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The value of Serum Anti-Chlamydia trachomatis Ig G Antibody testing in predicting Tubal Factor Infertility

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ABSTRACT

Infertility is increasingly becoming a significant health problem in many areas of the world. The purpose of this study was to investigate the role of Serum Anti Chlamydia Trachomatis Ig G antibody serology as a screening test for tubal infertility and to compare the result with hysterosalpingography (HSG). Prospective cross-sectional study, Serum Chlamydia trachomatis immunoglobulin G antibody test were determined prospectively in 192 infertile patients by using ELISA test, the results of testing will compared with the results of HSG with respect to their predictive value of tubal factor infertility. A positive CAT had 32.4% sensitivity and 83.2% specificity at detecting tubal disease with a positive predictive value of 31.6% and a negative predictive value of 83.8%. There was statistically significant difference between age and type of infertility. In secondary infertility women, the sensitivity of CAT 40.0% and 81.4% specificity for detecting tubal disease with a positive predictive value of 35.3% and a negative predictive value of 84.2%. The LR+ of CAT was 2.15 while the LR- 0.74. These finding highlighted a correlation between the tubal factor and the anti-chlamydial antibodies. CAT is poorly sensitive and highly specific.

1. INTRODUCTION

Genital Chlamydia trachomatis infection recognized as the most common cause of tubal damage. It is the most prevalent bacterial sexually transmitted infections (STI) recognized throughout the world and Chlamydial PID is an important preventable cause of infertility and adverse pregnancy outcome. The World Health Organization estimates that annually almost 101 million new cases occur worldwide every year [1]. More than two-thirds of these cases occur in the developing world where diagnostic and treatment facilities are almost absent [2]. Urogenital C. trachomatis infections have a broad spectrum of clinical manifestations, including urethritis, cervicitis, and pelvic inflammatory disease (PID) [3]. C. trachomatis can persist in the genital tract for a long time in a form that is resistant to immune destruction, and symptoms of infection go unnoticed in approximately 75–80% of women [4]. Current infection does not necessarily mean recent

infection, as the infection can persist for many years in the absence of treatment [5]. Genital C. trachomatis infection markedly increases the risk of reproductive tract sequelae in women, including tubal damage, ectopic pregnancy, and spontaneous abortion [6]. Chlamydial PID can cause tubal occlusion and subsequent infertility [7]. The pathology affects approximately 15 to 30% of sub fertile women. Chlamydia trachomatis however, is a slow (eliminate) growing intracellular organism. The growth cycle of chlamydia is 48 to 72 hours; therefore, several weeks to months are required for the growth to reach sufficient numbers to cause clinical symptoms [8]. C. trachomatis preferentially infects the columnar epithelium. Serious sequelae often occur in association with repeated or persistent infections. The precise mechanism through which repeated infection elicits an inflammatory response that leads to tubal scarring and damage in the female upper genital tract is not yet clear [9]. C. trachomatis may cause intraluminal adhesions, fibrosis, hydrosalpinx and pelvic adhesions. Due to the serious consequences of these conditions, C. trachomatis infection can affect a woman's fertility [10]. Chlamydia is now associated with at least 50% of the cases of acute pelvic inflammatory disease (PID) in developed countries. Due to the asymptomatic nature of C. trachomatis, the diagnosis of tubal disease cannot rely solely on the presence or absence of a history of PID. Since late sequelae of PID (chronic pelvic pain and tubal damage) have major health implications; therefore, it is important to screen this group of patients for chlamydial infection [9]. The purpose of this study was to investigate the role of Serum Anti Chlamydia trachomatis Ig G antibody serology as a screening test for tubal infertility and to compare the result with hysterosalpingography (HSG).

2. Method

Prospective cross- sectional study done in Al-Imamein Kadhimein Medical city, Department of infertility Clinic during a period of one year; from 1 April 2015 to 1 April 2016. 192 infertile female patients who presented consecutively in our clinic participated in the present study. The basic data on these 192 patients (age, duration of infertility, primary or secondary infertility), after giving written informed consent; routine hormonal assay, seminal fluid analysis and HCG were carried out on all patients.

Inclusion criteria, Age in years (17-39) years, Duration of infertility in months (≥ 12months), Type of infertility (primary or secondary).

Exclusion criteria: Patients with severe male factor infertility, Thyroid dysfunction, Hyperprolactinemia, Serum FSH ≥ 15 ml IU/ml, History of pelvic or abdominal surgery excluded from the study i.e Endometriosis. All the patients subjected to the following: They told about the nature of the study and only those who agreed to participate in the study were included. verbal consent obtained from all infertile women in the study. The demographic characteristics of each patient assessed including age, duration and type of infertility. Both partners medical history about sex life, any birth control methods, any STD, medicine use, and the use of tobacco, caffeine, alcohol or illegal drugs. Menstrual cycle for her checked. Both partners physical examination were done to all participants (A woman's physical exam usually includes a pelvic examination and Pap test and A man's physical exam usually includes a testicular examination by Urologist). A trans vaginal ultrasound examination were done to all patients to assess the overall condition of uterus and ovaries, the endometrium thickness, and follicle development on the ovaries and to exclude presence of fibroids, cysts, PCOS, endometriosis. HSG performed for all infertile women after menstruation, during the follicular phase and before ovulation, using Lipiodol (Laboratoire Guerbet, Aulnay-sous-Bois, France). An HSG considered abnormal if one or both tubes did not allow passage of contrast medium. A blood drawn for determination of the C. trachomatis Ig G antibody, 2 ml of venous blood drawn for laboratory measurement of the serum Chlamydia Ig G antibody. The blood collected from each participant, labelled and sent to the laboratory. The serum separated, frozen and stored. An ELISA test (DEMEDITEC Diagnostics GmbH company, Germany) was used according to manufacturer's instruction

for the qualitative immunoenzymatic determination of Ig G-class antibodies against Chlamydia trachomatis. Samples considered positive if the absorbance value is higher than 10% over the cut-off and negative if the absorbance value is lower than 10% over the cut-off. Statistical analysis done by SPSS22, the data presented as mean ± standard deviation (SD). Percentage of primary and secondary infertility presented by Pie circle graph. Un paired t-test was done for the comparison between age and duration according to type of infertility and Fisher Exact test was done for the comparison of HSG and CAT between primary and secondary infertility to show the level of significance. The spearman correlation (correlation coefficient) denoted by R value was done to measure of how well the relationship between two variables can be described by a monotonic function, A p value less than 0.05 considered statistically significant.

3. Results

Blood samples drawn from 192 patients to determine the C.trachomatis antibody who underwent HSG investigation identified to participate in the study. The women's ages range from 17 to 39 years (mean 28.24+5.94 years). The duration of infertility at the time of study ranged from 12-180 months (mean 51.16+36.12 months) as seen in table (1).

The positive Chlamydia trachomatis IgG class antibody was found in (38) of 192 patients (19.79%) and in (154) patients the Chlamydia trachomatis IgG class antibody was negative (80.21%) In (37) of 192 patients the hysterosalpingography was positive (19.27%) and negative in (155) of 192 patients (80.73%). In the present study, there were (118) of 192 (61.64%) have primary infertility and (74) of 192 (74) of 192 (38.54%) have secondary infertility as shown in table (2)

The mean age (yrs.) of patients with primary infertility was (27.12 +6.06) and the mean age of patients with secondary infertility was (30.03+5.32). There was statistically significant difference as P value was 0.0006. The mean duration of infertility (months) of patients with primary infertility was (49.97+37.56) and the mean duration of infertility of patients with secondary infertility was (53.05+33.86). There was no significant difference as P value was 0.5564 (Table 3)

In 118 patients with primary infertility and in 74 patients with secondary infertility, there was a discrepancy between HSG and CAT findings. Positive CAT was found in 21 patients with primary infertility (17.8%) and 17 patients with secondary infertility (22.97%). Negative CAT was found in 97 patients with primary infertility (82.2%) and 57 patients with secondary infertility (77.03%). Positive HSG was found in 22 patients with primary infertility (18.64%) and 15 patients with secondary infertility (20.27%). Negative HSG was found in 96 patients with primary infertility (81.36%) and 59 patients with secondary infertility (79.73%). CAT was significantly positive in those women who had conceived previously as compared with primary infertile women. In HSG results, in comparison between primary and secondary infertility, no significant difference as P value was 0.852. In CAT, in comparison between primary and secondary infertility, no significant difference as P value was 0.457. As show in table (4). The table (5) shows Sensitivity and specificity of CAT test in comparison to HSG in 192 infertile women, a positive CAT had 32.4% sensitivity and 83.2% specificity at detecting tubal disease with a positive predictive value of 31.6% and a negative predictive value of 83.8%. The LR+ for the CAT was 1.93; which indicated that a patient with tubal factor infertility was 1.93 times more likely to have a positive test result than a patient without tubal factor infertility.

The LR- for the CAT was 0.81, indicating a patient with tubal factor infertility to be 0.81 times as likely to have a negative test as a patient without the disease.

Sensitivity of CAT in comparison to HSG in primary infertility women was 27.3% and 84.4% specificity at detecting tubal disease with a positive predictive value of 28.6% and a negative predictive value of 83.5%. The LR+ of CAT was 1.75 while the LR- 0.86. In secondary infertility women, the sensitivity of CAT 40.0% and 81.4% specificity for detecting tubal disease with a positive predictive value of 35.3% and a negative predictive value of 84.2%. The LR+ of CAT was 2.15 while the LR- 0.74. (Table 6)

4. Discussion

In this study, we evaluated the efficiency of CAT to screen for tubal factor infertility and found that the prevalence of a positive CAT is higher in women with tubal factor infertility. Acute genital tract infections with C. trachomatis diagnosed by direct detection of the microorganism from the infected site. After the acute episode, the organism may no longer be detectable and chlamydia antibodies in serum may be the only indication of previous chlamydia involvement. So C. trachomatis infection may be primary or a chronic recurrence/reinfection. (i) Primary infection: A serial infection of the mucosal cells is seen during the primary infection. The infected epithelial cells secrete numerous pro-inflammatory chemokines and cytokines, including granulocyte - macrophage colony stimulating factor (GM-CSF), growth regulated oncogene, IL-1, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) 62. (ii) Chronic infection - recurrence/reinfection: Chronic infection, associated with persistence of Chlamydiain the host cells, recurrent infection is more dangerous. A delayed hypersensitivity reaction or rarely type 3 hypersensitivity reactions (Arthus reaction) observed in long term or recurrent stimulatory action of chlamydial antigens [11]. Antibodies are not involved in the delayed type of reaction developing within 24-48 h due to antigen interaction with specifically sensitized Th1 lymphocytes. Processes, which occur during these reactions, lead to tissue damage, fibrosis and cicatrization within the affected organs. Irreversible consequences like PID leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains and chronic urethritis may occur. [9], showed a significant increase in the degree of tubal damage in women with C. trachomatis MIF titers ≥1 in 128. The higher the titer the more likely there would be tubal damage and showed that Chlamydia antibody titers used as the sole test of tubal patency. Patients may have an unrelated cause for adhesions (e.g. endometriosis or salpingitis due to another microorganism). Chlamydia infection detected by a variety of methods. No single test has total diagnostic accuracy, so this agree with our study. [13], In their study they evaluated the efficiency of medical history taking, CAT testing and a combination of both in selecting women for laparoscopy to detect tubal pathology. They found that the discriminative capacity of the 'medical history' model did not differ from that of CAT testing alone. So our study being poor (so close to fair) due to small number of data patients (192 women). In study done by [14]. The study population consisted of 55 women with tubal damage and 55 parous women. CAT measured using the whole-cell inclusion immunofluorescence test and cervical chlamydial DNA detected by PCR. They found that Chlamydia antibody titers were associated with tubal occlusion, prior ectopic pregnancy, and with sexual behavior, suggesting that a chlamydia infection was the major contributor to the tubal damage in these women. The immunofluorescence test employed in the present study is highly sensitive. While in the present study, in 118 patients with primary infertility, 6 patients had abnormal HSG (i.e uni – or bilateral closed tubes) and positive CAT and 16 had abnormal HSG and negative CAT. Abnormal HSG in 74 patients with secondary infertility was seen in 6 patients with positive CAT and 9 patients with negative CAT. CAT by ELISA is poorly sensitive and highly specific. [15], showed the role of chlamydia serology as a screening test for tubal infertility with comparison of results with hysterosalpingography and laparoscopy.

The very high positive predictive value (94.8%) of Chlamydia serology makes it a good screening test before laparoscopy. However, the serology's low negative predictive value of 69.8% which this agree with our study, as the positive predictive value 31.6% and highly negative predictive value 83.8% and this

necessitates the continued use of HSG to find tubal damage from causes other than Chlamydia. [16], in this study The positive likelihood ratio for C. trachomatis antibody testing was 9.1, indicating a patient with tubal factor infertility to be 9.1 times more likely to have abnormal serology results than a patient without tubal factor infertility. This was superior to HSG, which had a positive likelihood ratio of 2.6. This disagree with our study, as the positive likelihood ratio of CAT in comparison to HSG is 1.93. In [17] study, for HSG testing, the sensitivity was 78% and the negative predictive value was 85%. For C. trachomatis titers, the sensitivity was also 78% and the negative predictive value was 82%. Indicating that there was no significant difference. While in our study, CAT in comparison to HSG in 192 infertile women, a positive CAT had 32.4% sensitivity and 83.2% specificity at detecting tubal disease with a positive predictive value of 31.6%, this mean poorly sensitive in comparison with previous study. [18] study, a negative correlation between CAT and age noted. In our study, the mean age (yrs.) of patients with primary infertility was (27.12 +6.06) and the mean age of patients with secondary infertility was (30.03+5.32). There was statistically significant difference as P value was 0.0006. [19] show the importance of C. trachomatis in infertility due to tubal occlusion and the need of C. trachomatis diagnosis in routine gynecologic analysis to prevent infertility and neonatal infection. This agree with our study [20], this study clearly demonstrated a statistically significant association between elevated C. trachomatis antibody titers as detected by ELISA and the presence of pelvic damage. [21] their data found on 32 of the 76 patients. Chlamydia serology in conjunction with the HSG had a sensitivity of 80% for tub peritoneal factor (tubal obstruction or pelvic adhesions), and a specificity of 82.3%. The positive predictive value was 80% and the negative predictive value was 82%. However, according to meta-analysis of [20] chlamydia serology is not a better screening test than HSG [22]. Also, studies by [10], [23], [24], indicated that the test of chlamydia antibodies alone or in combination with HSG were not cost-effective and beneficial. Therefore, in our study since Chlamydia trachomatis IgG serologic testing is simple, non-invasive, relatively inexpensive, and causes minimal inconvenience to the patient. We recommend combining it with hysterosalpingography as an infertility work-up.

5. Conclusion

These finding highlighted a correlation between the tubal factor and the anti-chlamydial antibodies. CAT is poorly sensitive and highly specific.

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6. References

[1] World Health Organization, Department of Reproductive Health and Research. Prevalence and incidence of selected sexually transmitted infections (Chlamydia trachomatis, Neisseria gonorrhoeae, syphilis andTrichomonas vaginalis). Methods and results used by WHO to generate 2005 estimates.2011;20-23.

[2] Gaydos CA, Theodore M, Dalesio N, et al. Comparison of three nucleic acid amplification tests for detection of Chlamydia trachomatis in urine specimens. J Clin Microbiol. 2004;42(7):3041–5.

[3] K. Sturm-Ramirez, H. Brumblay, K. Diop, et al. Molecular epidemiology of genital Chlamydia trachomatis infection in high-risk women in Senegal, West Africa. Journal of Clinical Microbiology.2000; 38 (1): 138–145.

[4] S.S. Witkin, L.M. Linhares. Chlamydia trachomatis in subfertile women undergoing uterine instrumentation. Human Reproduction.2002;17 (8): 1938–1941.

[5] Molano M, Meijer CJ, Weiderpass E, et al. The natural course of C. trachomatis infection in asymptomatic Colombian women: a 5-year follow-up study. Journal of Infectious Diseases. 2005; 191:907–916.

[6] L.M. Hafner, C. McNeilly. Vaccines for Chlamydia infections of the female genital tract. Future Microbiol. (2008); 3 (1):67–77.

[7] Honey E, Templeton A.Prevention of pelvic inflammatory disease by the control of Chlamydia trachomatis infection. Int J Gynaecol Obstet. 2002;78(3):257-261.

[8] Evers JL. Female subfertility. Lancet Medical journal. 2002;360: 151-159.

[9] Thomas K, Coughlin L, Mannion PT, et al. The value of Chlamydia trachomatis antibody testing as part of routine infertility investigation. J Hum Reprod .2000; 15(5):1079-1082

[10] Veenemans LM, Van der Linden PJ. The value of Chlamydia trachomatis antibody testing in predicting tubal factor infertility. J Hum Reprod. 2002;17(3):695-698.

[11] Morton RS, Kinghorn GR. Genitourinary chlamydial infection: a reappraisal and hypothesis. Int J STD AIDS. 1999;10: 765–75.

[12] Paavonen J. Immunopathogenesis of pelvic inflammatory disease and infertility - what do we know what shall we do? .J Br Fer Soc. 1996;1:42–5.

[13] Coopus SF, Opmeer BC, Logan S,et al. The predictive value of medical history taking and Chlamydia Ig G ELISA (CAT) in the selection of subfertile women for diagnostic laparoscopy: a clinical prediction model approach. J Hum Reprod.2007;22(5):1353-1358.

[14] Machado AC, Guimaraes EM, Sakurai E, et al. High titers of Chlamydia trachomatis antibodies in Brazillian women with tubal occlusion or previous Ectopic pregnancy. Infect Dis Obstet Gynecol. 2007: 24816.

[15] Keltz MD, Gera PS, Moustakis M. Chlamydia serology screening in infertility patients.Fertil Steril.2006;85(3):752-4.

[16] Debekausen YA, Evers JL, Land JA, et al. Chlamydia trachomatis antibody testing is more accurate than HSG in predicting tubal factor infertility. Ferti Steril. 1994; 61 :833-7.

[17] Meikle SF, Zhang X, Marine WM, et al. Chlamydia trachomatis antibody titers and hysterosalpingography in predicting tubal disease in infertility patients. Fertil Steril. 1994; 62: 305-12.

[18] Akande VA, Hunt LP, Cahill DJ, et al. Tubal damage in infertile women: prediction using Chlamydia serology. Hum Reprod. 2003; 18(9): 1841-7.

[19] Videla C, Carballal G, Kekiklian G, et al. Chlamydia trachomatis and tubal obstruction sterility. Medicina J (B Aires). 1994; 54(1): 6-12.

[20] Swart P, Mol BWJ, Van der veen F, et al. The value of hysterosalpingography in the diagnosis of tubal pathology, a meta-analysis. Fertil Steril.1995;64:486-491.

[21] Tanikawa M, Harade T, Katogiri C, et al. Chlamydia trachomatis antibody titers by enzyme - linked immunosorbent assay are useful in predicting severity of adnexal adhesion. Hum Reprod. 1996; 11: 2418-21.

[22] Bernstein RC, Yalcinkaya TM. Utilizing chlamydia trachomatis IgG serology with HSG diagnose tuboperitoneal factor infertility. W V Med J. 2003; 99(3): 105-7.

[23] Logan S, Gazvani R, Mc Kenzie H, et al. Can history, ultrasound or ELISA Chlamydia antibodies, alone or in combination predict tubal factor infertility in subfertile woman? .Hum Reprod. 2003; 18(11): 2350-6.

[24] Ficicioglu C, Api M. Chlamydial serology and hysterosalpangography in predicting tubal disease in infertility patients. Acta Eur Fertil J.1995; 26(3): 109-12.

Table (1): Age and duration of infertility

Parameters	Mean ± SD	Range
Age (yr.)	**28.24+5.94**	**17-39**
Duration (months)	**51.16+36.12**	**12-180**

Table (2): patients with positive and negative HSG and CAT (N=192)

Parameters		Number	%
HSG	**Positive**	**37**	**19.27**
	Negative	**155**	**80.73**
CAT	**Positive**	**38**	**19.79**
	Negative	**154**	**80.21**
Primary		**118**	**61.46**

Secondary	**74**	**38.54**

Table (3): Comparison of age and duration according to type of infertility by unpaired t-test

Parameters	**Primary** **Mean ± SD**	**Secondary** **Mean ± SD**	**P value**
Age (yrs.)	**27.12+6.06**	**30.03+5.32**	**0.0006**
Duration (months)	**49.97+37.56**	**53.05+33.86**	**0.5564**

Table (4): Comparison of HSG and C. trachomatis IgG Ab between primary and secondary infertility by Fisher Exact test

Parameters		**Primary** **N=118**		**Secondary** **N=74**		**P value**
		No.	**%**	**No.**	**%**	
HSG	**Positive**	**22**	**18.64**	**15**	**20.27**	**0.852**
	Negative	**96**	**81.36**	**59**	**79.73**	
CAT	**Positive**	**21**	**17.8**	**17**	**22.97**	**0.457**
	Negative	**97**	**82.2**	**57**	**77.03**	

Table (5): Sensitivity and specificity of CAT test in comparison to HSG in 192 infertile women.

	All infertile cases **N=192**
Sensitivity	**32.4%**
Specificity	**83.2%**
Positive predictive value	**31.6%**
Negative predictive value	**83.8%**
Positive likelihood ratio	**1.93**
Negative likelihood ratio	**0.81**

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Table (6): Sensitivity and specificity of CAT in comparison to HSG in primary and secondary infertility

	Primary N=118	Secondary N=74
Sensitivity	**27.3%**	**40.0%**
Specificity	**84.4%**	**81.4%**
Positive predictive value	**28.6%**	**35.3%**
Negative predictive value	**83.5%**	**84.2%**
Positive likelihood ratio	**1.75**	**2.15**
Negative likelihood ratio	**0.86**	**0.74**