Evaluation of Chlamydia Trachomatis Antibodies In Women with Infertility

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ABSTRACT

The obligate intracellular gram negative bacterium Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen worldwide, especially among young adults; infection with this agent can be asymptomatic in 80% of women, which can make diagnosis and detection of the bacterium difficult. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease (PID) lead not only to significant morbidity but far more importantly to infertility.

Fifty-two of infertile women (duration of infertility was approximately 1-15 years) with age ranged between (25-40) years (30 ± 4.12) and 50 of both unmarried women and mothers with age ranged between (10-30) years (28.6 ± 5.34) were studied for their antichlamydial antibodies levels using enzyme linked immunosorbent assay (ELISA). The study showed significant increase in serum IgM, IgG, IgA levels (P< 0.05) in (43.2%), (25%), (3.8%) respectively of infertile women as compared to control group; while the prevalence of antibodies in control were (10%), (4%), (2%) for IgM,IgG and IgA respectively. Also there was a high positive relation between these antibodies levels and the age of the range of (28-32) years in infertile women. The study showed high levels of C.trachomatis antibodies in both infertile and unmarried women also; we suggest that routine screening programs for C.trachomatis are needed to prevent the development of reproductive sequelae for women before marriage.

Key words: Chlamydia trachomatis, women infertility, antichlamydial antibodies, ELISA.
INTRODUCTION
The obligate intracellular gram negative bacterium *C. trachomatis* is the most common sexually transmitted bacterial pathogen worldwide, especially among young adults and the majority of pelvic infection caused by chlamydia is asymptomatic (1-3). Infection with this agent can be asymptomatic in 80% of women, which can make diagnosis and detection difficult (4-6). The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease (PID) lead not only to significant morbidity but far more importantly to infertility (3,7). Screening programs have been established in some industrialized countries to reduce the rate of PID and to prevent the development of reproductive sequelae (5).

The Center for Disease Control and Prevention estimate that 3 million people are infected annually with *C. trachomatis*, with 75% of infected women having few or no recognized symptoms (8). The bulk of infections remains undetected and untreated because most infected people are asymptomatic and do not seek medical attendance. If untreated, chlamydiae may reach the upper genital tract of affected women and cause PIDs with the risk of severe reproductive complications, such as tubal factor infertility and ectopic pregnancy (8-10). After one episode of PID, the ratio of infertility has been estimated at 11%, which increases to 23% and 54% after 2 and 3 episodes, respectively (11,12). Maternal–infant transfer of this disease occurs in approximately 23%–70% of infants born to infected mothers (13,14).

The above-mentioned hypothesis would initiate the concept that some of the microorganisms present in vagina cause disorder in sperm function which might lead to infertility in females. The role of infectious agent in infertility is not only due to creation of certain disorder in sperm function, but also infection in different parts of the female genital tract might induce infertility due to various reasons (15,16). That *C. trachomatis* infection not only affected fallopian tubes but also other genital tract sites; it might also affect ovarian function. An association between serum anti-*C. trachomatis* antibodies and low ovarian response to ovulation induction were detected also (17). Due to the higher prevalence of *C. trachomatis* infection infertile than fertile women and the importance of screening for this infection. This study was undertaken to evaluate antichlamydial antibodies by ELISA method in sera of infertile women.

MATERIALS AND METHODS
A total of 52 infertile women (who have no baby after 1-15 years of marriage) included in this study; aged between 25 and 40 years, mean±S.D. (30±4.12).
Questionnaire was made for those patients including name, age & duration of marriage.

Another healthy 40 mothers (fertile women) and 10 unmarried women; aged between 10 to 30 years mean± S.D. (28.6± 5.34) were also admitted in the study. All serum samples were collected from private laboratories, and the study was achieved in Biology department, College of Science, Baghdad University.

Three millimeters of blood samples were collected from patient and normal individuals in sterile plain tube. Serum was separated by centrifugation of the blood for 10 minutes at 1000 r.p.m. The serum was collected and stored at -20°C. Serum IgG, IgA and IgM levels were measured by Enzyme linked immunosorbent assay (ELISA); this was performed as described in (18).

Statistics
The data are analyzed using F-test; chi-square test ($x^2$) provided by SPSS statistical program. A $p$-value $\leq 0.05$ was considered statistically significant (19).

RESULTS AND DISCUSSION
The data in table 1 show the percentage of antichlamydial antibodies IgG, IgA, IgM in studied groups. Serum antichlamydial IgM index was the highest percentage among infertile women (42.3%) compared to 10% in controls; and the differences were statistically significant ($p<0.05$). Similarly; the antichlamydial IgG raised in 25% of infertile women, while controls showed only 4% positive IgG index. Finally, there was 3.8% of antichlamydial IgA index in infertile women compared to 2% in controls; but the differences were not significant ($p>0.05$).

The mean distribution of antichlamydial antibodies index in sera of infertile women and control are reported in table 2; it showed a significant elevation ($p<0.05$) of IgG and IgM index in sera of infertile women compared to the control; while there was no significant differences ($p>0.05$) in IgA index between the studied groups.

Figures 1 and 2 show the mean distribution of antichlamydial antibodies (IgG, IgA, IgM) index in sera of infertile women and controls according to age groups. When infertile women based age, were divided in to 4 groups (<28, 28-32, 32-36, 36-40) years old; we found that the most seropositive cases were in second age group (28-32) years followed by the first age group (<28) years.
Table-1: Percentage of antichlamydial antibodies (IgG,IgA,IgM) in studied groups (infertile and control).

<table>
<thead>
<tr>
<th></th>
<th>Infertile women</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive sera</td>
<td>No. of negative sera</td>
</tr>
<tr>
<td>IgG</td>
<td>13</td>
<td>39</td>
</tr>
<tr>
<td>IgA</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>IgM</td>
<td>22</td>
<td>30</td>
</tr>
</tbody>
</table>

Table-2: Mean distribution of antichlamydial antibodies (IgG,IgA,IgM) index in sera of the studied groups.

<table>
<thead>
<tr>
<th>Antichlamydial Ab. in studied groups</th>
<th>Mean ± S.D.</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG index</td>
<td>Infertile women</td>
<td>0.822±1.15</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0.489±0.37</td>
<td></td>
</tr>
<tr>
<td>IgA index</td>
<td>Infertile women</td>
<td>0.368±0.36</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0.383±0.21</td>
<td></td>
</tr>
<tr>
<td>IgM index</td>
<td>Infertile women</td>
<td>1.023±0.74</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0.557±0.39</td>
<td></td>
</tr>
</tbody>
</table>

Figure-1: Mean distribution of antichlamydial antibodies (IgG,IgA,IgM) index in sera of infertile women according to age group.
P= positive sera, N=negative sera
Chlamydial infections are highly prevalent infection and emerging as health problem in many countries of the world including Iraq. In our study, *C. trachomatis* infection found in a highly seropositive antibody index. We found a significantly higher percentage of IgM and IgG antibodies against *C. trachomatis* among women suffering from infertility (42.3% and 25 %,) respectively compared with control groups (10% and 4 %,) respectively. When infertile women based age, were divided in 4 groups (<28, 28-32, 32-36, 36-40) years old; the most seropositive cases were found in second age group 28-32 years followed by the first age group <28 years. This high percentage of *C. trachomatis* infection was similar to that reported by several studies; they reported about 60% of infertile patients had seropositive antichlamydial antibody compared to fertile population who had 8% to 49% (20, 21). Also in a separated study in which detection of *C. trachomatis* particles by the method of direct immunofluorescence indicates that in infertile females 8.8% were positive while only 0.8% was positive in control group (14). Similarly, another study showed that 39.6% of seropositive antichlamydial antibodies ranged between 29-33 years age group (22).

Specific IgM antibodies have been associated with acute inflammation and recent infection of both IgM seropositive participants, while specific IgG and IgA antibodies reflect chronic inflammation and infection (23, 24). It has been suggested that serum IgA antibodies may be more reliable marker for persistent chlamydial infections (25). Polymerase chain reaction (PCR) testing revealed presence of *C. trachomatis* IgG in 8.6% of infertile women (3). While 32.4% were
seropositive for the IgG to *C. trachomatis* in another study (26). However, it is difficult to estimate whether the presence of specific IgG and IgA antibodies reflects an acute, chronic or past *C. trachomatis* infection because little is known how long specific antibodies may persist in individuals with resolved infections. This could indicate the most of the women with positive IgG and IgA antibodies might have become previously infected with *C. trachomatis*, for example as adolescents or young adults (12). Also, previous study showed that 56% of patients undergoing in vitro fertilization (IVF) had IgG antibodies anti *Chlamydia trachomatis* in serum (17). However, the incidence of *C. trachomatis* infection was more common in women with second infertility, this increased susceptibility could be due to their longer period of active sexual life; thus enhancing their exposure to chlamydial infection (27).

Our study suggests that all infertile women should be screened for *C. trachomatis*. The index of suspicion should be higher in asymptomatic women in whom our study revealed a larger chlamydial positivity. Also; Screening of infertile women for *C. trachomatis* is recommended in the first year of infertility so that early therapeutic intervention can be instituted to conceive naturally; and prevent development of reproductive sequelae for women before marriage. Further studies are needed to clarify the problem of *C. trachomatis* infection among adult females and the situation of immunity in our country.

**REFERENCES**


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