CHLAMYDIA ANTIBODY TITRE AS A PREDICTOR OF TUBAL FACTOR INFERTILITY

Oghenefegor E. OLOKOR[a] (MBBS, FWACS)
Michael E. AZIKEN[a] (MBBS, MPH, FWACS, FMCOG, FICS, DMAS).
[a]Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria

ABSTRACT

BACKGROUND: Genital *Chlamydia trachomatis* is major cause of tubal factor infertility. Assessment of tubal patency by hysterosalpingography (HSG) and laparoscopy are invasive and expensive. In low resource setting, there may be a place for Chlamydia Antibody Titre (CAT) in infertility work up.

Objective: To determine the predictive value of Chlamydia antibody titre in tubal factor infertility.

Materials and Methods: This was a prospective cross-sectional study of women presenting with infertility at the Human Reproduction Research Programme unit of the University of Benin Teaching Hospital, Benin City, Nigeria. All the women had serum Chlamydia antibody titre and hysterosalpingography and where indicated, laparoscopy and dye test. The predictive value of Chlamydia Antibody was assessed using likelihood ratios.

Results: The prevalence of Chlamydia antibody among women with infertility was 32.1%. This was significantly higher among women with HSG diagnosed tubal damage (72.1% vs 27.9%; P = 0.000). The sensitivity and specificity of Chlamydia Antibody were 84.6% and 66.7% respectively while the sensitivity and specificity of Hysterosalpingogram were 85.7% and 50% respectively. The positive likelihood ratio (LR+) and negative likelihood ratio (LR-) of CAT were 2.58 and 0.22 respectively while the LR+ and LR- of HSG were 1.72 and 0.28 respectively. Both tests showed comparable predictive value in screening for tubal damage.

Conclusions: The predictive value of CAT was comparable to that of HSG. Chlamydia antibody titre determination is non-invasive and inexpensive and may be used in infertility screening to select patient for HSG and laparoscopy.

Key Words: Chlamydia Antibody Titre, Tubal Factor Infertility, Tubal Damage, Hysterosalpingogram, Predictive value

Corresponding Author: DR. OGHENEFEGOR E. OLOKOR
Department of Obstetrics and Gynaecology,
University of Benin Teaching Hospital, P.M.B. 1111,
Benin City, Edo State, Nigeria. Phone: +234 803 8295249
E-mail: ofegor@yahoo.com

INTRODUCTION

Infertility is a significant public health problem in our environment where a high premium is placed on the ability to bear children. Its prevalence has been estimated to be 6.9 – 9.3% in developing countries[1].

Tubal damage is a major cause of infertility with a reported incidence of 13.5% to 38%[2]. At the University of Benin Teaching Hospital, it was responsible as the sole cause of infertility in 14% of patients[3]. The damage is most commonly due to ascending genital tract infection[4] and the two organisms frequently implicated are *Neisseria gonorrhoea* and *Chlamydia trachomatis*. It has been shown that most cases are caused by *Chlamydia trachomatis*[5]. Genital *Chlamydia trachomatis* infections are often asymptomatic with potential long term consequences for female reproductive health[6-7].

Among infertile women, evidence of previous Chlamydia infection is common[8]. Some women who have tubal damage may have no history of pelvic inflammatory disease (PID)[9]. This is often due to previous silent Chlamydia infection as most cases of Chlamydia PID are asymptomatic even when it has caused tubal damage discovered only in the course of investigation[10].

Chlamydia apparently causes more severe subclinical tubal inflammation and ultimately

tubal damage than other agents despite its more benign presentation[11]. Superficial infection is considered to provide a weak stimulus for antibody formation, whereas infiltrating disease leading to upper genital infection is associated with sero-conversion[12]. Immunoglobulin M (IgM) production is often transient, whereas serum IgG antibodies persist for years and can be used as a marker of previous infiltrating Chlamydia infection[4].

A number of studies have investigated the antibody responses produced by *Chlamydia trachomatis* and have generally found a good correlation between serum antibodies to *Chlamydia trachomatis* and pelvic inflammatory disease and tubal factor infertility[6,12,13]. Mol *et al* demonstrated a positive correlation between Chlamydia antibody titre and tubal factor infertility[14].

Tuboperitoneal disease can be diagnosed by hysterosalpingography (HSG) or laparoscopy. These are both invasive and expensive procedures. Laparoscopy, though still the gold standard for the diagnosis of tubal disease,[4,12,15,16] requires general anaesthesia, hence it is not a suitable method for screening for tubal disease on a large scale. More so, it is not readily available in low resource setting like ours. Hysterosalpingogram is the most widely used screening test for tubal factor infertility. The overall sensitivity of HSG for the detection of tubal damage is only 65% with a specificity of 83%[17]. HSG is a painful procedure, has risk of infection, ionizing radiation and is poor at diagnosing peritubal adhesions[15,18]. False positive results can occur due to tubal spasm, dissimilar tubal filling pressure, excessive viscosity, faulty technique or misinterpreted films[17]. In contrast to laparoscopy or HSG, serological detection of past Chlamydia infection is non-invasive, simpler and a faster test to perform. It has similar sensitivity to that of HSG[14].

Several studies in Nigeria have reported high prevalence of Chlamydia antibodies among Nigerian women. However, data is scarce on the predictive value of CAT for tubal factor infertility. The aim of this study is to determine the value of *Chlamydia trachomatis* antibody as a screening test for tubal factor infertility. Findings from this study will be a useful guide in selecting patients for hysterosalpingography or laparoscopy in infertility work up.

MATERIALS AND METHODS

This was a prospective cross-sectional study of women who presented for infertility treatment at the Human Reproduction Research Programme (HRRP) unit of the department of Obstetrics and Gynaecology, University of Benin Teaching Hospital. Exclusion Criteria included women with previous pelvic or abdominal surgery, and women with contraindications to laparoscopy. Approval for this study was obtained from the ethical committee of the hospital. All participants gave written informed consent.

A woman was considered to have tubal factor infertility if she had bilateral tubal occlusion on HSG or at laparoscopy.

Socio-economic class was as described by Olusanya *et al*[19].

Upon recruitment, data was obtained using pretested data retrieval form after clients were assured of confidentiality. Data extracted included socio-demographic characteristics (age, parity, level of education, occupation, ethnic group, social class), type of infertility (primary or secondary), duration of infertility, previous history of PID. Thereafter each woman had serological assay for Chlamydia antibody as well as hysterosalpingography done while laparoscopy was done when indicated.

For Chlamydia antibody assay, 5ml of venous blood was collected from the volar surface of the forearms of all patients into clear sterile plain bottle. The blood specimen was allowed to clot, and then centrifuged to obtain serum. The serological assay was done using the immunocomb *Chlamydia trachomatis* immunoglobulin G kit (orgenics, Isreal), an indirect solid phase enzyme immunoassay (EIA) test that quantitatively measures antibodies to *Chlamydia trachomatis* in human serum. The reagent test kit was

brought to room temperature and then about 3.5ml pipette serum analysed with reagent control samples for *Chlamydia trachomatis* IgG antibodies. The results were recorded with the aid of calorimetric calibration scale in titres. The tests were validated with the control samples and significant titre (positive result) was recorded as 1 in 32 or greater[15].

All patients had hysterosalpingography done in the proliferative phase of their menstrual cycle. Patients with suspected endometriosis or in whom tubal spasm was suspected as the reason for lack of passage of contrast on HSG were selected as the cases for laparoscopy and dye test. Patients were classified as having tuboperitoneal disease if evidence was encountered during laparoscopy of fimbrial, peritubal and or periovarian adhesions or proximal or distal occlusion of one or both fallopian tubes, or a combination of these.

Data Management and Analysis

Data entry and analysis was performed using SPSS for Windows Version 16.0 (SPSS Inc. Chicago, IL). The prognostic value of CAT in predicting tubal factor infertility was compared with that of HSG by calculating sensitivity, specificity and likelihood ratios (LRs). The positive likelihood ratio (LR+) is calculated as [sensitivity/(1-specificity)]. The negative likelihood ratio (LR-) is calculated as [(1-sensitivity)/specificity][15]. Calculations of LRs yield a score that allows categorization of test results: an LR+ of 2-5 indicates a fair clinical test, 5-10 is good, and >10 is excellent[15,20]. A LR- of 0.5-0.2 indicate a fair clinical test, 0.2-0.1 is good, and <0.1 is excellent[15,20]. Statistical test of association was done using c^2 – test or Fisher exact test when appropriate and level of significance of $P < 0.05$.

RESULTS

Of the two hundred women recruited for this study, 190 (95%) had complete clinical information and investigation results and these formed the basis of analysis for this study. Four were lost to follow up and 6 had incomplete investigation results. The mean age of the study population was 35.83 years (standard deviation {SD – 5.61}, range 25-49). The mean duration of infertility was 5.55 years (standard deviation {SD – 3.56}, range

TABLE 1:SOCIODEMOGRAPHIC CHARACTERISTIC OF STUDY POPULATION

Variables	Positive CAT† n = 61 (%)	Negative CAT‡ n = 129 (%)	P. value
Age (Years)			
≤ 30	8(13.1)	23(17.8)	0.004*
31 – 35	21(34.4)	44(34.1)	
36 – 40	11(18.0)	42(32.6)	
41 – 45	13(21.3)	18(14.0)	
≥ 46	8(13.1)	2(1.6)	
Mean	37.07 ± 6.67	35.25 ± 4.95	0.037*
Marital status			
Single			
Married	61	129	
Level of education			
No formal education	3(4.9)	0(0.0)	0.089
Primary	7(11.5)	15(11.6)	
Secondary	13(21.3)	27(20.9)	
Tertiary	38(62.3)	87(67.4)	
Parity			
Nulliparae	42(68.9)	80(62.0)	0.359
Multipara	19(31.1)	49(38.0)	

* Statistically significant
† Chlamydia antibody titre ≥ 1:32
‡ Chlamydia antibody titre = 1:16

TABLE 2: COMPARISON OF CLINICAL CHARACTERISTICS WOMEN WITH POSITIVE AND NEGATIVE CAT

Variables	Positive CAT† n = 61 (%)	Negative CAT‡ n = 129 (%)	P. value
Type of infertility			
Primary	10(16.4)	18(14.0)	0.658**
Secondary	51(83.6)	111(86.0)	
Duration of infertility (months)			
≤ 12 months	5(8.2)	9(7.0)	
13 – 36	13(21.3)	41(31.8)	
37 – 60	13(21.3)	32(24.8)	
≥ 61	30(49.2)	47(36.4)	
Mean duration of infertility(years)	6.3 ± 4.11	5.19 ± 3.23	0.046
History of previous PID	6(9.8)	13(10.1)	0.95**
HSG Result			
Patent	17 (27.9)	107 (82.9)	0.000*
Occlusion	44 (72.1)	22 (17.1)	

* Statistically significant
** Statistically not significant
† Chlamydia antibody titre = 1:32
‡ Chlamydia antibody titre = 1:16

TABLE 3: COMPARISON OF CHLAMYDIA ANTIBODY TITRE AND HSG WITH LAPAROSCOPY AND DYE TEST

	Abnormal laparoscopy n = 13 (%)	Normal laparoscopy n = 6 (%)	P value
C. trachomatis antibody titre			
Positive†	11(84.6)	2(33.3)	0.046*
Negative	2(15.4)	4(66.7)	
Hysterosalpingography			
Abnormal	12(92.3)	3(50)	0.071
Normal	1(7.7)	3(50)	

[n] A total of 19 women had laparoscopy and dye test
* Statistically significant
† Chlamydia antibody titre ≥1:32

TABLE 4: COMPARISON OF C. TRACHOMATIS ANTIBODY AND HYSTEROSALPINGOGRAPHY

	C. trachomatis antibody titre	Hysterosalpingography
Sensitivity (%)	84.6	85.7
Specificity (%)	66.7	50
LR +	2.58	1.72
LR –	0.22	0.28

1-18).

All the women had Chlamydia antibody titre as well as hysterosalpingography result. Nineteen women (10%) had laparoscopy and dye test. Sixty-one women tested positive for Chlamydia antibody giving a prevalence of 32.1%.

Table I compared the socio-demographic characteristics of women with positive CAT and those with negative CAT. The mean age of patients with positive CAT was 37.07 years (standard deviation {SD} – 6.67) while the mean age of patients with a negative CAT was 35.25 years (standard deviation {SD} – 4.95). This was statistically significant (P = 0.037). There was no significant difference in the social class of patient with positive and negative Chlamydia antibody titre (P = 0.16). There was also no significant difference in the parity distribution between those with positive CAT and negative CAT. Table II compared the clinical characteristics of women with positive and negative CAT. The type of infertility (primary or secondary) was not different among patients with positive or negative CAT. The duration of infertility was significantly longer among women with positive CAT (6.3 4.11 vs 5.19 3.23 years, p = 0.046). Only 10% of women reported a history of previous pelvic inflammatory diseases and there was no significant difference between the two groups. Women with positive CAT were significantly more likely to have tubal occlusion on HSG than women with negative CAT (72.1% vs 17.1%; p = 0.000).

Table 3 shows the comparison of the CAT and HSG with laparoscopy findings among the 19 patients that had laparoscopy. There was a significant association between positive CAT and abnormal laparoscopy as against positive CAT and normal laparoscopy (84.6% vs 33.3%, p = 0.046). There was no significant association between tubal occlusion on HSG and abnormal laparoscopy. (92.3% vs 50%, p = 0.071).

Table 4 shows the comparison of CAT and HSG using sensitivity, specificity, positive and negative likelihood ratios. The sensitivity of a positive CAT was 84.6% with specificity of 66.7% at detecting tubal disease. The LR+ of CAT was 2.58, indicating that a patient with tubal factor infertility is 2.58 times more likely to have a positive test result (i.e. titre $\geq$ 32). HSG had a sensitivity of 85.7% and a specificity of 50% at detecting tubal disease. The LR+ of HSG was 1.72. The LR – of CAT was 0.22, indicating a patient with tubal factor infertility to be 0.22 times as likely to have a negative CAT (i.e. $\leq$ 32) compared to a patient without the disease. The LR – of HSG was 0.28.

DISCUSSION

Tubal damage due to *Chlamydia trachomatis* infection is an important cause of infertility[6,9]. This study reports a prevalence of 32.1% of positive Chlamydia antibody titre among infertile patients. This is lower than the 65.8% reported by Omo-Aghoja *et al*[6] but comparable with 30.3% reported by Veenemans *et al*[15] in Netherlands. It was however higher than 24.5% reported by Peivandi *et al* in Iran[21] and 16% by Logan *et al*[16]. The observed difference in prevalence rate may be due to the difference in population studied. The study by Omo-Aghoja *et al* used women with proven tubal factor infertility confirmed by hysterosalpingogram and laparoscopy. This may have accounted for the high prevalence in their study. Our study included all women with infertility irrespective of the aetiology and further analysis revealed that the prevalence of Chlamydia antibody titre among women with tubal occlusion on HSG is 72.1% showing that the prevalence of positive CAT was higher among women with tubal damage. The differences may also be due to differences in diagnostic test for Chlamydia. Logan *et al* used enzyme immunoassay and subsequently nucleic acid amplification assay. All positive EIA test were confirmed by direct immunoflourescence and all positive samples were further retested. These series of tests as well as the use of nuclieic acid amplification test with specificity approaching 100%[22-24] may have been responsible for the low

prevalence reported from their studies.

Results from this study show that CAT and HSG have comparable sensitivity (84.6% vs 85.7%). The clinical implication of this is that both will have similar false negative rates. However, HSG is less specific at detecting tubal disease (66.7% vs 50%) and will have a higher false positive rate compared to CAT. The sensitivity and specificity of CAT found in this study is comparable to that reported by Veenemans *et al*[15]. However, Peivandi *et al*[21] reported a sensitivity of 66.7% and a specificity of 93.5% while Dabekausen *et al*[25] reported a sensitivity of 74% and a specificity of 92% for CAT. HSG in this study has specificity different from what has been reported probably because of the small proportion of patients with normal HSG result that had laparoscopy and dye test (2.1%).

The diagnostic value of CAT was compared with the value of HSG in tubal pathology, using likelihood ratios (Lrs). The likelihood ratio of a positive test result (LR+) indicates the likelihood of a positive test in a patient with the disease over the likelihood of a positive test in a patient without the disease. The LR- indicates the likelihood of a negative test in a patient with the disease over the likelihood of a negative test in a patient without the disease. The LR+ of CAT from this study is 2.58 while the LR+ of HSG is 1.72. The higher LR+ of CAT over that of HSG indicate that it is better at ruling in tubal damage than HSG, though both LR+s are low. The clinical implication of the low LR+ is that a proportion of patients with tubal disease could be missed or have their laparoscopy delayed. Nevertheless, the simplicity and cost effective advantage of CAT makes it a good screening tool for tubal damage. The LR- of CAT from this study is 0.22 while the LR- of HSG is 0.28. Though both of them showed they are poor tests, the smaller LR- of CAT shows it is better at ruling out tubal damage than HSG.

Dabekausen *et al* reported a better performance of CAT with LR+ of 9.1 and LR- of 0.3 at a threshold level of <1:8 for CAT indicating a good clinical test[25]. Peivandi *et al* also showed CAT as a good clinical test with LR+ of 10.26 while that of HSG was 3.0[21]. The differences noted between result from this study and that of Dabekausen *et al* as well as Peivandi *et al* is most probably because of difference in sample size. In the study by Peivandi *et al*, all the women (110) had HSG and laparoscopy irrespective of the result of HSG. In this study, only 19 patients (10%) had laparoscopy and only 4 patients who had normal HSG had laparoscopy. This small number may have affected the result from this study. Therefore, a larger study with more women undergoing laparoscopy would have helped to appreciate this aspect of this study that was constrained by limited number of women who had laparoscopy.

Antibody titre decline over time is a possible reason for false negative results. However, this issue may be controversial. Previous studies have suggested a chronological decline in titers[26,27]. This may suggest that older patients will have less positive rate than younger patients. This is however at variance with the findings from this study which showed that older patients are more likely to have positive CAT than younger patients. A more recent study has also revealed no significant decline in Chlamydia antibody titre with age[28]. Another explanation for false negative results is the immune-mediated reaction responsible for adhesion; or, for unknown reasons, tubal occlusion may not have occurred in these women[29]. Therefore false negative test results may lead to expectant management. However, the strength of using this study is the fact that the decision to perform a diagnostic laparoscopy was irrespective of the result of CAT.

CONCLUSION AND RECOMMENDATIONS

This study shows evidence that CAT test is useful in selecting patients that are more likely to benefit from early recourse to the use of HSG or laparoscopy to exclude or rule in tubal factor infertility. We however recommend a larger population study to validate these findings.

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