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Chlamydia Trachomatis and Recurrent Spontaneous Abortion in Iraqi Pregnant Women

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Abstract

Background Certain infectious agents have been identified more frequently in cultures from women who have had a spontaneous pregnancy loss; these include *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia*.

Objective The aim of the study was to evaluate the frequency of *Chlamydia trachomatis* infection among women who experienced recurrent spontaneous abortion.

Methods A total of 119 women, age ranged from 23.9−28.5 years were enrolled in the current study and were classified into: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5±0.68); Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4±0.85) and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9±0.88). From each patient and control blood and urine samples were collected. Urinalysis test strips including Leukocytes esterase in urine was done, and estimation of IgM levels against ***Chlamydia trachomatis*** in sera of patients was done using ELISA method.

Results Based on ELISA screening assay, results showed a significant difference in the level of circulating *C.trachomatis* specific IgM antibody between group A and group C ($p< 0.05$) as well as between group B and group C ($p< 0.01$). Also highly significant positive correlation (r=0.401, $p<0.001$) between *C.trachomatis* acute infection and urine level of leukocyte esterase.

Conclusion *C.trachomatis* infection is an important causative agent of miscarriages in women. *C.trachomatis* infection diagnostic procedures should be considered in screening tests during pregnancy.

Key words *Chlamydia trachomatis*, RSA, ELISA, Leukocytes esterase

Introduction

The increased risks of viral and intracellular bacterial infections suggest that there is reduced Th1 cell activity against pathogens during pregnancy because of the Th1 cytokines are important for continuing pregnancy, the shift away from Th1 cells is consistent with this increased risk of maternal infection due to intracellular organisms, the more sever risk to the fetus [(1)]. Although sporadic pregnancy loss has been associated with such organ-isms as *Ureaplasma urealyticum, Mycoplasma hominis, Chlmydia trachomatis*, TORCH (*Toxoplasma gondii,* rubella, human cytomegalovirus and herpes) there is no convincing association with repeated miscarriage. The mere presence of an organism at the time of the loss can not be assumed to be proof of cause [(2,3)].

Bacterial vaginosis, which refers to an imbalance in the polymicrobial vaginal flora, is more commonly associated with mid-trimester losses [(4,5)]. Lower genital tract infection with *Chlamydia trachomatis* is currently the most commonly diagnosed sexually transmitted disease, *Chlmydia trachomatis* infection is an important causative agent of miscarriages in women [(6,7)]. However there are also investigations that were unable to

prove any relationship. More recently it has been shown that only women with evidence of recent infection were at a higher risk of developing premature rupture of membranes and preterm labor [4]. Others postulated that an immune response to an epitope shared by a Chlamydial and a fetal antigen is responsible for recurrent miscarriage [8].

Hence this study was designed to study was the frequency of *Chlamydia trachomatis* (C.t.) infection among women who experienced recurrent spontaneous abortion.

Methods

One hundred and nineteen women attending the Obstetrics and Gynecology department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of this study. They comprised 62 pregnant ladies all of whom gave a history of previous 3-6 consecutive abortions. (Recurrent spontaneous abortion; RSA) (groupA); non-RSA(first and second abortion)(groupB) included 34 pregnant ladies ,and 23 pregnant ladies(full term) had at least two previous normal pregnancies as a control group(groupC).

Sample collection

Blood: Five ml of venous blood was collected from each patient and control group. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at 4^{o}C at 450 x g for serum collection. The serum was then aspirated by using a Pasteur pipette and dispensed into sterile glass tubes (1 ml in each) and stored at -20 ^{o}C until used.

Urine: A mid stream urine specimen was collected in a sterile container; External and preineal area were cleaned, washed thoroughly and dried before collecting the specimens. These samples were used for strip test. These urinalysis test strips including Leukocytes esterase are simple, easy to use reagent strips for the detection of key diagnostic chemical markers in human urine

Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of *Chlamydia trachomatis* /IgM (NovaTec Immundiagnostica Gmb H. Germany), the test was done according to the manufacture instructions.

Statistical analysis: - The ANOVA analysis program **was used.**

Results

As shown in table 1, the current study investigated the possible existence of acute *C. trachomatis* infections among the three patient's groups based on IgM antibody detection assay. Accordingly, group A gave 16.1% positive reactive and group B showed 29.4% positive finding while group C gave 100% negative reaction.

Table 1. Prevalence of acute infection *C. trachomatis* in studied groups

Variable	Result	Groups						Total	Chi-Square *P* value
		A		B		C			
		No.	%	No.	%	No.	%		
C.trachomatis **(IgM)**	Negative	50	80.6	24	70.6	23	100	97	0.034*
	Equivocal	2	3.2	0		0		2	
	Positive	10	16.1	10	29.4	0		11	
Total		62		34		23			

*=significant difference (p<0.05)

Interestingly, the current study showed a highly significant positive correlation (r=0.401, *p*<0.001) between *C.trachomatis* acute infection and urine level of leukocyte esterase, as shown in Figure 1.

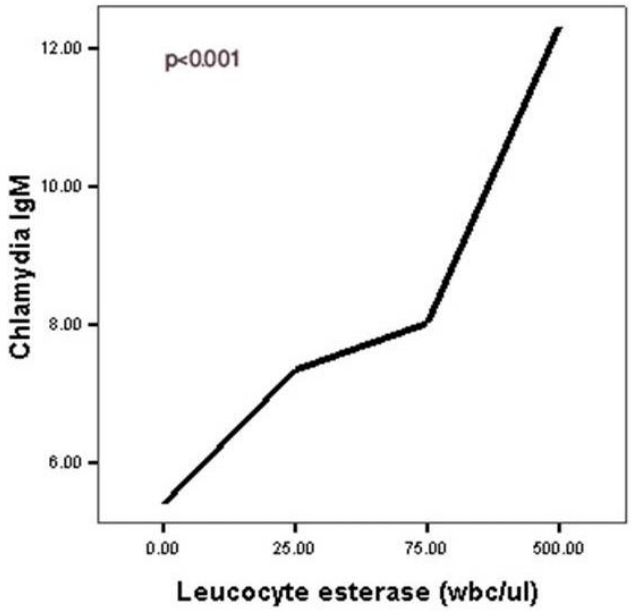

Figure 1. Correlation between *Chlamydia trachomatis* and leukocyte esterase

The ANOVA test analysis in table 2 shows significant difference (*p*<0.05) in the mean of *C.trachomatis* infection between group A (RSA) and group C (successful pregnancy), and a highly significant difference (*p*<0.001) between group B (non-RSA) and group C. In addition, the data showed marginally significant difference (*P*<0.05, *p*<0.1) between the mean value of *C.trachomatis* infection in group A and B (6.4±0.4 and 7.8± 0.6, respectively).

Table 2. Comparison of acute *C.trachomatis* infection in studied groups

Variable	Group	No.	Mean ± SE	F test *P* value	Significance between groups	
					Group	*P* value
***C.trachomatis* (IgM)**	**A**	62	6.4±0.4	<0.01	A & B	0.055[a]
	B	34	0.6±7.8		A & C	0.030*
	C	23	0.6±2.9		B & C	0.000**

[a]= marginally significant difference (0.05>*p*>0.1); *=significant difference (*p*<0.05); **= highly significant difference (*p*<0.01); SE= standard error.

On the other hands acute *C.trachomatis* infections showed no significance difference (*p*>0.05) in the mean value of infection in first and second trimester abortion, but statistically significant difference (*p*<0.05) in the mean value was found between first trimester abortion (6.7±0.5) and control (4.6±0.6) and highly significant difference (*p*<0.001) between acute infection in second trimester abortion (7.2±0.5) and control (full term), as shown in table 3.

Table 3. Comparison between *C.trachomatis* infection in first, second trimester abortion and control

Variable	Group	No. (119)	Mean ± SE	F test *P* value	Significance between groups	
					Group	*P* value
***C.trachomatis* (IgM)**	1st	53	6.7±0.5	<0.05	1st -2nd	0.485
	2nd	43	7.2±0.5		1^{st}-C	0.016*
	C	23	4.6±0.6		2^{nd}-C	0.000**

*=significant difference (p<0.05); **= highly significant difference (p<0.01); SE= standard error; 1st= first trimester abortion; 2nd=second trimester abortion.

Discussion

Acute C.trachomatis infections showed no significance difference (p>0.05) in the mean value of infection in first and second trimester abortion. However, a statistically significant difference (p<0.05) in the mean value was obtained when compared between first trimester abortion and control, and highly significant difference (p<0.001) between second trimester abortion and control. This result agreed with the study done by Oakesshott and colleagues [4], that showed chlamydial infection associated with second trimester abortion.

It has been shown no significant correlation (p>0.05) between gestational age and acute infection with *C.trachomatis*. This result might indicate that in this study gestational age was not a risk factor in *C.trachomatis* infection. In the present study, there was a significant difference (p<0.05), in the serum level of *C.trachomatis* specific IgM among the three investigated groups. The prevalence of positive acute infection of *C.trachomatis* was 10/62 (16.1%) in group A (RSA) and 10/34 (29.4%) in group B (non-RSA). These results agreed with studies stated by [8,9] who showed a significantly high titers of chlamydial antibodies found in the sera of women with habitual abortion.

Also, it was found a significant difference (p<0.05) in the mean of *C.trachomatis* infection between group A (RSA) and group C (successful pregnancy), and highly significant difference (p<0.001) between group B (non-RSA) and group C. In addition, the data showed marginally significant difference (0.05<p<0.1) between the mean value of *C.trachomatis* infection in group A and B (6.4±0.4 and 7.8± 0.6, respectively). Qublan [6] postulated that an immune response to an epitope shared by a Chlamydia and a fetal antigen is responsible for recurrent miscarriage. There were, however, no data available to confirm the role of intervention in improving the outcome of pregnancy. Interestingly, the current study showed a highly significant positive correlation (r=0.401, p<0.001) between *C.trachomatis* acute infection and urine level of leukocyte esterase as shown in figure 1. This result agreed with study of O'Brien et al [10], which utilized leukocyte esterase dipstick to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* urethritis in asymptomatic adolescent male detainees, they further explained that detection of leukocyte esterase as 100% sensitive, 83 % specific, and 54 % predictive for the presence of either organism.

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