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■ Original Research Article

# Chlamydia Trachomatis Antibody Titre Association with Tubal Pathology among Infertile Women in a Tertiary Care Facility in Nigeria

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**Abstract:**

**Context:** Tubal pathology has been implicated as the leading cause of infertility in sub-Saharan Africa and has been linked with Chlamydia trachomatis infection. However, there is limited reports on the relationship between Chlamydial antibody titre and tubal infertility in our environment. **Aim:** To study the relationship between quantitative serum Chlamydia trachomatis antibody titre and tubal factor infertility. **Study Design:** Case control study. **Methods and Materials:** Fifty infertile women with tubal pathology were matched with 100 pregnant women. Blood samples were collected and analysed using ELISA for quantitative determination of anti-chlamydial antibody. **Statistical analysis Used:** Data management was with SPSS with significant p-value set at < 0.05. **Results:** The prevalence of positive Chlamydia antibody titre in the infertile women with tubal pathology was 72% and 23% in the pregnant women (P < 0.001). The median (IQR) chlamydial antibody titre of 2.5 (0.89-4.41) seen in the women with tubal infertility was significantly higher than 0.8 (0.41-0.92) seen in the pregnant women (P<0.001). Patients with tubal infertility were two times more likely to test positive for chlamydia antibody when compared with the pregnant patients. There was no statistically significant relationship between serum levels of chlamydia trachomatis IgG titre and severity of tubal pathology (P=0.293). **Conclusion:** Chlamydia trachomatis infection was highly prevalent among patients with tubal infertility, with significantly higher titres in them compared with the controls. There was no significant association between elevated chlamydial antibody titre and extent of tubal damage.

**Keywords:** Chlamydia trachomatis, antibody titre, tubal factor infertility, tubal pathology

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## Introduction

Infertility is defined as failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse[1]. It is a worldwide problem estimated to affect 1 in 10 couples and leading to increasing number of patients seeking specialist fertility care[2,3,4]. The prevalence of infertility is particularly high in sub-Saharan Africa, due to an increasing prevalence of sexually transmitted diseases, post-abortal and puerperal sepses[2]. Infertility causes severe emotional and social distress for couples in African societies due to high premium placed on childbearing, it is also a cause of marital disharmony and physical violence against women[2,4].

Tubal disease is the most common cause of female factor infertility in sub-Saharan Africa[2]. This is linked to tubal damage from pelvic inflammatory disease[2,5]. Most common microbial aetiology of pelvic inflammatory disease are Chlamydia trachomatis and Neisseria gonorrhoea[2,6]. Close to two-thirds of people with Chlamydia trachomatis infections are asymptomatic[7]. This is an important factor in the spread of the disease and the associated complications such as chronic intraluminal and fimbria adhesions, fibrosis, hydrosalpinx and pelvic adhesions.

Incidence of Chlamydia infection as documented in western literature is 4.2% [8]. In Nigeria, incidence of 9.6% has been reported in a study done in Kano, North western Nigeria[9]. Prevalence of 38.3% was reported in a study done in Ahmadu Bello University teaching hospital Zaria by Tukur and colleagues[10] which is comparable with the prevalence of 38.6% reported in another study done in Calabar[11]. Studies have shown that infertile women with tubal factor infertility are more likely to have elevated antibodies to Chlamydia trachomatis than either infertile women with normal tubes or pregnant women[7,11,12,13]. The pathogenic process of Chlamydia infection is thought to be partly immunologic and an association between Chlamydia trachomatis heat shock protein 60 (HSP60) antibodies and sequel of infection has been observed[12].

In developed countries, screening programmes for Chlamydia have been set up to reduce its transmission and reproductive tracts morbidity[13]. United State Centre for Disease Control and Prevention recommends universal screening of adolescent and adult females up to 35 years of age (or based on local institutional prevalence data)[14]. This is not so in developing countries especially in Nigeria where there is no structured screening programme for Chlamydia trachomatis infection. This may explain the reason for higher incidence of chronic infection with Chlamydia trachomatis and its long-term sequelae in form of tubo- peritoneal infertility seen in this country.

There are several methods used to diagnose tubal infertility. These include hysterosalpingography (HSG), laparoscopy and dye hydrotubation, and hysterocontrastsonography. Unlike HSG and laparoscopy, chlamydia testing is non-invasive, simpler and faster to perform, with a comparable sensitivity and specificity to that of HSG[15,16,17]. Chlamydia trachomatis antibody testing may therefore serve as a simple non-invasive screening test for previous chlamydial infection and suspected tubal disease.

Studies have shown that Chlamydia trachomatis infection is associated with tubal damage and infertility[18,19]. Some have even advocated the incorporation of Chlamydia antibody titre in the fertility work up of high risk patients[2,18]. However, there are limited data supporting the role of quantitative chlamydial antibody testing in tubal factor infertility in this country. This underscores the need for more studies to accurately define the role chlamydia trachomatis antibody titre plays in the management of infertile patient with tubal disease. This may be useful in optimizing the fertility work-up of at-risk patients in my centre and can also provide room for treatment where necessary. Therefore, the aim of this study was to identify the relationship between tubal factor infertility and quantitative chlamydial antibody titre.

## Subjects and Methods:

This was a case control study conducted at the department of Obstetrics and Gynaecology, Federal Medical Centre Katsina between 26th August, 2018 and 3rd June, 2019. The study population consisted of 50 consecutive women between ages 20 and 45 with primary or secondary tubal factor infertility diagnosed by HSG. Thirteen out of the fifty patients had laparoscopy and dye test for further confirmation of their tubal pathology. The control were 100 healthy pregnant women of the same age group attending antenatal clinic within the study period. Basic evaluation for infertility was done for all patients presenting with infertility in line with departmental protocol and those with tubal factor infertility as determined by HSG or laparoscopy that met the inclusion criteria and consented to the study were selected and enrolled for the study. Participants' socio-

demographic and other data were collected using a structured questionnaire.

About 4 millilitre of venous blood was collected from the patients arm into a plain bottle and taken to the laboratory within 1 hour of collection. Serum was separated by centrifugation of the blood for 10 minutes at 1000 rpm and the samples were stored in the refrigerator at temperature below -20$^0$C until assay in batches. The Chlamydia IgG Enzyme linked immunosorbent assay (ELISA) MBS494135, was used in this study to determine the Chlamydia trachomatis antibody titre. The Chlamydia IgG Enzyme linked immunosorbent assay (ELISA) MBS494135 is a micro well strips used for quantitative analysis of IgG antibodies to Chlamydia trachomatis in human serum. The buffer was prepared by adding the content of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature. The desired number of coated strips was placed into the holder, 1:21 dilution of the test sample was prepared by adding 10ul of sample to 200ul of sample diluents and mixed well. One hundred microlitres of diluted sera, calibrator and control were dispensed into the appropriate well and were incubated for 20 minutes at room temperature, then the liquid was removed, and the wells washed with 300ul of wash buffer and blotted. One hundred microlitres of enzyme conjugate was dispensed into each well and incubated for another 20 minutes at room temperature. The enzyme conjugate was removed and washed with 300ul of wash buffer and blotted. One hundred microlitres of tetramethylbenzidine (TMB) substrate was dispensed into the well and incubated for 10minutes at room temperature after which 100ul of stop solution was added. It was then read within 15minutes using the automated ELISA reader calibrated at 450nm. Cut off value was calculated using calibrator factor, with value of ≥1.2 reported positive and result was recorded based on the titre level.

The data was statistically presented using descriptive statistics of means, percentages, range and standard deviation. The Statistical Packages for Social Sciences (SPSS), version 20.0 was employed. Student's t- test was used to compare means between normally distributed continuous variables. Median inter-quartile range was used to summarize skewed data which was compared with Mann Whitney U test. Chi-square and Fisher-Exact tests was used to determine the association between categorical variables. P value less than 0.05 was considered as statistically significant in this study. The study was undertaken after due approval from the Ethics and Research Committee of Federal Medical Centre, Katsina.

## Results

A total of 150 patients consisting of 50 cases and 100 controls were recruited for this study. The mean age of patient with tubal infertility was 32.4±5.6 while that of their pregnant counterpart was 31.5±4.7. There was no statistically significant difference between the mean ages of the two groups. (P =0.287). Majority of the patients in the study were married in monogamous family setting (64% and 75% for the infertile and pregnant women respectively). There was no statistically significant difference in the family setting of the two groups (P =0.160). Twenty-two (44%) of

Table I. Socio-demographic and gynaecological history of the cases and controls

| Variables | Cases (n=50) | Controls (n=100) | Statistical Test | P-value |
|---|---|---|---|---|
| Mean age ± SD (years) | 32.4± 5.62 | 31.5 ± 4.74 | t = 1.07 | 0.287 |
| Educational status | | | | |
| Primary | 7 (14.0%) | 14(14.0%) | | |
| Secondary | 18(36.0%) | 38(38.0%) | $\chi^2$ = 7.35 | 0.062 |
| Tertiary | 23(46.0%) | 30(30.0%) | | |
| No Formal education | 2(4.0%) | 18(18.0%) | | |
| Family settings | | | | |
| Monogamous | 32(64.0%) | 75(75.0%) | | |
| Polygamous | 18(36.0%) | 25(25.0%) | $\chi^2$ = 1.97 | 0.160 |
| Past pelvic surgeries | | | | |
| Yes | 15(30.0%) | 15(15.0%) | | |
| No | 35(70.0%) | 85(85.0%) | $\chi^2$ = 4.69 | 0.030* |
| Previous STDs | | | | |
| Yes | 22(44.0%) | 9(9.0%) | | |
| No | 28(56.0%) | 91(91.0%) | $\chi^2$ = 24.91 | < 0.001* |
| Past multiple sexual partners | | | | |
| Yes | 9(18.0%) | 3(3.0%) | Fishers exact | 0.003* |
| No | 41(82.0%) | 97(97.0%) | | |
| Previous pregnancy loss | | | | |
| No | 24(48.0%) | 62(62.0%) | | |
| Spontaneous | 19(38.0%) | 37(37.0%) | $\chi^2$ = 11.71 | 0.003* |
| Induced | 7(14.0%) | 1(1.0%) | | |

*Statistically significant

the infertile women had history of sexually transmitted diseases (STDs) while only 9(9%) of the pregnant controls volunteered this history, this was statistically significant (P<0.001).

**Table II. Chlamydia trachomatis antibody (IgG) titre in the cases and controls**

| Variables | Cases (n=50) | Controls (n=100) | Statistical Test | P-value |
|---|---|---|---|---|
| **Chlamydia Ab. titre(IgG)** Positive | 36(72.0%) | 23(23.0%) | $\chi^2$ = 33.54 | < 0.001* |
| Negative | 14(28.0%) | 77(77.0%) | | |
| **Median Chlamydia IgGtitre (IQR)** | 2.5(0.89-4.41) | 0.8(0.41-0.92) | U= 5403.0 | <0.001* |

*Statistically significant

**Table III. Association between severity of tubal damage and chlamydia antibody titre among the cases**

| Variables | Unilateral tubal damage | Bilateral tubal damage | Statistical Test | P-value |
|---|---|---|---|---|
| Chlamydia Ab. titre(IgG) Positive | 19(70.4%) | 17(73.9%) | $\chi^2$ = 0.08 | 0.781 |
| Negative | 8(29.6%) | 6(26.1%) | | |
| Median Chlamydia (IgG) titre (IQR) | 2.49(0.90-4.60) | 2.45(0.83-2.95) | U= 743.00 | 0.293 |

**Table IV. Relationship between chlamydia antibody (IgG) titre and duration of infertility.**

| Variables | < 4years (n= 9) | ≥4 years (n=41) | Statistical Test | P-value |
|---|---|---|---|---|
| **Chlamydia Ab. titre(IgG)** Positive | 6(66.7%) | 30(73.2%) | Fisher's Exact | 0.697 |
| Negative | 3(33.3%) | 11(28.8%) | | |
| **Median Chlamydia IgGtitre (IQR)** | 2.90(1.46-4.60) | 2.49(0.88-4.49) | U= 162.0 | 0.573 |

Secondary infertility was the commonest type of infertility with 29(58%) women while 21(42%) women presented with primary infertility.

Binary logistic regression was used to determine the association between tubal disease and chlamydia antibody titre reactivity. The result showed that controlling for all other factors, tubal disease was significantly associated with chlamydia antibody titre reactivity (p-value <0.001). Also, patients with tubal disease were 2 times more likely to have positive antibody titre than those without tubal disease (Odds ratio (OR) =2.0).

**Table V. Relationship between Antibody (IgG) titre and types of infertility.**

| Variables | Primary (n= 21) | Secondary (n=29) | Statistical Test | P-value |
|---|---|---|---|---|
| **Chlamydia Ab. titre(IgG)** Positive | 16(76.2%) | 20(69.0%) | $\chi^2$ = 0.32 | 0.574 |
| Negative | 5(23.8%) | 9(31.0%) | | |

Binary logistic regression was used to determine the association between the severity of tubal disease and Chlamydia antibody reactivity. The result showed that controlling for other factors, the severity of the tubal disease was not significantly associated with Chlamydia antibody titre reactivity (p-value =0.781), even though patients with bilateral tubal disease were 1.2 times more likely to have positive chlamydia antibody titre than those with unilateral tubal disease (OR =1.2).

## Discussion

Serological testing for past and recent chlamydia trachomatis infection can serve as a non-invasive and inexpensive screening test for infertile women with tubal pathology because of the relationship between the infection and its long-time sequela of tubal damage. This test may be very useful in Sub-Sahara Africa where there is high burden of sexually transmitted diseases and tubal factor infertility. This study employed the use of ELISA technique to detect the anti-chlamydial IgG in infertile women with tubal blockage and their pregnant controls.

In this study, the prevalence of positive anti-chlamydial IgG was 72% in the patients with tubal factor infertility and was 23% in the pregnant women, the difference of which was statistically significant (p-value < 0.001). The prevalence in those with tubal infertility was comparable with 65.8% to 74% reported in the previous studies done in Nigeria and Democratic Republic of Congo[20-23]. However, this was

higher than 30%- 38.6% reported by other studies done within and outside the country[7,10,11,24,25]. The high prevalence seen in this study can be explained by high incidence of sexually transmitted infections in our country, the population of the patient used and the ability of the test to pick patient with both recent and past exposure to chlamydia trachomatis infection. This also shows that despite the widespread antibiotics use with regimen including doxycycline in the treatment of both confirmed and suspected cases of genital tract infection, the incidence of chlamydia trachomatis infection has not drastically reduced in my centre.

Chlamydia trachomatis IgG antibody titre was higher in infertile women with tubal blockage than in the pregnant women and this was statistically significant (p-value < 0.001).This shows a strong association of chlamydia infection with tubal factor infertility. The strong association of chlamydia antibody titre (CAT) with tubal blockage seen in this study, support the fact that chlamydia trachomatis infection is implicated in the aetiology of tubal damage among the women with infertility and this finding was similar to previously published studies both within and outside the country[26,27,28,29,30]. This however is in variance with the study done in Iran, which reported no relationship between chlamydia IgG antibodies and tubal factor infertility[31]. Based on the association of CAT with tubal pathology, some studies have suggested that patients who present with suspected tubal infertility with positive or elevated CAT, can just be triaged for laparoscopy and dye test rather than HSG [11,22,32].

There was no relationship between chlamydia antibody titre and extent of tubal blockage in this study. The high titre could also not differentiate patient with unilateral from bilateral tubal blockage. This same finding was similar to the report of the study done by van Ess and colleagues[33], where chlamydial positivity did not depict severe tubal pathology. This finding differs from another study that reported linear relationship between chlamydia antibody titre and severity of tubal pathology[27]. The lack of relationship with the severity of tubal blockage seen in this study may be due to the method used in classifying the severity of tubal blockage which just classified the severity into unilateral and bilateral tubal blockage primarily based on HSG findings without considering other pathologies like tubal fibrosis, tubal distension and dense pelvic adhesions which can be seen on laparoscopy.

This study shows no significant relationship between chlamydia trachomatis infection and the type of infertility as was reported by other authors[26,34]. However, another study done in Nigerian reported that Chlamydia trachomatis infection plays a prominent role in the aetiology of primary infertility than secondary infertility[25] while Malik et-al.[35]noted a strong association between CAT and secondary infertility. There was also no relationship between the chlamydial antibody titre and the duration of infertility. This was similar to the findings of a study done in Egypt[7], this implies that the titre level was not affected by the duration of tubal infertility. The findings may be in line with the fact that chlamydia trachomatis antibodies detection decrease with time since last infection and most patient in this study is expected to have had the infection long time before the development of tubal pathology. Also, most patients in this study presented with prolong duration of infertility, during which chlamydial antibody titre is expected to have reduced.

## Conclusion

The prevalence of chlamydia trachomatis infection was very high among the infertile women with tubal disease in this study. The chlamydia antibody titre was also found to be significantly higher in infertile patients with tubal pathology compared to the pregnant women, though there was no significant association between the elevated chlamydia antibodies and severity (extent) of tubal damage.

## Recommendations

Due to strong association of chlamydia trachomatis infection with tubal pathology found in this study, this test may be an important part of fertility work-up of infertile patients with suspected tubal pathology. This test may also serve as a useful tool for early triage of infertile women for laparoscopy and dye test.

## Limitations

Laparoscopy and dye test being the gold standard for assessing tubal patency was not used for majority of the participants which limits the test of tubal patency in all infertile participants to Hysterosalpingography (HSG). Classification of severity of tubal blockage was based on HSG findings which could have missed some tubo-peritoneal pathologies that could be picked during laparoscopy.

## Line of future research

The incidence of chlamydia trachomatis infection recorded among the infertile women in this study was high, despite widespread antibiotics use in the treatment of genital infections. This calls for the need to access more ways of preventing chlamydia infection. There is also need for larger multicentre based study to give a stronger conclusion on the

association between chlamydia trachomatis antibody titre and tubal factor infertility

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