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Prevalence of the Anti-Chlamydial Antibodies in Infertile Women and Its Association with Tubal Factor Infertility

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Abstract

The aim of this study is to determine the prevalence of the antichlamydial antibodies in patients with tubal factor infertility and to investigate the role of antichlamydial antibodies as a screening test for tubal factor infertility. An observational case control study. Setting: Ain Shams Maternity University Hospital. Patients and methods: The study population consisted of 70 women of the reproductive ages (17- 40yrs), who had tubal factor infertility diagnosed by hysterosalpingogramor laparoscopy, and attended the infertility clinic of Ain Shams Maternity Hospital for evaluation of their fertility problems. 30 healthy women, of a similar age group who attended the antenatal clinic during the study period, were enrolled as a control group. All women were investigated for the presence of antichlamydial IgG antibodies in the serum by E.L.I.S.A. 21(30%) women had positive serum antichlamydial IgG in group I, while in the control group 2 (6.66%) women only were positive with highly significant difference between both groups. There was no significance correlation of serum antichlamydial IgG with age, or duration of infertility.

Keywords

Anti-Chlamydial Antibodies – Infertility

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I. Introduction

Tubal factor infertility is one of the main causes of involuntary childlessness in women [1]. Sexually Transmitted Diseases (STDs) are believed to play an important role in the increase of the infertility, particularly when it is caused by tubal factors [2]. Female infertility is attributed to the tubal factors in about 14- 38% of the cases. The tubal damage is presumed to be secondary to salpingitis, with a two-third of the subjects being asymptomatic while the remaining third present with symptoms. The C. trachomatis infection is the most common sexually transmitted infection worldwide, especially among young adults [3]. C. trachomatis, an obligate intracellular bacterium, is one of the most common sexually transmitted infections with 89 million new cases thought to occur globally per annum [4]. The obligate intracellular pathogen Chlamydia trachomatis belongs to the most common sexually transmitted bacterial organisms, worldwide. According to the Centers for Disease Control and Prevention (CDC), about one million reports of C. trachomatis infections occur annually among sexually active young people in the United States. Based on the antigenic reactivity of the major outer membrane protein, C. trachomatis is divided into 15 serovariants whereby the serovariants D through K typically cause nongonococcal urethritis in men and cervicitis in women [5]. The chlamydial infection produces less severe symptoms than other sexually transmitted infections [6]. The bulk of infections remains undetected and untreated because most infected people are asymptomatic and do not seek medical attendance. If untreated, chlamydia may reach the upper genital tract of affected women and cause pelvic inflammatory disease (PID) with the risk of severe reproductive complications, such as tubal factor infertility and ectopic pregnancy [5]. These deceptively mild symptoms allow the infection to go unnoticed, with minimal patient awareness, until the secondary or the tertiary symptoms develop. Serious sequelae like acute salpingitis and pelvic inflammatory disease often occur in association with repeated or persistent infections [7]. C.trachomatis may cause intraluminal adhesions, fibrosis, hydrosalpinx and pelvic adhesions. Due to the serious consequences of these conditions, the C. trachomatis infection can lead not only to significant morbidity, but it can also affect a woman's fertility. In addition, C. trachomatis-specific antibodies have been associated with tubal damage and infertility. In Europe, the reported incidence of Chlamydial infections has increased, some of which may be accounted for through increased testing and the availability of more sensitive tests, but may also reflect an increase in risk-taking behavior. In 2006, there was 112,473 C. trachomatis diagnoses identified from laboratory reports in England and Wales and 17,962 from Scotland [8]. Several studies have demonstrated that tubal factor infertility was significantly associated with the serum antibodies to C. trachomatis, which resulted in infertility [9]. A better understanding of the role of persistent C. trachomatis infections in

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tubal factor subfertility may be useful in optimizing the fertility work-up by incorporating screening tests for persistent C. trachomatis infections, aiming to accurately estimate the risk of persistence and identify those women who are at highest risk of tubal pathology. Due to the high prevalence rates of C. trachomatis and the asymptomatic course of infection, screening programs have been established in some industrialized countries to reduce the rate of PID and prevent development of reproductive sequelae. A large follow-up study (over 13000 participants) has evaluated the risk of subfertility following a positive C. trachomatis test on samples obtained from the cervix and/or urethra [10]. Serological testing of uncomplicated C. trachomatis infections of the lower genital tract has not been recommended, but antibody titers are especially high in women with PID. The infertility which was caused by C. trachomatis represented a preventable type of infertility, if it was detected early. Hence, the present study aims to evaluate the chlamydial infection in women who suffer from tubal factor infertility and to investigate the possible role of the chlamydial serology as a screening test for tubal infertility, by the detection of the anti-chlamydial IgG antibodies by using E.L.I.S.A. and study the benefit of using non-invasive methods to diagnose chlamydial infection.

II. Patients and Methods

Study population consisted of 70 women of the reproductive ages (17- 40yrs), who had primary or secondary infertility with tubal factor diagnosed by HSG or Laparoscopy, and attended the infertility clinic of Ain Shams Maternity Hospital for the evaluation of their fertility problem. Thirty healthy women of a similar age group who attended the antenatal clinic during the study period were the control group. Each patient had a recently done semen analysis for husband or was advised to have a recent one if a previous semen analysis revealed abnormal or subnormal results in one or more of the semen parameters, or if more than one year has elapsed since the last test, and to have tubal assessment reports of hysterosalpingography and /or laparoscopy and hormonal profile.

Exclusion criteria were those whose husbands had abnormal semen analysis parameters, cases with galactorrhoea, hirsutism or proved to have anovulatory cycles, cases with poor progesterone challenge tests, polycystic ovarian disease and abnormal hormonal profile, patient who took recent antibiotic within previous two months, patients with acute PID and patient with male cause of fertility.

A detailed history was obtained from these patients including age, marital status, occupation, duration and type of infertility, investigations and treatment received before. Menstrual and obstetric history was taken with special attention to spontaneous or induced abortions and the postoperative period. History also included detailed information about the contraceptive methods used, its duration, and complications, chronic pelvic pain, dyspareunia and chronic vaginal discharge. We asked also about history of treated pelvic infections. Complete examination of these patients was performed with special attention to the presence of pelvic mass, chronic cervicitis and purulent or mucopurulent endocervical discharge. All women were

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further investigated for the presence of serum antichlamydial IgG antibodies.

Specimen Collection and Preparation

Three millimiters of Blood were withdrawn. Serum was separated by centrifugation of the blood for 10 minutes at 1000 r.p.m. The serum collected and stored at -20°C till used.

The Enzyme Linked Immunosorbent Assay Test (ELISA) for the Detection of IgG Antibodies to Chlamydia trachomatis.

(A) Principle of the Test

The Chlamydia IgG ELISA, which is an enzyme immunoassay for the qualitative determination of IgG antibodies to human Chlamydia trachomatis, was used in this study. Strips of microtiter wells coated with Chlamydia antigen are incubated with control sera as well as with patient's samples. During this incubation, specific antibodies (if present in the patient's samples) are bound to the immobilized antigen. After removal of the unbound material by a washing procedure, the antigen-antibody complex in each well is detected with peroxidase-labeled anti human IgG antibody. After removal of the unbound conjugate by proper washing, the strips of microtiter wells are incubated with a substrate solution containing 0-phenyl-enediamine and hydrogen peroxide. A yellow-orange color develops, the depth of which is in proportion to the amount of Chlamydia-specific IgG bound to the wells of the micro titer strips. The enzymatic reaction is stopped by the addition of 2'N H2S04 and the absorbance values are determined at 492nm. The cut-off value is calculated taking into account the optic density values of positive and negative control sera. The presence of IgG antibodies to Chlamydia in patient's samples is detected by comparison of the sample's optical density value to the cut-off.

(B) Materials

la- Positive control serum (human serum with specific IgG antibodies to trachomatis.

Ib- Negative control serum (human serum devoid of specific IgG antibodies.

2- Washing buffer.

3- Specimen dilution buffer.

4- Antigen coated microtiter strips (1 plate with 12x8 strips coated with Chlamydia antigen).

5- Control antigen coated microtiter strip (1 plate with 12x8 strips coated with control antigen).

6- Conjugate diluent solution.

7- Conjugate (concentrated antihuman IgG peroxidase).

8- Chromogen (6 OPD enzyme substrate tablets).

9- Substrate buffer.

10- Stopping solution (2 N sulphuric acid).

11- Microtiter plate reader capable of measuring absorbance at 492nm.

(C) Assay Procedure

Before starting the procedure, all the reagents and specimens, are allowed to come to room temperature. Mixing of the reagents was done without foaming in a separate disposable tip for each sample transfer to avoid cross contamination. The procedure passed in the following steps:

1. The test strips were secured in the frame by placing two antigen coated strips and two control antigen coated strips next to each other.

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2. The strips were washed three times with diluted washing buffer allowing a soak time of one minute. The diluted washing buffer was prepared by adding 50 ml of the concentrated washing buffer to 1000 ml distilled water.
3. The moist microtiter plate was left upside down on absorbent paper.
4. Patients' sera were diluted to 11300 with the working specimen diluent solution in clean glass test tubes. The working specimen diluent solution was prepared by dilution of the concentrated specimen solution buffer 1/20 times with the diluted buffer.
5. 100 m1 of the control sera and diluted patient samples are pipette into 4 adjacent wells (2 wells of specific antigen and 2 wells of control antigen).
6. The microtiter plates were then incubated for 60 minutes at 37°C in a moist atmosphere.
7. The conjugate solution was prepared 5 to 10 minutes prior to the end of the incubator. It was prepared by diluting the enzymatic conjugate with the ready to use conjugate diluents solution. The exact dilution rate is indicated on the label of the conjugate vial.
8. After the end of the first incubation, the microtiter plate was inverted over a suitable container and briskly shacked to evacuate the contents. The plate was then washed as described under 2 and 3.
9. 100 ml of the conjugate solution was then added to each well.
10. The microtiter plate was incubated again for 30 minutes at 37°C in a moist atmosphere.
11. Toward the end of this second incubation, the substrate solution was prepared by adding 1 OPD tablet to 6 ml substrate buffer.
12. The washing step was repeated as described in 2 and 3.
13. 100 ml of the substrate solution was dispensed into each well.
14. A third incubation, for 20 minutes at room temperature with avoidance of light exposure, was done.
15. 100 ml of the stopping solution was added into each well, following the same sequence and in the same time interval used to dispense the substrate.
16. Within 30 minutes after addition of the stopping solution, the absorption value was read at 492 nm.
17. The value obtained for each serum, both with the specific antigen and the control antigen, was recorded.

(D) Reading and Interpretation of the Results

- To accept the test, the mean absorbance value (A) for the positive reference serum against antigen must be higher than 0.7, and (A) for the negative reference serum against antigen must be below 0.2.
- To calculate the cut-off value, we added the mean absorbance values of positive (P) and negative (N) reference sera against specific antigen. This value was then multiplied by the K coefficient as indicated on the label of the reference sera. The value obtained in that way represented the cut-off value that was used in the test.
- The cut off value obtained was 0.45 i.e. values higher or equal to 0.45 were considered positive while lower values were considered negative.

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STATISTICS

Data were statistically represented in terms of range, mean and standard deviation (±SD) and percentage. Comparison between different groups in the present study was done using student t-test for comparing parametric data with 2 groups and using ANOVA test for when groups 'were more than 2. For comparing non parametric data, Chi square test was performed. Yates correction was used instead when the frequency is less than 10. Correlation was done using the Pearson correlation coefficient (r) and was represented graphically using the linear regression curve. A probability value (p value) less than or equal 0.05 was considered significant. All statistical calculation were done using computer programs Microsoft Excel version 5 and SPSS (Statistical Package for the Social Science) statistical program. The sample size was calculated at N>61 based on the following: 95% confidence limit, 80% power of the study and expected prevalence of chlamydia antibodies among infertile women of 20-30%. Approval of the ethical committee of Ain Shams Maternity Hospital was obtained.

III. Results

This observational case control study involved 70 women of the reproductive ages (17-40yrs), who had tubal factor infertility diagnosed by HSG or laparoscopy, and attended the infertility clinic of Ain Shams Maternity Hospital for evaluation of their fertility problems. 30 healthy women, of a similar age group who attended the antenatal clinic during the study period, were enrolled as a control group. All women were investigated for the presence of antichlamydial IgG antibodies in their serum by E.L.I.S.A.

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Table (1): The values and Standard Deviation of the ages of infertility and control cases:

	Infertility cases	Control cases
Total No.	70	30
Max. Age	40	40
Min. Age	17	17
Mean Age	26.37	27
SD±	5.13	6.79
P (t-test)	**0.613 (Non-significant)**	

Table (1) shows that the maximum age of patients in both the infertility and control group was 40 years. The minimum age of both groups was 17 years. The Mean age of the infertility group was 26.27 years and 27 years for the control group. The standard deviation for the infertility group was 5.13 and 6.79 for the control group. The P-value for the result was 0.613. There was no significant difference between case & control as regards the age.

Table (2): Distribution of infertility cases according to age groups:

Group	Number of Patients	Percent (%)
< 20 years	9	12.86%
21-30 years	42	60 %
31-40 years	19	27.4%
Total cases	**70**	**100%**

Table (2) shows that the distribution of infertility cases according to age, revealed that 12.86% were < 20 years old, 60% were between 21-30 years and 27.4% were between 31-40 years.

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Table (3): Distribution of infertility cases according to the duration of infertility:

	Number of Patients	Percent (%)
< 3 years	33	47.14%
≥ 3 years	37	52.86%
Total cases	**70**	**100%**

Table (3) shows that distributing the infertility cases according to duration of infertility taking the 3 years duration as a cut-off value, gave rise to two groups: a group of duration of infertility of less than 3 years was 47.4% of cases while those of equal or more than 3 years duration of infertility was 52.86% of cases.

Table (4): The values of duration of infertility among infertility cases:

	Duration
Max. Duration	**7**
Min. Duration	1
Mean	2.76
SD±	1.29

Table (4) shows that maximum duration of infertility in infertility patients studied was 7 years and minimum duration was one year with a mean of 2.76 years and a standard deviation of 1.29 years duration.

Table (5): Distribution of patients according to type of infertility:

	Number of Patients	Percent (%)
Primary infertility	46	65.71%
Secondary infertility	24	34.28%
Total cases	**70**	**100%**

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Table (5) shows that 65.71% of cases had primary infertility while 34.28% of cases had secondary of infertility.

Table (6): The values and Standard Deviation of Antichlamydial IgG between cases and control:

	Infertility cases	Control cases
Max. IgG	2.100	1.332
Min. IgG	0.103	0.103
Mean IgG	0.534	0.214
SD±	0.599	0,243
P (t-test)	0.000 Highly significant	

Table (6) shows that comparing the antichlamydial IgG values between the cases and the control was highly significant P 0.000. i.e High significant difference between both groups (case & control) as regard IGg level.

Table (7): Frequency of Antichlamydial IgG among infertility cases:

	Number of Patients	Percent (%)
Positive IgG cases	21	30%
Negative IgG cases	49	70%
Total cases	70	100%

Table (7) shows that 30% of patients with tubal infertility had antichlamydial IgG for Chlamydia trachomatis in their serum, compared to 70% who did not show antichlamydial IgG for Chlamydia trachomatis in their serum.

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Table (8): Frequency of Antichlamydial IgG in the control group:

	Number of Patients	Percent (%)
Positive IgG cases	2	6.66%
Negative IgG cases	28	93.33%
Total cases	30	100%

Table (8) show that 6.66% of the control group had antichlamydial IgG in their serum compared to 93.33% who did not show antichlamydial IgG in their serum.

Table (9): Correlation of Antichlamydial IgG to age groups in group I:

*** Patients < 20 years age:**

	Antichlamydial IgG	
	Value	Result
Mean	0.384	
SD±	0.476	
No. of +ve cases		3
No. of -ve cases		6

*** Patients 20 - 30 years age:**

	Antichlamydial IgG	
	Value	Result
Mean	0.542	
SD±	0.622	
No. of+ve cases		12
No. of -ve cases		30

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*** Patients 31-40 years age:**

	Antichlamydial IgA	
	Value	**Result**
Mean	0.589	
SD±	0.618	
No. of +ve cases		7
No. of -ve cases		12

- P (ANOVA) 0.699 Non significant
- X2 0.549 Significant
- P (x2) 0.724 Non significant

Table (9) Shows that there was no significance correlation between the antichlamydial IgG in the serum of women in group I and their age. P 0.699, %2 0.549 (px2) 0.724.

Table (10): Shows percent of case and control patients with positive antichlamydial IgG.

	Positive IgG	Percent (%)
Case patients	21	30%
Control patients	2	6.66%
P (t-test)	**0.000 Highly significant**	

Table (10) shows that number of positive IgG patients from case group were 21 cases, (30% of the cases) and number of positive IgG from control group were 2 (6.66% of control patients). There was a high significant difference between both groups (case & control) as regards the percentage of positive IGg.

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Table (11): Correlation of Antichlamydial IgG to duration of infertility in infertility cases:

*** Duration of infertility < 3 years:**

	Antichlamydial IgG	
	Value	Result
Mean	0.479	
SD±	0.565	
No. of +ve cases		9
No. of -ve cases		24

*** Duration of infertility more than or equal 3 years:**

	Antichlamydial IgG	
	Value	Result
Mean	0.584	
SD±	0.632	
No, of +ve cases		12
No. of -ve cases		25

- P (t-test) 0.466 Non significant
- X2 0.044 Significant
- P (x2) 0.834 Non significant

Table (11) shows that there was no significance correlation between the antichlamydial IgG in the serum of the case group and their duration of infertility.

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IV. Discussion

Chlamydia trachomatis is a prevalent and virulent pathogen in the genital tract of sexually active women. In fact, Chlamydia trachomatis infection is now the most common bacterial sexually transmitted disease, with asymptomatic carrier rates in the general population of 2% to 5%. Although, many chlamydial infections of the lower genital tract in women are asymptomatic they can progress into the upper genital tract to produce serious complications as PID and tubal factor of infertility and tubal adhesions [6]. The initial evidence that Chlamydia trachomatis is an important agent in acute salpingitis came from European investigations predominantly Scandinavians, however the rate at which it occurs is not known. The first direct evidence of an association with Chlamydia trachomatis in acute salpingitis was documented by Eilard and colleagues who isolated Chlamydia trachomatis from tubal specimens, in 2 of 22 women with acute salpingitis undergoing laparoscopy [11].

C. trachomatis is a mucosal pathogen that establishes infection within epithelial cells in both the lower and upper genital tracts of women. Up to 70% of women with Chlamydia1 genital tract infection have silent infection and in the majority of all women with such an infection the disease is self-limited. This spontaneous cure is very suggestive of that a specific immunity is established [12].

In the current study 70 women were evaluated. They had tubal factor infertility whether unilateral or bilateral, whether primary infertility or secondary infertility, and were compared to a control group of 30 patients who attended the outpatient clinic and had no infertility problem. Twenty one patients out of 70 (30%) of the infertility group showed Chlamydia trachomatis infection as proved by the presence of antichlamydia IgG in their serum in relation to 2 out of 30 (6.66%) of the control group. This agreed with Ingrid et al. (2014) who reported that the IgG antibody in the serum of the infertile women in his study was found to be high [13]. Also Israel et al. (2011) reported that antibodies were present against Chlamydia trachomatis in 74% of patients, compared to 51% of the control group [14]. Whereas Broeze et al. (2011) demonstrated antichlamydial antibodies in 35% of infertile patients with tubal infection [15]. In a study carried out by Jorn et al. (2008) the seropervelance rate among women suffering from infertility was 39.3% [16].

Luke et al. (2006), found that 84 out of 210 (40%) of infertile women in the study were seropositive for Chlamydia IgG antibodies [17]. Another study for Hartog et al. (2004) showed that the prevelance of IgG Antibody was significantly higher in women with tubal pathology (54.2%), while the rate was (7.9%) in the women without tubal pathology [18]. Cohen et al. (2003), stated that 33 (47%) of 70 women with tubal infertility were seropositive [19]. Also Veenemans et al. (2002), tested 277 patient with tubal infertility and found that only 84 (30.3%) of them were seropositive for IgG with titre >1:32 [20]. Higher titres results were reported by EL-Shourbagy et al. (1996) who investigated genital Chlamydia infection incidence among high risk clinical conditions in Egyptian

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women [21]. It was a case control study in Ain Shams University Maternity Hospital involving 501 patients with cervicitis (n=58), abnormal cervical smear (n=256), tubal infertility (n-85), ectopic pregnancy (n=22), preterm labor (n=8) and 192 controls. Active cervical Chlamydia infection was diagnosed using direct immunofluorescent technique. Results reported significant increase of Chlamydia infection among different clinical conditions compared to controls. The percentage of positive Chlamydia infection was 79.3% among cervicitis group, 33.3% among subjects with inflammatory smear, 75.2% among those with cervical condyloma, 82.6% among those with cervical intraepithelial neoplasia, 1.8% among tubal infertility subjects, 77.2% among ectopic patients and 56.3% among subjects with preterm labor.

El-Shourbagy et al 1996 concluded that chlamydia infection in these high risk Egyptian patients was relatively high, empirical treatment was recommended as the diagnosis was costly and usually not available [21].

Another study was done by Ashish et al. (2012), a high seropositivity for the anti-chlamydial antibody in 60% of the patients. 52% of the females in the study showed bilateral tubal block mostly in the ampullary part [22]. Clude et al. (2011) reported that the prevalence of Chlamydia antibody were 90.9% of the cases and 19.9% of the control group [23]. In 2003 study by Sharma et al. (2003) enrolled forty women with tubal infertility (verified at hysterosalpingography and laproscopy), 20 women with infertility due to variety of other reasons and 20 healthy fertile women of reproductive age in a study. It was found that the presence of Chlamydia specific IgG antibody was significantly higher (70%) in women with infertility of tubal origin as compared to 35% seropositivity in healthy fertile women and 55% seropositivity in infertile women with cause of infertility other than tubal factor [24].

Morhason-Bello et al., 2014 reported that the prevalence of asymptomatic Chlamydia trachomatis was 20.5% [25]. May and Amer, (2012) concluded that the antichlamydial IgG was positive in 25% of infertile women, while controls showed only 4% positive IgG index [26].

Ficicioglu and Api (1995) concluded that tubal factor has a major role among causes of infertility but in its etiology Chlamydia trachomatis infection does not seem to play a major role [27].

By far the most effective method to prevent tubal disease infertility due to chlamydia is to prevent acquisition of the organism (primary prevention). If the organism has been acquired but remains at the level of the endocervix, eradication of the organism using effective antimicrobial therapy will also prevent tubal disease (secondary prevention). Prompt diagnosis of early chlamydial salpingitis may prevent extensive tubal damage and subsequent infertility (tertiary prevention). All these findings suggest that gynecologists should be aware of the importance of Chlamydia trachomatis in acute pelvic inflammatory disease.

In conclusion, the antichlamydial antibodies IgG testing is a simple, rapid and inexpensive test that can be used to detect such infection in patients with

infertility and thus adequate treatment can be prescribed for such patients.

V. Conclusion

It is recommended for the infertile women to perform routine screening for Chlamydia trachomatis for early treatment of those found to have positive C. Trachomatis antibodies. Such patients should proceed to tests for tubal patency.

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