



## Seropositivity of Chlamydia Trachomatis among Iraqi Pregnant Women in Al-Najaf Governorate.

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### Abstract

**Background:** Chlamydia trachomatis infection is an upper genital tract infection that can be found in pregnancy and lead to subsequent harm on the outcome.

**Aim of study:** To determine the seroprevalence rates of immunoglobulin G (IgG) and immunoglobulin M (IgM) to Chlamydia trachomatis in Al- Najaf Iraqi pregnant women.

**Design of study:** A prospective cross sectional study.

**Patients and Method:** Using enzyme-linked immunosorbent assay (ELISA), a total of 200 serum samples from pregnant women were tested for antibodies to Chlamydia trachomatis known to cause a variety of clinical syndromes in women and newborn infants.

**Results:** Chlamydia trachomatis IgG antibodies were detected in 17(8.5%) and IgM antibodies were found in 9 (2.18%) of different age groups, peak incidence was around age groups between 20-30 years old 52.38%.

**Conclusion:** Pregnant Iraqi women in al-najaf have low prevalence rate of Chlamydia trachomatis IgG antibodies and lower prevalence for Chlamydia trachomatis IgM.

### Introduction

Chlamydia trachomatis is one of the most commonly diagnosed bacterial sexually transmitted infections worldwide, with over 100 million cases estimated annually [1]. Chlamydia is caused by biovars of the bacterium *C. trachomatis* that primarily infect the genital tract, with transmission occurring through vaginal, anal, or oral sex [2-3]. While often asymptomatic, untreated chlamydial infections can lead to serious reproductive health consequences in women, especially those in their reproductive years [4].

When chlamydia infects the cervix, it can ascend through the reproductive tract and cause pelvic inflammatory disease (PID) [5]. PID occurs in 10-15% of untreated chlamydial infections and can damage the fallopian tubes, ovaries, and other pelvic structures [5]. This may result in chronic pelvic pain, ectopic pregnancy (when fertilized eggs implant outside the uterus), and tubal factor infertility. Studies have found that up to 40% of women with a history of PID experience long-term pelvic pain, with 10-20% eventually developing clinical manifestations of tubal factor infertility [6-7].

Pregnancy represents an important opportunity to screen for and treat chlamydial infections in order to prevent potential harm to both mother and developing fetus [8]. If a mother is infected but untreated during pregnancy, chlamydia can transmit to the newborn during vaginal delivery, causing neonatal conjunctivitis or pneumonia in approximately 50% and 10% of cases, respectively [9]. Additionally, some research suggests chlamydial infection may be associated with preterm birth and low birth weight, though the data remains inconclusive [10-14]. While national guidelines recommend screening all pregnant women for chlamydia at their first prenatal visit, the true burden of infection during pregnancy is not fully understood [15]. Many studies have relied on nucleic acid amplification tests (NAAT) that detect active

infection but cannot account for prior, treated exposures [16-18]. Seroprevalence studies measuring antibodies to *C. trachomatis* may provide a more comprehensive picture of exposure history [19]. However, few such studies exist specifically within pregnant populations. Better characterizing chlamydia risk factors among childbearing women has the potential to guide efforts addressing disparities and optimizing screening practices [20]. This study aimed to determine the seroprevalence rates of immunoglobulin G (IgG) and immunoglobulin M (IgM) to *Chlamydia trachomatis* in Al-Najaf Iraqi pregnant women.

## Patients and Method

A prospective Cross-sectional study on pregnant women attending the labour room at term in Al-Zahraa teaching hospital, in Al-Najaf, Iraq, between May and September 2011 were included in the survey. These women were from different socioeconomic classes and educational status. The study was done Cross-sectional where a history was taken from each woman including details of age, educational state, obstetrical history including abortion and symptoms arising during pregnancy mainly discharge. A total of 200 conveniently selected Iraqi term pregnant women were included. The age range of the patients was 16-40 years. A 5ml clotted blood sample was obtained from each patient after obtaining informed consent. Serum was separated, aliquoted into one tube and tested within less than 5 days and stored 2-8°C in the laboratory of the hospital.

## Principle of the Assay

The qualitative immunoenzymatic determination of IgM and IgG class antibodies against *C. trachomatis* is based on the ELISA technique. Microtiter strip wells are precoated with *C. trachomatis* antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase labelled anti-human IgG or IgM conjugate is added. This conjugate binds to the captured *Chlamydia* – specific antibodies. The immune complex formed by the bound conjugate is visualized by adding tetramethylbenzidine substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of *Chlamydia* –specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450nm is read using an ELISA microwell plate reader. The results for both ELISA tests were interpreted by calculating the antibody Index (AI) of each sample. This was determined by dividing the Optical Density (OD) value of each sample by the cut-off value. Specimens giving an AI value of less than 0.9 were regarded as negative. An antibody index of between 0.9-1.1 was considered a borderline positive or equivocal and the sample was repeatedly tested. Specimens with an AI of greater than 1.1 were considered positive for *C. trachomatis*.

## Statistical Analysis

This was done by using SPSS (Statistical Package for Social Sciences). In which we use chi-square( $X^2$ ) because it is categorical data. P-value <0.05 regarded as significant.

## Results

A total number of 200 sera were tested, only 17 (8.5%) sera were positive for IgG to *C. trachomatis* they were all negative to IgM. The remaining 183 sera only 4 (2.18%) were positive for IgM which indicate recurrent or current infection with the organism, this is shown in table -1. Regarding table -2- which demonstrates *C. trachomatis* positive IgG and IgM among different age group, the total sera positive was 21(IgG 17, 4 IgM). The peak incidence of cases was among age group between 20-30 years old, 9(4.5%) cases were positive for IgG antibody to *C. trachomatis*, however, at same age group there were to (1.09%) with positive IgM sera. On the other hand, table -3- showed that sera positive is higher among illiterate and primary school but higher educational state showed low prevalence 4(8.88%). While table -4- showed the percentage of discharge among women with positive sera, 5(23.8%) had discharge who were sera positive. While discharge was absent in patients with positive sera in 16(76.2%). This demonstrate that higher percentage were asymptomatic, which is consistent with non-pregnant population. Finally, table -5- demonstrated the percentage of foetal loss among those with positive sera 3(14.3%) only had positive history, the remaining sera positive 18(85.7%) with no history of foetal loss. All tables showed insignificant difference in comparison between sera positive and sera negative women because p-value more than 0.05.

**Table (1) shows the number and the percentage of women with sera positive IgG or IgM**

No. of women tested	POSITIVE IgG		POSITIVE IgM	
	200	17	8.5%	0
183		0	4	2.18%

**Table (2) chlamydia trachomatis with sera positive IgG and IgM among different age groups**

Age group	POSITIVE IgG		POSITIVE IgM		Total percent
	No.	%	No.	%	
<20	6	28.57	1	4.76	33.3
20-30	9	42.85	2	9.52	52.38
31-40	2	9.52	1	4.76	14.28

**Table (3) Chlamydia trachomatis with sera positive IgG and IgM among different educational state**

Educational state	total	Sera Positive for Chlamydia	Percent in total sera positive
Illiterate and primary school	69	9	42.85
Secondary school	86	8	38.09
Higher education	45	4	19.04
total	200	21	

$X^2=0.220$

$p=0.974$

**Table (4) shows number of patients with discharge among those with sera positive IgG and IgM**

Discharge	Total	Sera Positive for Chlamydia	Percent in total sera positive
present	24	5	23.80
absent	176	16	76.19
Total	200	21	

$X^2=3.099$

$p=0.078$

**Table (5) shows number of patients with previous history of foetal loss among those with sera positive IgG and IgM**

H <sub>x</sub> of foetal loss	Total	Sera Positive for Chlamydia	Percent in total sera positive
Present	37	3	14.28
Absent	163	18	85.71
Total	200	21	

$X^2=0.219$

$p=0.640$

**Table (6) shows the percentage of discharge among women with sera positive Chlamydia test**

History of discharge	Total	Sera Positive for Chlamydia	Percent in total sera positive
Present	37	5	23.80
Absent	163	16	76.19
Total	200	21	

## Discussion

The World Health Organization estimated that 89 million cases of *C. trachomatis* infection occurred worldwide [21]. The sequelae of *C. trachomatis* infections in women, namely pelvic inflammatory disease (PID), infertility and ectopic pregnancy, are the most costly outcome of any STD (except HIV/AIDS). Neonates usually become infected with *C. trachomatis* during birth. Conjunctivitis, pneumonia, myocarditis, otitis media and other diseases may develop in neonates born to mothers infected with Chlamydia [22]. The prevalence of *C. trachomatis* infection in pregnant women ranges from 2 to 35% worldwide [22-23]. In two studies, it was found that women with recent or invasive infection indicated by significant immunoglobulin M (IgM) antibody titers against *C. trachomatis* were at higher risk for preterm delivery and premature rupture of membranes [21]. In our study, 8.5% of the women were positive for IgG antibodies to *C. trachomatis* with. Only 4/183 (2.18%) as shown in table -1-. of the women were positive for *C. trachomatis* specific IgM antibodies. The low prevalence rate in our patient population may be due to the adherence of strict moral principles and code of ethics in Iraq. For comparison with other study made in the same way, a study made in Saudia Arabia in Makkah, 2006 where 1600 pregnant women were taken and the results were 8.7% of the women were positive for IgG antibodies to *C. trachomatis* and 1.5% of the women were positive for *C. trachomatis* IgM antibodies. There is some sort of similarity between Iraqi and Saudi Arabia social culture and this is why the results are approximated [24]. In other hand study was done in India by method of Antigen detection from Endocervical specimens in pregnant women showed

that 19% were positive which is about two folds of our result (table-1) [25]. Regarding signs and symptoms 27.7% were positive in patients with previous spontaneous abortion which is also about two folds of our result for foetal loss (table-5-). On the other hand, 16.6% were positive for vaginal discharge which is less than our result (table-6-). Finally, the peak incidence among age group of less than twenty years (33.3%) while in our study the peak incidence was between 20-30 years old (52.38%) and this is mainly because of extra marital relationships that started even before marriage like in this country. In UK study was done 2009 showed that the range of positive Chlamydia test in pregnant women is between 2-4% for Chlamydia, and this low percent may be due to early screening in the young age group and good antenatal care with screening test in first visit for each pregnant woman for Chlamydia [26]. In contrast, in the Nepal study done in 2015 showed that 17.8% of pregnant women were positive for Chlamydia and this is may be due to large number of immigrants and more sexual relationships among the population [26]. The ELISA test tends to have a lower sensitivity and Negative predictive value NPV, and more false negatives may be seen. Additionally, tests based on highly specific peptides may be so specific that they are not able to detect all relevant antigens [28]. Consequently, highly specific tests may not be able to identify all serotypes involved in Chlamydia infections thus causing false negative Chlamydia IgG antibody titer results. Previous Chlamydia infection frequently produces long –standing anti bodies that cannot be easily distinguished from anti bodies produced from current infection and this cause one of the limitations of ELISA test screening. However, I made my study by this test because it's objective, quicker, easy to perform and the last important cause it's available and not costly. More specific than the ELISA test is the micro immunofluorescence (MIF) test, which uses type specific antigens and is nowadays regarded as the “gold serological standard” to which other serological tests should be compared [28]. Recently, several enzyme-linked immunosorbent assays (ELISAs) have been commercially developed with *Chlamydia* recombinant antigens, some of them known to be *C. trachomatis* specific.

## Conclusion

In my study the percent of positive test to Chlamydia is low and could be less than this result if more accurate test has been done like NAATs. Current BASHH (British Association for Sexual Health and HIV) guidelines for Chlamydia screening do not recommend the use of Enzyme immuno assay, point of care test for diagnosis owing to their inferior sensitivity and specificity compared with NAATs and also the only test recommended for confirming a positive NAAT is a different NAAT. A closer attempt should be made to correlate risk factors and disease entity when screening for *C. trachomatis* and the choice of laboratory investigations. Thus, in the populations at high risk of the disease, it would be more effective to detect antigen especially in sexually active young women. In our country it is difficult socially to do this test before marriage; in addition to that the disease is low because of code of ethics in Iraq. However, the disease should be kept in mind in the antenatal care for each pregnant woman.

## Recommendation

There is statically difference ELISA and MIF technique, therefore, it is suggested that any ELISA positive IgG titer samples to be rechecked and reconfirmed by MIF method. NAATs are the recommended test for all population groups in UK because of high sensitivity and specificity and it is the test of cure in pregnant women.

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