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ANTI-CHALMYDIAL ANTIBODY AS A PREDICTIVE TEST FOR TUBAL FACTOR INFERTILITY

By

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ABSTRACT

Background: Infertility is a common gynaecological problem that has a multi factorial aetiology. Conception and pregnancy depend on complex physiological, anatomic and immunological factors.

Objective: to evaluate the prevalence of chlamydial infection, especially subclinical cases, in a population of Egyptian tubal infertile women and to relate it to history, symptoms, clinical, and laparoscopy findings. Finally, to find any advantage of detecting antichlamydial antibodies in serum of these patients and evaluate its importance in prediction of tubal factor of infertility.

Patients and Methods: This study includes 50 primary or secondary infertile females (patients group) their age between 20-30 years, and 50 random fertile females (control group) and Blood sample for IgG, Chlamydia trachomatis antibodies were drawn from all cases of the study for ELISA test.

Results: The prevalence rate of Chlamydia trachomatis IgG antibodies was significantly higher in infertile group than that of control group. There was significant higher rate and ratio of positive results in infertile group than that of control group concerning anti-chlamydial IgG. There was a strong correlation between serum levels of anti-chlamydial IgG in the infertility patients. There was a significant correlation between serum anti-chlamydial IgG levels and the duration of infertility. There was no relation between the serum level of anti-chlamydial IgG and the age of the patients of the type of infertility. The results of this study are matched with most of the previous published studies yet there are some differences in the positive and negative ratios.

Conclusion: Chlamydia trachomatis plays a major role in the occurrence of tubal factor of infertility. Subclinical chlamydial salpinigits was an important cause of tubal infertility. Serological test for Chlamydia trachomatis namely anti-chlamydial antibodies IgG are sensitive, simple, and inexpensive tests even if compared by using direct methods for detection and should be done as a routine part of infertility investigations. The serological test could be an accurate non-invasive predictor of tubal status especially if combined with other methods as HSG, good history taking and examination.

Key words: Anti-chlamydial antibody, tubal factor, infertility.

INTRODUCTION

Tubal factor infertility is one of the main causes of involuntary childlessness in women *(Tabong and Adongo, 2013)*. 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. Female infertility is attributed to the tubal factors in about 14-38% of the cases *(Tsevat et*

al., 2017). 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 *(Surana et al., 2012).*

The obligate intracellular pathogen Chlamydia trachomatis belongs to the most common sexually transmitted bacterial organisms, worldwide *(Bastidas et al., 2013).* According to the Centers for Disease Control and Prevention (CDC), about one million reported 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 serovars whereby the serovars D through K typically cause nongonococcal urethritis in men and cervicitis in-women *(O'Connell and Ferone, 2016).*

The C. trachomotis infection is the most common sexually transmitted infection worldwide, especially among young adults *(Cárcamo et al., 2012).*

The chlamydial infection produces less severe symptoms than other sexually transmitted infections *(O'Connell and Ferone, 2016).*

The bulk of infections remains undetected and untreated because most infected people are oligo- or asymptomatic and do not seek medical attendance. If untreated, chlamydiae 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 *(Bastidas et al., 2013).*

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 *(Peivandi et al., 2009).*

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 *(Surana et al., 2012).*

In addition, C. trachomatis-specific antibodies have been associated with tubal damage and infertility *(Budrys et al., 2012).*

Several studies have demonstrated that tubal factor infertility was significantly associated with the serum antibodies to C. trachomatis, which resulted in infertility *(Hjelholt et al., 2011).*

A better understanding of the role of persistent C. trachomatis infections in 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 *(Seth-Smith et al., 2013).*

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 *(Joolayi et al., 2017).*

The present study aimed to evaluate the chlamydial infection in women who suffered from tubal infertility by the detection of the anti-chlamydial IgG antibodies using E.L.I.S.A.

PATIENTS AND METHODS

This case control study was done between September 2018 to May 2019. The study population consisted of 50 women of the reproductive ages (17yrs-40yrs), who has lyr or 2yr infertility specially tubal factor infertility diagnosed by HSG or Laparoscopy, and attended the infertility clinic of Al-Hussein University Hospital, Al-Azhar University, for the evaluation of their fertility problem. Fifty healthy women of a similar age group who attended the family planning clinic during the study period were the control group for receiving suitable contraceptive method.

The study excluded patients with male cause of fertility as 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 Patients with clinical symptoms of acute PID and infertility due to other causes rather than tubal factor.

Informed consents were taken from all patients after explaining the purpose and nature of the study.

A detailed history was obtained from these patients including details of age, marital status, occupation, duration and type of infertility, investigations and treatment received before. Menstrual history and obstetric history were taken in details 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 (including dysparunia) and chronic vaginal discharge. We asked also about history of treated pelvic infections with special attention to those who entered a fever hospital and those with history of admission to chest hospital. Complete examination of these patients was performed with special attention to the presence of pelvic mass, chronic cervicitis and purulent or mucopurulent end cervical discharge.

Serological Studies:

All the 50 patients who proved to have a tubal factor as the only or the major cause for their infertility, as diagnosed by HSG and DL, were further investigated for the presence of anti-chlamydial IgG antibodies in their serum. The same tests were done for serum obtained from the control women.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Probability (P-value): P-value <0.05 was considered significant.

RESULTS

The distribution of infertility group according to age, revealed that 12% were <20 years old, 60% were between 21-30 years and 28% were between 31-40 years. It also shows that the distribution of infertility group 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 48% of infertility group while those of equal or more than 3 years duration of infertility was 52% of cases. Maximum duration of infertility in infertility patients studied was 8 years and minimum duration was one year with a mean of 3.04 years and a standard deviation of 1.42 years duration. 66% of cases had primary infertility while 34% of cases had secondary of infertility (table 1).

Table (1): Distribution of age, duration of infertility, type of infertility in infertility group

Age (years)	**No.**	**%**
<20 years	6	12.0%
21-30 years	30	60.0%
31-40 years	14	28.0%
Total	50	100.0%
Duration of infertility	**No.**	**%**
<3 years	24	48.0%
≥3 years	26	52.0%
Total	50	100.0%
Type of infertility	**No.**	**%**
Primary infertility	17	34.0%
Secondary infertility	33	66.0%
Total	50	100.0%

The anti-chlamydial IgG values between the infertility group and the control was highly significant p-value <0.001. i.e. high significant difference between both groups (infertility group and control group) as regard IgG level (table 2).

Table (2): Comparison between the two groups according to anti-chlamydial IgG

Groups / **Anti-Chlamydial IgG**	**Control Group (n=50)**	**Infertility Group (n=50)**	**t-test**	**p-value**
Range	0.114-1.465	0.113-2.310	3.501	<0.001**
Mean±SD	0.235±0.267	0.587±0.659	3.501	<0.001**

30% of patients with tubal infertility patients has anti-chlamydial IgG for chlamydia trachomatis in their serum, compared to 70% who did not show anti-chlamydial IgG for chlamydia trachomatis in their serum, which is significant. It also shows that 6% of the control group had anti-chlamydial IgG in their serum compared to 94% who did not show anti-chlamydial IgG in their serum (table 3).

Table (3): Frequency of Anti-chlamydial IgG among infertility group

Anti-chlamydial IgG (Control group)	**No.**	**%**
Positive IgG	3	6.0%
Negative IgG	47	94.0%
Total	50	100.0%
Anti-chlamydial IgG (Infertility group)	**No.**	**%**
Positive IgG	15	30.0%
Negative IgG	35	70.0%
Total	50	100.0%

Number of positive IgG patients from case group were 15 cases, (30% of the cases) and number of positive IgG from control group were 3 (6% of control patients), comparing the anti-chlamydial IgG values between the cases and the control was highly significant p 0.004*, i.e. high significant difference between both groups (infertility group & control group) ads regard IgG level (table 4).

Table (4): Percent of infertility group and control group with Positive Anti-chlamydial IgG and their percent

Groups / **Anti-chlamydial IgG**	**Control group *(n=50)***		**Infertility group *(n=50)***		**Chi-square test**	
	No.	**%**	**No.**	**%**	**x^2**	**p-value**
Positive	3	6.0%	15	30.0%	8.198	0.004*
Negative	47	94.0%	35	70.0%	8.198	0.004*

DISCUSSION

In this study 15 out of 50 (30%) of patients of the infertility group showed Chlamydia trachomatis infection as proved by the presence of anti-chlamydia IgG in their serum in relation to 3 out of 50 (6%) of the control group.

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 *(Bastidas et al., 2013).*

Serious sequelae like acute salpingitis and pelvic inflammatory disease often occur in association with repeated or persistent infections *(Peivandi et al., 2009).*

In addition, C. trachomatis-specific antibodies have been associated with tubal damage and infertility *(Budrys et al., 2012).*

The study has demonstrated that tubal factor infertility was significantly associated with the serum antibodies to C. trachomatis, which resulted in infertility.

A previous study agreed with our study, done in Baghdad 2011 by *May and Amer, (2012)* the antichlamydial IgG raised in 25% of infertile women, while controls showed only 4% positive IgG index.

In a study carried out by *Jorn et al. (2008)* found that the sero pervelance rate among women suffering from infertility was 39.3%.

Keltz et al. (2006) found that 84 out of 210 (40%) of infertile women in the study were seropositive for Chlamydia IgG antibodies.

Another study for *Jeremiah et al. (2011)* disagree with current study showed that the prevalence 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. In 313 sub fertile women, serological test results and laparoscopy reports were available for analysis. Of those 313 women, 59 (18.8%) met the definition of distal tubal pathology (extensive peri-adnexal adhesions and/or distal occlusion of at least one tube), whereas 254 women (81.2%) did not have distal tubal pathology and served as controls. Of those 254 women without distal tubal pathology, 94.9% had patent tubes and 5.1% had proximal occlusion of at least one tube. Since proximal tubal occlusion is considered not to be related to chlamydia disease, all 254 women without distal tubal pathology served as controls. In women with and without distal tubal pathology, mean age (30.6 and 31.2 years respectively) and duration of subfertility (2.4 and 2.3 years respectively) were comparable.

This was in disagreement with *Amadi et al. (2019)* in a study done on 125 women with infertility who met the inclusion criteria were enrolled into the study. Relevant information on their socio-demographic characteristics, gynecological symptoms and risks factors for infertility were obtained. Participants had Hysterosalpingography (HSG) as part of their fertility work-up while 5ml of venous blood was withdrawn to check for Immunoglobulin G antibody to Chlamydia trachomatis using rapid test kits. The HSG findings were correlated with the result of Chlamydia serology. Data was analyzed using the computer software, Statistical Package for Social Science (SPSS) version 20. The level of significance (p value) was set at 0.05.

A total of 120 infertile women completed the study, 5 had incomplete investigations and were excluded from the analysis. The prevalence of TFI was 47.5%, while that of positive chlamydia serology was 36.5%. The prevalence of chlamydial seropositivity was 59.6% for patients with TFI but 15.9% for non-TFI. There was a significant association between positive chlamydia serology and TFI $p< 0.05$. The study revealed moderate sensitivity 59.6%, and negative predictive value 69.7% but high specificity 84.1% and positive predictive value of 77.2%. In this study the odds for diagnosing tubal infertility was 7.8 Chlamydia serology is useful in predicting TFI and should be incorporated in the routine work up for infertility.

There are other studies that disagree with the study as *Israel et al. (2011)* reported that antibodies were present against Chlamydia trachomatis in 74% of patients, compared to 51 % of the control group. This difference may be due to large cases size, age, socioeconomic level, and sexual practice which considerable influence on the prevalence of c.trachomatis.

This study clearly demonstrate that sexually active women who didn't use condoms where more likely to acquire chlamydia infection.

These differences are due to large sample size, many clinical conditions.

Another study in 2012 has done by *Surana et al. (2012)* found a high seropotivity in 60% for the anti-chlamydial antibody and 52% of the females in the study show bilateral tubal block mostly in the ampullary part.

Higher titre was reported in a study done by *Claude et al. (2011)* show that the prevelance of Chlamydia antibody were 90.9% of cases and 19.9% of control group.

Gorwitz et al. (2017) aiming to find the prevalence of chlamydia trachomatis seropositivity among women with infertility of tubal origin. 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 were enrolled in the 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.

Singh et al (2016) disagreed with the results of the current stuidy, in a prospective study was conducted on 200 consecutive patients undergoing laparoscopy as a part of infertility work-up. Preoperatively, serological determination of Immunoglobulin G (IgG) specific antibodies against Chlamydia Trachomatis was done by Enzyme linked immunosorbant assay (ELISA). Findings of laparoscopy were evaluated against presence or absence of chlamydial antibodies in serum.

Out of 200 patients, 10 patients tested positive for chlamydial antibody. Chlamydial antibody was found positive in 20% and 22.7% of patients with tubal pathology and peri-hepatic adhesions of patients, respectively. The sensitivity of chlamydial antibody for diagnosing tubal pathology was found to be 20%, while specificity was 100%. The positive chlamydial antibody test was not statistically associated with involvement of one or both tubes and site of tubal block.

Chlamydia antibody test did not appear to be good screening test for tubal pathology especially in Indian subcontinent. In view of its high specificity, this test can be used to identify patients with higher chances of tubal pathology requiring operative intervention.

These results were different, possibly because our demographic profile is different too. Our study population comprised of only those women who underwent laparoscopy. Though the percentage of possible tubal factor infertility was higher, but other factors like unexplained, ovarian or uterine factor infertility were also parts of this cohort. The methodologies used to detect antibodies vary in their utility and populations studied may vary in their genetic predisposition to immune response and antibody production and persistence. Therefore, laboratory and regional differences could exist in chlamydial.

This variability was found to be subjected to how the tubal pathology was

verified and type of chlamydia antibody titre assay.

A previous study has also considered seropositivity for chlamydial antibody in relation to type and sites of tubal block. They found that seropositivity for chlamydia IgM antibody was the highest among the subjects with a fimbrial blockage (80%), followed by those with an ampullary blockage (66.6%). *Results of Surana et al. (2012)* are different from current study. These prospective studies were carried out at a tertiary care hospital in north India.

CONCLUSION

Chlamydia trachomatis plays a major role in the occurrence of tubal factor of infertility. Subclinical chlamydial salpinigits was an important cause of tubal infertility. Serological test for Chlamydia trachomatis namely anti-chlamydial antibodies IgG, are sensitive, simple, and inexpensive tests even if compared by using direct methods for detection and should be done as a routine part of infertility investigations.

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الأجسام المضادة للكلاميديا كعامل تنبؤ فى حالات العقم الناتج عن إصابة فى قنوات فالوب

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خلفيـــة البحـــث: يُعتبـر العقـم أحـد المشـكلات الشـائعة فـي مجـال أمـراض النسـاء ويـنجم عـن بعـض المسـببات المرضـية متعـددة العوامـل. ويعتمـد الحمـل علـى عوامـل فسيولوجية وتشريحية ومناعية معقدة.

الهـــدف مـــن البحـــث: تقيـيم مـدى انتشـار عـدوى الكلاميـديا، وخاصـة الحـالات دون الإكلينيكيـة، فـي مجموعـة مـن السـيدات المصـريات المصـابات بـالعقم الأنبـوبي وعلاقتـه بالتـاريخ والأعـراض والنتـائج الإكلينيكيـة والتنظيـر البطنـي. كمـا يهـدف إلـى العثـور علـى أي ميـزة فـي اكتشـاف الأجسـام المضـادة لمضـادات الكلاميـديا فـي مصـل هؤلاء المرضى وتقييم أهميتها في التنبؤ بالعقم الناتج عن إصابة بالبوقين.

المرضـــى وطـــرق البحـــث: شـملت هـذه الدراسـة 50 سـيدة مـن المصـابات بـالعقم الابتـدائي أو الثـانوي (مجموعـة المرضـى) تتـراوح أعمـارهن بـين 17-40 عامـاً و50 مـن السـيدات الأصـحاء (كمجموعـة الضـابطة) وعينـة دم لفحـص الأجسـام المضـادة للكلاميـديا (المتـدثرة الحثريـة) وتـم استخلاصـها مـن جميـع الحـالات المشاركة فى الدراسة عن طريق تقنية الإليزا.

نتـــائج البحـــث: كـان معـدل انتشـار الأجسـام المضـادة المتـدثرة الحثريـة IgG أعلـى بكثيـر فـي مجموعـة العقـم مـن تلـك المجموعـة الضـابطة وهنـاك نسـبة ذات دلالـة إحصـائية مرتفعـة فـي مجموعـة الإصـابة بـالعقم مـن تلـك المجموعـة الضـابطة فيمـا يتعلـق بمضـادات الالتهـاب. هنـاك علاقـة معنويـة بـين مسـتويات IgG المضـادة للكلاميـديا فـي الـدم ومـدة العقـم. لا توجـد علاقـة بـين مسـتوى الأجسـام المضـادة للكلاميـديا بمصـل الـدم وبـين عمـر مرضـى العقـم. تتوافـق نتـائج هـذه الدراسـة مـع معظـم الدراسـات المنشـورة السـابقة، لكـن هنـاك بعـض الاختلافـات فـي النسـب الإيجابية والسلبية.

الاستنتاج: تلعب المتدثرة الحثرية دورًا رئيسيًا في حدوث العقم البوقي للعقم. وتعد الكلاميديا سبباً هاماً للإصابة بالعقم الناتج عن إصابة بالبوقين. والاختبار المصلي لداء المتدثرة الحثرية، أي الأجسام المضادة للكلاميديا ، اختبار حساس وبسيط وغير مكلف حتى لو تمت مقارنته باستخدام طرق مباشرة للكشف وينبغي إجراؤه كجزء روتيني من فحوصات العقم. ويمكن أن يكون الاختبار المصلي مؤشراً دقيقا غير تدخلى للكشف عن البوق خاصة إذا تم دمجها مع طرق أخرى مثل الأشعة بالصبغة على البوقين وأخذ تاريخ جيد وعمل الفحوصات اللازمة.