



Relationship between Chlamydia trachomatis infection and Repeated Abortion in Iraqi pregnant Women

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ABSTRACT

Background: Chlamydia trachomatis (*C. trachomatis*); was even a little reason for the frequent abortion in women.

Objective: The study aimed to know the relationship between *C. trachomatis* infection and women who suffer from repeated abortion.

Methods: A total of 104 women, ages ranging from (15-50) years, were enrolled in the current studies, and each patient and control blood samples were collected. And estimation of IgG and IgM levels against *C. trachomatis* in sera of patients was done using ELISA method.

Results: Based on the ELISA screening assay, the level of circulating *C. trachomatis* -specific IgG and IgM antibodies between the patients is 4.8%. of total infections in three Iraqi cities, there is a relation between age and repeated abortion. In most abortion cases, more than 46 years old in 15.3%, and the abortion in pregnant women in the first period of gestation with three positive and the result shows there is no relation between abortion and area of life.

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.

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INTRODUCTION

Because cytokines are necessary for maintaining pregnancy and the increased risks of viral and intracellular bacterial infections imply reduced cell activity against pathogens during pregnancy, the shift away from Th1 cells is consistent with this increased risk of maternal infection from intracellular organisms and the more serious risk to the fetus.¹ Although organ-isms like Urea plasma unrealistic, Mycoplasma hominis, Chlamydia trachomatis, and TORCH (Toxoplasma gondii, rubella, human cytomegalovirus, and herpes) have been linked to sporadic pregnancy loss, there is no conclusive evidence to support such an association with repeated miscarriage. One cannot presume that the mere existence of an organism at the time of a loss proves its causation.^{2,3}

A polymicrobial vaginal flora imbalance known as bacterial vaginosis is more frequently linked to mid-trimester losses.^{4,5} The most prevalent sexually transmitted disease is now lower genital tract infection caused by Chlamydia trachomatis, and this infection is a major factor in female miscarriages.^{6,7} Investigations have occasionally failed to find a connection, though. More recently, it was discovered that the only women who had signs of a recent infection had a higher risk of early membrane rupture and preterm labor (4). Others hypothesized that recurrent miscarriage is caused by an immunological reaction to an epitope shared by a Chlamydial and a fetal antigen.⁸

The purpose of this study was to determine how frequently women who had repeated spontaneous abortions were infected with Chlamydia trachomatis (C.t.). According to several research, C. trachomatis antibodies are more common in investigations of spontaneous.¹¹

MATERIALS AND METHODS

Specimen Collection

A total of 104 women from three provinces in Iraq were collected, and blood samples were taken from them. Eleven samples were from Martyr Muhammad Baqeri Al-Hakim hospital, Maternity Department in Baghdad city, 29 samples from Women's Hospital and obstetrics in Karbala city; and 12 samples from AL-Batool Hospital, Department of birth. In

Table 1: percentage of infection by *Chlamydia trachomatis* in three Iraqi cities.

City	No. of Sample	Positive		Negative		Percentage
		IgG	IgM	IgG	IgM	
Karbala	54	2	0	52	52	3.7%
Baghdad	25	1	0	24	24	4.1%
Diyala	25	2	1	22	24	8%
Total	104	5		99		4.8%

The normal value IgG Normal value The normal value IgM Normal value
 Positive / >11 NTU Positive / >15 NTU
 Equivocal/ 9-11 NTU Equivocal/ 14-15 NTU
 Negative/ < 9 NTU Negative/ < 9 NTU

Dayle city, December 2020 and May 2021 were the subjects of this study. They comprised 104 pregnant ladies, all of whom gave a history of repeated abortion the test on ELISA automatic systems, we recommend increasing the washing steps from three to five and the volume of washing solution from 300µL to 350µL to avoid washing effects. Before commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Please select the required microtiter strips or wells and insert them into the holder.

Sample Dilution

All samples must be diluted 1+100 times with IgG Sample Diluent before analysis. To obtain a 1+100 dilution, dispense 10L sample and 1mL IgG Sample Diluent into tubes, then thoroughly mix with a vortex.

Procedure

Before beginning the test run, it is crucial to bring all reagents, samples, and controls to room temperature (20–25°C).

- Snap-off strips with coating *C. trachomatis* antigen are coated on the break-apart snap-off strips that are ready for use. Keep items at 2...8°C. As soon as the strips are removed, the remaining strips should be resealed in aluminum foil with the provided desiccant and kept at 2...8 °C; stability until the expiration date.
- IgG conjugate of *C. trachomatis* Anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives, and an inert blue dye are all included in the 20 mL solution in the bottle. The answer is available for use. Keep items at 2...8°C. Stability from the initial opening up to the expiration date when kept at 2...8°C.
- Controls An already prepared control solution can be found in the bottles marked Positive, Cut-off, and Negative Control. It must be kept between 2 and 8°C and contains 0.1% Kathon. Stability from the initial opening up to the expiration date when kept at 2...8°C.
- Sample IgG Diluent 100 mL phosphate buffer, stabilizers, preservatives, and an inert yellow color are all present in the bottle. For diluting the patient specimen, it is employed. Store this ready-to-use solution between 2 and 8 degrees Celsius. Stability from the initial opening up to

Table 2: percentage of infection by *Chlamydia trachomatis* in three Iraqi cities After 14 days from the first test.

City	No. of Sample	Positive		Negative		Percentage
		IgG	IgM	IgG	IgM	
Karbala	54	2	0	52	52	3.7%
Baghdad	25	1	0	24	24	4.1%
Diyala	25	2	1	22	24	8%
Total	104	5		99		4.8%

The normal value IgG Normal value The normal value IgM Normal value
 Positive / >11 NTU Positive / >15 NTU
 Equivocal/ 9-11 NTU Equivocal/ 14-15 NTU
 Negative/ < 9 NTU Negative/ < 14 NTU

Table 3: Relationship between Age and number of infections with *Chlamydia trachomatis*.

Age of patient	No of sample	Positive sample	Negative sample	Percentage%
(15-25)	49	0	49	0%
(26-35)	24	1	23	4.1%
(36-45)	18	2	16	11.1%
More than 46	13	2	11	15.3%
total	104	5	99	4.8%

the expiration date when kept at 2...8°C.

- A cleaning agent (20xconc.), a concentrated buffer, detergents, and preservatives total 50 mL in the bottle. Washing Solution 1+19 should be diluted; for example, 10mL Washing Solution + 190 mL fresh, redistilled water should be used. At room temperature, the diluted buffer is stable for 5 days. When the solution is heated in a water bath to 37°C, the crystals in the solution vanish. The concentrate remains stable after the initial opening till the expiration date.
- TMB Substrate Solution, sixth Tetramethylbenzidine/hydrogen peroxide system in 15 mL container. The reagent must be stored at 2...8°C, away from light, after it is ready for use. The mixture must be colorless or it could have a little blue tint. The substrate should be discarded if it turns blue because it may have become polluted. Stability from the initial opening up to the expiration date when kept at 2...8°C.
- Stop the Problem 15 mL of a 0.2 M sulfuric acid solution (R 36/38, S 26) is contained in the container. Store this ready-to-use solution between 2 and 8 degrees Celsius stability following first opening through expiration.

Statistical Analysis

The ANOVA analysis program was used.

RESULTS AND DISCUSSION

104 sample was taken from a woman with repeated abortion history in three Iraqi cities, Karbala, Baghdad, and Diale. The sample was transported to Karbala gynecology hospital to make an ELISA test, and results were as follows. Blood five ml of venous blood was collected from each patient, and 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. The serum was stored at -20 o C until used. Enzyme-Linked Immune Sorbent Assay (ELISA) for the detection of *Chlamydia trachomatis* /IgG and IgM, the test was done according to the manufacturer's instructions.

The result is shown in Table 1. A 4.8% infection by *Chlamydia trachomatis* in three Iraqi cities, Karbala, Baghdad, and Diyala in arrangement, also showed 3.7% in Karbala, 4.1% in Baghdad, and 8% in Diyala. The test was done by detection of IgG and IgM antibodies in the blood of women with a history of repeated abortion.

Table 4: Relation of infection *Chlamydia trachomatis* and the gestation period.

Period of abortion	No. of abortion	Positive	Negative	Percentage
First gestation period	65	3	62	4.6%
Second gestation period	22	2	20	9%
Last three months	17	0	17	0%
Total	104	5	99	4.8%

Table 5: Relation between infection *Chlamydia trachomatis* and geographical area.

Region	City	No. sample	Positive	Negative	Percentage%
Elaskary area	Karbala	3	1	3	33.3%
Elamel area	Karbala	6	0	6	0%
Elantafada area	Karbala	1	0	1	0%
Elsamod area	Karbala	1	0	1	0%
Imam Ali area	Karbala	1	0	1	0%
Elghdir area	Karbala	1	0	1	0%
Elmowazafin area	Karbala	1	0	1	0%
Elhur area	Karbala	4	1	4	25%
Almorakazeyah	Karbala	1	0	1	0%
Elbana elgahes	Karbala	1	0	1	0%
Elmamelgy	Karbala	1	0	1	0%
Elnasr area	Karbala	2	0	2	0%
Alabbasiyah	Karbala	1	1	0	0%
Alatarat	Karbala	1	0	1	0%
Al salaam area	Karbala	1	0	1	0%
Durra complex	Karbala	1	0	1	0%
Alaskan	Karbala	1	0	1	0%
Al sholah	Baghdad	6	0	6	0%
Elbanok area	Baghdad	3	1	3	33.3%
Om Nagem area	Baghdad	2	0	2	0%
Ibrahim River	Diyala	11	2	10	18.2%
Alkalis	Diyala	1	0	1	0%
Total		104	5	99	4.8%

The test was repeated after 14 days to confirm a diagnosis and the result shown in Table 2 is 4.8% infection by *Chlamydia trachomatis* in three Iraqi cities, Karbala, Baghdad, and Diyala in arrangement, also shown 3.7% in Karbala, 4.1% in Baghdad and 8% in Diyala. The test was done by the detection of IgG and IgM antibodies. The test was repeated to know the titration of antibodies in the blood, and the level of antibodies remained constant because the infection old.

Also, the result (Table 3) shows the most age period of women infected with *Chlamydia trachomatis* in the age period (45-50) 15.3%, then patients with age (35-45) 11.1% and the age between (26-35) 4.1% and on infection in age period (15-25).

Table 6: Number of infections and presence of congenital malformation of the fetus

city	No. of infection <i>Chlamydia trachomatis</i>	Presence of congenital malformation infection <i>Chlamydia trachomatis</i>	malformation type	Per% Presence of congenital malformation in total infection
Diyala	2	1	Head deformity	50%
Karbala	2	1	Head deformity	50%
Baghdad	1	0	0	0%
Total	5	2		40%

The result in Table 4 shows the relation between infection with *Chlamydia trachomatis* and the period of gestation, so most abortion in the first period of gestation. There are three positive abortion cases with pregnant women. Then the second period of gestation, there are two positive abortion cases, and there is no infection in the third period of pregnancy.

The result in Table 5 shows there is no relation between the infection *Chlamydia trachomatis* and geographical area; there is one positive case in each different area in Karbala, also this same in Baghdad and in Diyala, Nahr Ibrahim it is a farming area with two infected case although a small number of sample.

Table 6 result shows congenital malformation in Kerbala and Diyala city with Head deformity (neural defect tube), While there is only abortion in Baghdad city.

DISCUSSION

We discovered a correlation between *C. trachomatis* infection and abortion ($p = 3.8\%$) in three cities in Iraq. A prolonged, asymptomatic *C. trachomatis* infection that spreads to the fetal tissue or endometrium may cause miscarriage. The majority of patients had *C. trachomatis*- positive serology, even though relatively few miscarriages happen during *C. trachomatis* primary infection can be brought on by chlamydial damage or chronic *C. trachomatis* antibodies that could obstruct the development of embryonic antigens.¹² A prolonged, asymptomatic *C. trachomatis* infection that spreads to the fetal tissue or endometrium may cause miscarriage. The absence of a connection between miscarriage and IgA is explained by the fact that *C. trachomatis* primary infection causes relatively few miscarriages. Miscarriage may occasionally be brought on by damage from past chlamydial infection or residual *C. trachomatis* antibodies that may interfere with embryonic antigens, as several individuals had *C. trachomatis*- positive serologic results without *C. trachomatis* DNA.¹³

It appears that variations in the findings regarding the link between *C. trachomatis* and abortion depend on the type of sampling, diagnostic techniques, sexual behavior, hygiene, the use of contraception during sexual activity, coinfection

with viruses or other microbes, treatment practices, and population.¹⁴

Syphilis and gonorrhea are two other sexually transmitted illnesses (STIs) that are dropping globally due to identification and treatment, but new infectious agents like *C. trachomatis* have taken their place. Early *C. trachomatis* infection detection and treatment may lower the risk of preterm birth as well as perinatal morbidity and mortality.¹⁵

Age, irregular sexual activity and having several sexual partners, failure to use or irregular use of a barrier during intercourse, and a lack of information about sexual health have all been determined to be the main risk factors for *C. trachomatis* infection. Our findings increase understanding of *C. trachomatis* identification in high-risk populations and community screening.¹⁶

The prevalence of *C. trachomatis* varies depending on the population being studied and the testing procedure's sensitivity. According to a recent study, *C. trachomatis* should be checked on all women who have had abortions. Asymptomatic women with a higher chlamydial positive level in our study should have a higher index of suspicion. Lacking the necessary resources and expertise for direct fluorescence testing and cultivation, ELISA can be very helpful in detecting *C. trachomatis* in infertile women using the direct fluorescent test. It is advised to screen abortion patients for *C. trachomatis* in the first year of the procedure itself so that early therapeutic intervention can be put in place to enable women to conceive naturally.¹⁷ The extent of abortion brought on by *C. trachomatis* in Iraq should be further clarified by studies with bigger sample sizes.

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