Chlamydia Trachomatis Infection as a Risk Factor of Infertility in Women

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SBEB and HAH managed the analyses of the study. Author DGEK managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: *C. trachomatis* is known to cause damage to the female reproductive tract, primarily due to adhesions or obstructions of the fallopian tubes secondary to the inflammatory response. Tubal Factor of Infertility (TFI) is the main cause of infertility in 10-30% of cases in developed countries and *C. trachomatis* is the most common causative agent to TFI in the developed world. The aim of this study was 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.
Materials and Methods: This prospective randomized clinical study was conducted on a (50) women complaining of Infertility due to Tubal Factor .The patient was randomly classified into two groups: (50)Group I: complaining primary or secondary infertility. Group II: including (25) patients pregnant and came for Antenatal care
Results: Prevalence of different types of tubal factor in the study population was 12% in Distal block (DB), 10% Distal block plus peritubal adhesions (DB + PTA), 40% Hydrosalpinx (HS), 8%

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Hydrosalpinx plus peritubal adhesions (HS + PTA), 26% Proximal block (PB), 4% Peritubal adhesions (PTA). serum antichlamydial IgG level was significantly higher among group I (8.62 ±1.4) than group II (2.11±0.82). Also, 46% of group I had serum antichlamydial IgG positive vs. (12%) in group II with significant difference. Comparing positive *C. trachomatis* IgG with type of infertility, a total of 13 (57.7%) of those with primary infertility and 10 (42.3%) of those with secondary infertility tested positive. The difference was of no statistical significance (P=0.725).
Conclusion: We can conclude that, Chlamydia trachomatis is a major factor in female infertility especially for tubal factor of infertility, serum Chlamydia trachomatis IgG assay could be used a predictive factor for tubal factor infertility.

1. INTRODUCTION

Genital Chlamydial infection incidence among high risk clinical conditions in Egyptian women was 79.3% among cervicitis group, 33.3% among subjects with inflammatory smear, 75.2% among those with cervical condyloma, [1] 82.6% among those with cervical intraepithelial neoplasia, 51.8% among tubal infertility subjects, 77.2% among ectopic patients and 56.3% among subjects with preterm labor. (2) *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 [3]. The obligate intracellular pathogen *Chlamydia trachomatis* considered as the most common sexually transmitted bacterial organisms, worldwide [2]. 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 non-gonococcal urethritis in men and cervicitis in women [4]. The chlamydial infection produces less severe symptoms than other sexually transmitted infections [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 [6]. The precise mechanism through which the repeated infection elicits an inflammatory response that leads to tubal scarring and damage in the female upper genital tract, is not yet clear [7]. *C. trachomatis* is known to cause damage to the female reproductive tract, primarily due to adhesions or obstructions of the fallopian tubes secondary to the inflammatory response.

Reduced chances to achieve an intrauterine pregnancy is the result [8].

The aim of this study was 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.

2. SUBJECTS AND METHODS

This prospective randomized clinical study was conducted on a (75) women complaining of Infertility due to Tubal Factor. The patient will be selected from the department of Obstetrics and Gynecology, Tanta University Hospital. The patient will be randomly classified into two groups: Group I: including (50) patients complaining primary or secondary infertility. Group II: including (25) patients pregnant and came for Antenatal care. All patients were selected from outpatients clinic, Obstetrics and Gynecology department, Tanta University Hospital.

Exclusion criteria: Whose husbands had abnormal semen analysis parameters Abnormal (male cause of fertility?). Polycystic ovarian disease and abnormal hormonal profile. Diagnose to have Endometriosis by Laparoscopy. Abnormal hormonal profile. Uterine cause such as fibroid, uterine anomalies as uterine septum & adenomyosis diagnosed by 3D ultrasound or hystro-salpingography. Systemic diseases such as diabetes, hypertension, renal disease & systemic lupus. This study was carried out without any external funds. If the patient refused to complete the study, she was excluded and replaced by another one from who are fulfilling the inclusion criteria of the study. General and gynecological examination:

General examinations which included psycho-logical condition of the patient, body built, secondary sexual characters, pulse temperature, blood pressure, chest, breast, heart, abdominal and lower limbs examinations. Gynecological examination of the vulva, vagina, cervix.

Blood sample will be collected for: The determination of IgG antibody titer of ***Chlamydia trachomatis*** through ELISA assay procedure.

a) The required number of microtiter strips or wells were Selected and inserted into the holder.
  - 1well (e.g. A1) for the substrate blank, 1well (e.g. B1) for the Neg. Control, 2wells (e.g. C1+D1) for the Cut-off Control and 1well (e.g. E1) for the Pos. Control.
b) Dispense
  - 100 µL of Neg. Control into well B1
  - 100 µL of Cut-off Control into wells C1 and D1
  - 100 µL of Pos. Control into well E1 and
  - 100 µL of each diluted sample with new disposable tips into appropriate wells.
  - well A1 was Leaved for substrate blank!
c) wells were covered with foil supplied in the kit and Incubated for 60 minutes at 37 °C.
d) The contents of the wells were briskly shake out.
  - The wells were rinsed 5 times with diluted Wash Solution (300 µL per well). The wells were strike sharply on absorbent paper to remove residual droplets.
e) 100 µL Enzyme Conjugate was dispensed into each well, except A1.
f) Wells were covered with foil and Incubated for 30 minutes at room temperature (20 °C to 25 °C) Do not exposed to direct sun light.
g) The contents of the wells were briskly shake out.
  - The wells were rinsed 5 times with diluted Wash Solution (300 µL per well). The wells were strike sharply on absorbent paper to remove residual droplets.
h) 100 µL of Substrate Solution was added into all wells.
i) Wells were covered with foil and Incubated for exactly 15 minutes at room temperature (20°C to 25°C) in the dark.
j) The enzymatic reaction was stopped by adding 100 µL of Stop Solution to each well.
  - Any blue color developed during the incubation turn into yellow.

k) The optical density was readied at 450/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.
5. Results Calculation
  - Mean absorbance value of Cut-off Control (CO)
  - Calculated the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1). Example: (0.44 +0.46): 2 = 0.45 = CO
  - Results in DRG Units [DU].
  - Sample (mean) absorbance value x 10/CO = [DRG Units = DU]. Positive results more than 9 DU.

2.2 Statistical Analysis

The sample size was calculated using Epi-Info software statistical package created by World Health organization and center for Disease Control and Prevention, Atlanta, Georgia, USA version 2002. The criteria used for sample size calculation (n>33) were 95% confidence limit, 80% power of the study.

Analysis of data were performed by SPSS v25 (SPSS Inc., Chicago, IL, USA). Quantitative parametric variables (e.g. age) were presented as mean and standard deviation (SD). Pearson's rho coefficient of correlation (r) was used to calculate the degree of correlation between 2 variables. P value < 0.05 was considered significant.

3. RESULTS

As regards the women age ranged from 21 up to 40 years old with mean (29.2 ± 5.9) and BMI ranged from 20 up to 43 kg/m^2 with mean (29.3 ±4.3). Duration of infertility in group 1 ranged from 1 up to 17 years with median (interquartile range) 5.18 (3.14-7.42) where 40% 1ry infertility and 60% 2ry infertility. 54% of women had history of previous abdominal surgery in group I vs. 56% in group II with insignificant difference. Table 1.

Prevalence of different types of tubal factor in the study population was 12% in Distal block (DB), 10% Distal block plus peritubal adhesions (DB + PTA), 40% Hydrosalpinx (HS), 8% Hydrosalpinx plus peritubal adhesions (HS + PTA), 26% Proximal block (PB), 4% Peritubal adhesions (PTA) as in Fig 1.

According to serum antichlamydial IgG level was significantly higher among group I (8.62 ±1.4) than group II (2.11±0.82) (p<0.001). Also, 46% of

group I had serum antichlamydial IgG positive vs. (12%) in group II with significant difference (p<0.001). Figs. 2-3.

Comparing positive *C. trachomatis* IgG with type of infertility , a total of 13 (57.7%) of those with primary infertility and 10 (42.3%) of those with secondary infertility tested positive. The difference was of no statistical significance (P=0.725) Table 2.

3. DISCUSSION

Infection with *Chlamydia Trachomatis* is an important bacterial cause of sexually transmitted disease worldwide with extensive consequences for fertility resulting from damage to fallopian tube [8].

Chlamydia trachomatis infection (chlamydia) prevalence has remained high with an estimated annual number of infections of 130 million worldwide in 2012 [9]. Studies indicate that 10%–30% of women experience one or more chlamydia episodes.

In Egyptian women, the incidence of Chlamydial 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, 51.8% among tubal infertility subjects, 77.2% among ectopic patients and 56.3% among subjects with preterm labor [10].

Chlamydia trachomatis is a leading cause of sexually transmitted infection (STI) worldwide. C. trachomatis infection may lead to ascending infection, causing complications such as ectopic pregnancy and tubal factor infertility. However, not all women infected with *C. trachomatis* develop tubal damage. Women with normal fallopian tubes on laparoscopy or with normal fertility can have high titers of antibodies to *C. trachomatis*. It is unclear what determines whether a woman exposed to *C. trachomatis* will develop tubal pathology. Host immune responses to *C. trachomatis* infection are thought to contribute significantly to tubal damage [11].

Table 1. Demographic data, obstetric history, and surgical history

Variable	Group I(n=50)	Group II(n=25)	p-value
Age (yr)	29.17 ±5.91	26.85± 5.84	0.412*[1]
Range	(21-40)	(21-40)	
BMI (kg/m^2)	29.3 ±4.3	27.2 ±4.7	0.703*[1]
Range	(20-42)	(20-43)	
Duration of infertility (yr)	5.18 (3.14-7.42)	0	<0.001*[1]
Range	(1- 17)		
Obstetric history			
Gravidity	0.62± 0.06	2.85± 1.08	<0.001*[1]
Parity	0.25± 0.04	1.32± 0.72	<0.001*[1]
Type of infertility			
Primary	20 (40%)	0	<0.001*[2]
Secondary	30(60%)	0	<0.001*[2]
Vaginal discharge			
Nil	25(50%)	16 (64%)	0.005*[2]
Whitish discharge	15 (30%)	7 (28%)	
Yellowish discharge	10 (20%)	2 (8%)	
Previous abdominal surgery	27 (54%)	14 (56%)	0.609*[3]

*Independent t test used; 2. Fisher exact test use, *statistical significant as p<0.05.*

Table 2. Comparing positive Chlamydia trachomatis IgG with type of infertility

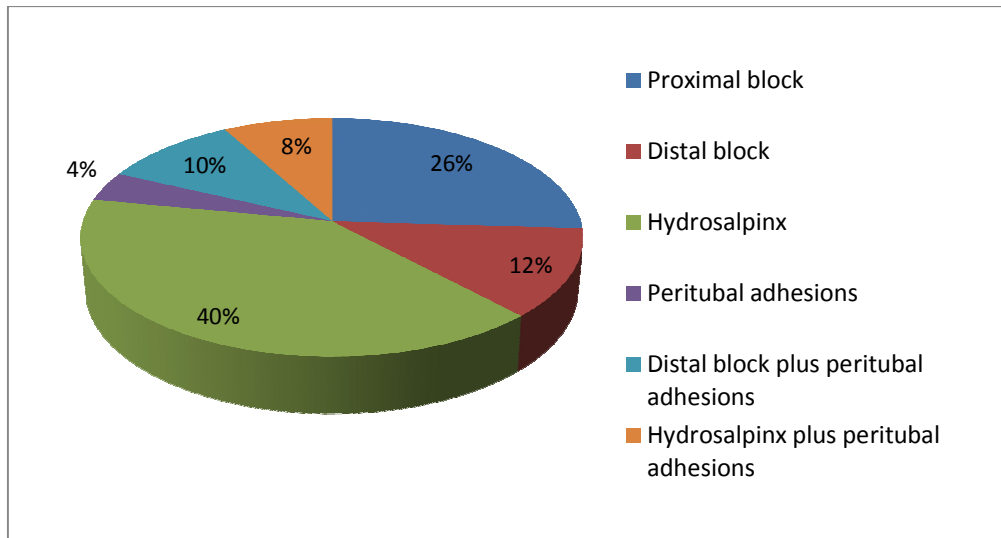| Type of infertility	Group I	Group II	P value
Primar	13 (56.5)	2 (66.7)	0.725
Secondary	10 (43.5)	1 (33.3)	
Total	23 (100)	3 (100)	

Fig. 1. Prevalence of different types of tubal factor in the study population
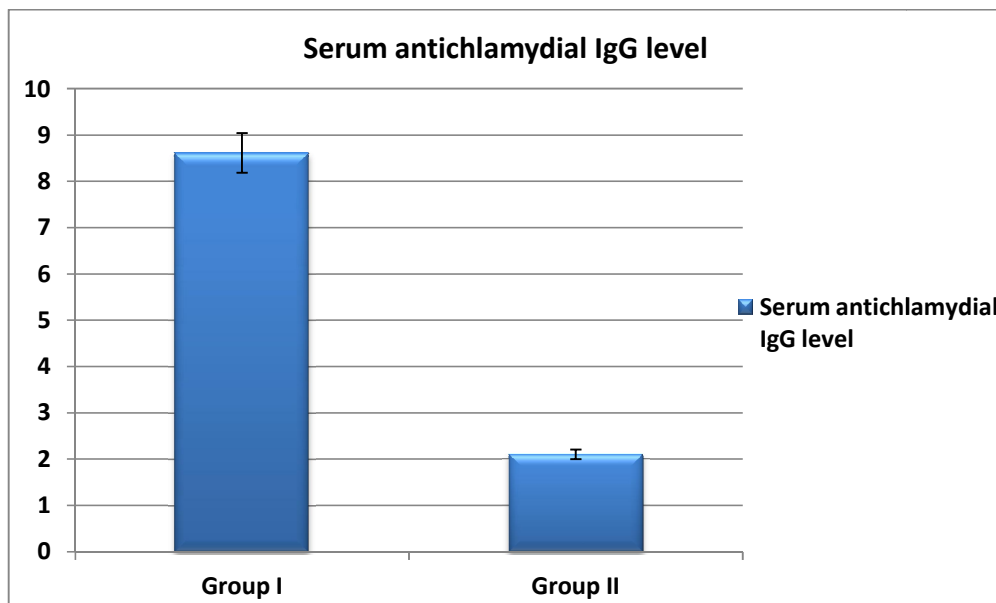

Fig. 2. Mean of antichlamydial IgG among both groups

Damage to the fallopian tubes, or tubal pathology, is a common cause of infertility in women. Tubal damage is thought to occur when pathogenic microorganisms, such as *Chlamydia trachomatis*, ascend from the lower genital tract and infect the tubes, inducing inflammation. This may cause scarring of the fallopian tubes, resulting in tubal factor infertility (TFI). It has recently been estimated that 45% (28%-62%) of confirmed TFI cases are caused by urogenital *C. tracho matis infection* [12].

Chlamydia trachomatis infections are most prevalent in young adolescents, but complications such as TFI can become evident more than 10 years later when these women fail to conceive and present with infertility. By that time, the *C trachomatis* bacterium has been cleared by the immune system, and *C. trachomatis* DNA is not detectable any more by PCR. *S. erum C. trachomatis* IgG antibodies however may remain detectable for many years after infection, even after antibiotic treatment [13].

Serum antichlamydial IgG positive
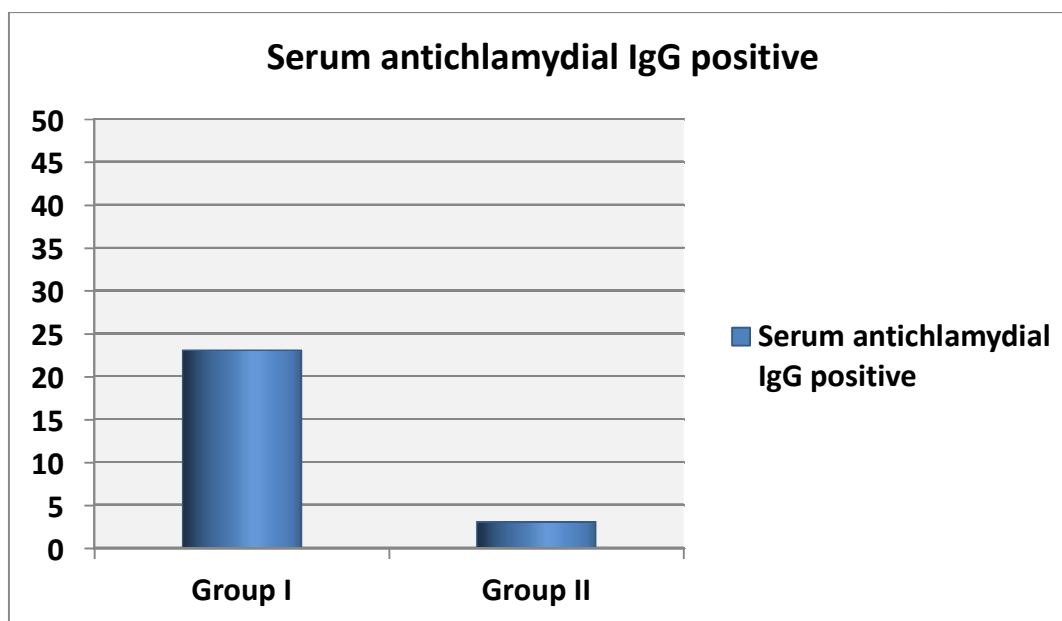
Fig. 3. Frequency of antichlamydial IgG positive among both groups

Infertility affects approximately 5-26% of couples in the reproductive age-group, a figure that is fairly similar between less and more developed countries. This figure could, however, rise in the near future as increasing numbers of women delay childbearing, resulting in decreased oocyte quality, raised chance of exposure to sexually transmitted diseases, and a secular decli ne in sperm counts [14].

C. trachomatis is known to cause damage to the female reproductive tract, primarily due to adhesions or obstructions of the fallopian tubes secondary to the inflammatory response. Reduced chances to achieve an intrauterine pregnancy is the result. Tubal Factor of Infertility (TFI) is the main cause of infertility in 10-30% of cases in developed countries and *C. trachomatis* is the most common causative agent to PID and TFI in the developed world [15]. The aim of our study is 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. In the study of Peivandi S and his coworkers [6] a total of 150 infertile women who underwent laparoscopic investigation were identified to participate in the study. After laparoscopy, 40 women were excluded from analysis because of severe endometriosis which was due to tuboperitoneal abnormalities not caused by *C. trachomatis* .

The womens' ages ranged from 18 to 42 years (Mean 27.5 ± 5.5 years). The duration of infertility at the time of laparoscopy ranged from 1 to 13 years (Median 3.9 ± 2 years).

There was not a significant relation between CAT and age, and or between CAT and duration of infertility [6].

In our study, As regard prevalence of different types of tubal factor in the study population was 12% in Distal block (DB), 10% Distal block plus peritubal adhesions (DB + PTA), 40% Hydrosalpinx (HS), 8% Hydrosalpinx plus peritubal adhesions (HS + PTA), 26% Proximal block (PB), 4% Peritubal adhesions (PTA).

In the study of Singh and his colleagues [16] regarding tubal pathology analysis and Site of tubal block, agglutinated fimbria was the most common finding was the most common site of block (41%), while hydrosalpinx was only in (25%). Which isn't consistent with our results [17].

A number of sero-epidemiological studies have examined the prevalence of antibodies to C trachomatis and chlamydial heat shock protein in women with laparoscopically or hysteron-salpingographically confirmed fallopian tube damage and ectopic pregnancies (120).

The results of these studies indicate that history of C trachomatis infection is associated with a significantly increased risk of tubal infertility in women, regardless of the infection invoking clinical symptoms. Chlamydial antibody testing may therefore continue to become a valuable predictor of not only tubal patency, but also of ectopic pregnancy, intrauterine insemination failure, and embryo and pregnancy wastage, independent of tubal damage [18].

5. CONCLUSION

The study concluded that, *Chlamydia trachomatis* could be one of the major factor causing female infertility especially caused by tubal factor. Serum *Chlamydia trachomatis* IgG assay using ELISA could be used a predictive factor for detection of tubal factor infertility.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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