice and associated with higher rates of chlamydial infection. In addition, the presence of chlamydia infection was correlated with nulliparous women, but non significant association was recorded between chlamydial infection and parity. Our finding was not in agreement with others (William *et al*., 1997) they found that, chlamydial infection is inversly related to parity. 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. Those were in agreement with others. They reported that C. trachomatis is a symptomatic infection in both women and men and transfere of infection can occur easily between husband and wife (Okoror *et al*.; 2007). In this study the frequency of C. trachomatis recovered from control cervix was (5%), while other authors (Gorander *et al*.; 2008) 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 asymptomatic sexual transmitted disease, also 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 mucopurulant cervicites 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 studied showed variable percentage of chlamydial infection (Kanki;

2000). 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 restricted sexual activity to one partner the husband, while other countries no restriction and there are several partners (van Valkengoed *et al*.; 2004)

As regard to C. trachomatis infection in abortion, our results revealed that 30% of spontaneous abortion had C. trachomatis infection, the prevalence was comparable to that of the previous studies (Cram *et al*.; 2002) which revealed C. trachomatis infection with 17.6% and 21.7% in spontaneous abortion cases. Chronic silient chlamydial infection may results in pregnancy loss. 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-abortal pelvic inflammatory disease is a well recognised complication of termination of pregnancy, with its attendant risks of tubal dysfunction and either infertility or subsequent ectopic pregnancy. The result of this study revealed the prevalence of oral contraceptive tablets among infected women; this finding was confirmed with others (Ness *et al*., 2000).

Conclusion:

Chlamydial trachomatis has an important role especially in infertile women and spontaneous abortion. IgA and ELISA testes are sensitive methods of diagnosis C. trachomatis. Detection of IgA gave a good sensitivity and specificity results. There is a high rate of maternal C. trachomatis and incomplete testing for the infection in pregnant women. These findings highlight the need to instigate 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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