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Original Research Article

Chlamydia trachomatis infection and tubal ectopic pregnancy in Jos, Plateau State, Nigeria

Onyeji John[1]*, Kingsley C. Ozele[2], Lucious C. Imoh[3], Samuelson C. Keneolisa[1], Didamson D. Gloria[1], Chimezie N. D. Nwachukwu[4], Amaka N. Ocheke[5]

[1]Department of Obstetrics and Gynecology, College of Medicine and Allied Health Sciences, Bingham University, Jos Campus, Nigeria
[2]Department of Medical and Health Services, National Institute for Policy and Strategic Studies (NIPSS) Kuru, Jos, Plateau State, Nigeria
[3]Department of Chemical Pathology, Jos University Teaching Hospital, Jos Plateau State, Nigeria
[4]Department of Obstetrics and Gynaecology, Maitama District Hospital, FCTA Abuja, Nigeria
[5]Department of Obstetrics and Gynaecology, Jos University Teaching Hospital, Jos Plateau State Nigeria

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***Correspondence:**
Dr. Onyeji John,
E-mail: jononyeji@gmail.com

ABSTRACT

Background: Chlamydia trachomatis is an obligate intracellular organism that is sexually transmitted and mostly asymptomatic. It is capable of tubal damage with subsequent tubal ectopic pregnancy. IgG anti-Chlamydia antibody can be measured in women with past or latent Chlamydia trachomatis infection. This study aimed to determine association between prior Chlamydia trachomatis infection and tubal ectopic pregnancy, and predictive value of Chlamydia trachomatis IgG for tubal ectopic pregnancy.
Methods: This was a case control study carried out within Jos metropolis within 6 months period (November 2018 to April 2019). Study groups comprised of 40 women with tubal ectopic pregnancy and 40 uncomplicated second trimester pregnant women recruited from Jos University Teaching Hospital, Plateau State Specialist Hospital Jos, and Faith Alive Hospital Jos by convenience sampling. The subjects' socio-demographic factors, obstetric history, sexual and reproductive risk factors were obtained using study pro forma. Five milliliter of venous blood was collected from subjects to determine proportions and serum levels of IgG anti-Chlamydia antibody in cases and controls using BIOS Chlamydia T. IgG ELISA kits. Data obtained were analyzed using IBM-SPSS version 26.
Results: Patients with tubal ectopic pregnancy demonstrated significant evidence of prior Chlamydia trachomatis infection compared with intrauterine pregnant controls (45.0% versus 15.0%, p value =0.003), 4.6-fold risk association for tubal ectopic pregnancy (OR =4.63, 1.593-13.494; p value =0.005), and 75% positive predictive value.
Conclusions: This study revealed an association between prior Chlamydia trachomatis infection and tubal ectopic pregnancy with Chlamydia IgG having good predictive value for tubal pregnancy.

Keywords: Chlamydia trachomatis infection, JUTH, Tubal ectopic pregnancy

INTRODUCTION

Chlamydia trachomatis is the commonest sexually transmitted disease among women worldwide, most damaging, and it is about 2.5 times greater than the number of cases of gonorrhea.[1-4] The Center for Disease Control and prevention (CDC) reported over 1.6 million cases of Chlamydia trachomatis infections in 2021, with the highest incidence seen in women.[5] The prevalence of Chlamydia trachomatis in Jos Nigeria was reported as 56.1%.[3] Two-

third of the infection remains undetected because most infected women are asymptomatic and do not seek medical attention.[6] Chlamydia trachomatis is an obligate intracellular gram-negative bacterium with 15 serotypes. Serotypes A, B, Ba and C cause trachoma associated with chronic conjunctivitis; serotypes D, E, F, G, H, I, J and K cause genital tract infections; and serotypes L1, L2 and L3 causes Lympho-granuloma venereum (LGV) associated with genital ulcers.[5-7]

The virulence factor of this pathogen depends on the serotype, while others are spontaneously cleared in the cervix (serotype F and G), some are associated with persistent infection (serotype I, J and K).[8-10]

Genital infection caused by Chlamydia trachomatis may cause mucopurulent cervicitis in female and non-gonoccocal urethritis in male.[3,7] This infection when it is unrecognized and poorly treated may ascend to the upper reproductive tract resulting in pelvic inflammatory disease, and consequently leading to chronic pelvic pain and tubal damage with resultant tubal ectopic pregnancy and tubal factor infertility.[7,11,12]

The exact mechanism by which Chlamydia trachomatis causes tubal pathology is not yet fully understood. However, two mechanisms are assumed to be responsible for the development of the tubal damage. The first and the most important, is by persistent infection causing chronic low grade immune response, which attacks and destroys the host epithelial cells of the fallopian tubes with distortion of the luminal architecture leading to ectopic pregnancy. This leads to failure of transport mechanism to move the embryo through the tubes and into the uterine cavity for implantation.[13] The 50-kDa Chlamydia protein, which is a member of 60-kDa heat shock proteins, plays a role in this immunopathogenesis of Chlamydia disease.[13-15] Secondly, Chlamydia trachomatis itself can damage the host tubal epithelial cells directly when its replication cycle has been completed and elementary bodies are released by cytolysis of the host epithelial cells.[16,17]

Following Chlamydia trachomatis infection of the upper genital tract, anti-Chlamydia trachomatis IgG antibody is produced that may persist for a long time in serum even after antibiotic treatment.[12,16,18] The presence of this antibody has been strongly associated with poor reproductive outcome such as tubal ectopic pregnancy and tubal factor infertility.[19,20]

Hence, undiagnosed and untreated Chlamydia trachomatis infection will result to major epidemiological, social and economic problems. The current diagnosis for Chlamydia trachomatis infection utilizes gene amplification technique such as polymerase chain reaction (PCR) and, Ligase chain reaction (LCR) which has now replaced cell culture technique.[2-6,8] Other tests for Chlamydia trachomatis are direct fluorescent antibody and enzyme immunoassay. These various methods of testing Chlamydia trachomatis infection have a wide variation in the cost, sensitivity and specificity.[4]

Tubal ectopic pregnancy is a form of abnormal pregnancy in which the fertilized ovum implants outside the endometrial cavity, with the ampullary region of the fallopian tube being the most common site of implantation.[12] It is one of the most serious complications of acute salpingitis, and genital Chlamydia trachomatis.[13-15]

Tubal ectopic pregnancy remains a major public health problem especially in many developing nations where it is a significant contributor to pregnancy related morbidity and mortality.[14-16] It affects approximately 1-2% of all pregnancies in Europe and USA, prevalence in Jos is 1.74%.[17] The risk factors include previous ectopic pregnancy, pelvic inflammatory disease (PID) including acute salpingitis, endometriosis, previous pelvic surgery, smoking, multiple sexual consorts, early age of sexual debut and history of infertility.[12-15] However, a third of women with tubal ectopic pregnancy have no known risk factor.[18] The incidence of ectopic pregnancy is rising and this is attributable to increase in pelvic infections and improvement in diagnostic techniques.[16,19,20]

This study aimed to determine the association between prior Chlamydia trachomatis infection and tubal ectopic pregnancy, and the predictive value of Chlamydia trachomatis IgG for tubal ectopic pregnancy in Jos, Nigeria.

METHODS

Study design

This was a matched case-control study of 40 women with tubal ectopic pregnancy and 40 uncomplicated second trimester pregnant women as control, recruited from the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital (PSSH) Jos and Faith Alive Hospital Jos over 6 months period (November 2018 to April 2019). These three health institutions were selected by purposive sampling technique. This was based on the assumption that these three health facilities in Jos metropolis should be able to attract at least 50% of women with tubal ectopic pregnancy in Jos to represent the reference population.

Study place

This study conducted at Gynaecological emergency units and antenatal care units of Departments of Obstetrics and Gynaecology in JUTH, PSSH and Faith Alive Hospital Jos.

Study participants

The study participants comprised consented 40 women with tubal ectopic pregnancy confirmed by histology who had presented at gynaecological emergency units of the

three health facilities within Jos metropolis compared with 40 uncomplicated second trimester pregnant women matched for age.

Inclusion criteria

Consenting tubal ectopic pregnant woman diagnosed at laparotomy and confirmed by histological evaluation of extirpated tube, and uncomplicated second trimester pregnant women attending antenatal clinic without history of previous ectopic pregnancy, infertility, miscarriage or tubal surgery were included.

Exclusion criteria

Exclusion in this study includes using IUCD at the time of ectopic gestation, having blood transfusion within the last six months, being on any form of antibiotic in the last three months, using any hormonal contraception at the time of conception or declined consent. Controls that were excluded include those with past history of tubal ectopic pregnancy, infertility, miscarriage, tubal surgery or being a primigravida

Sample size estimation

The sample size was estimated using the formula

$$n = \frac{\{P_1(1-P_1) + P_2(1-P_2)\} \times (Z_{\alpha} + Z_{\beta})^2}{(P_1-P_2)^2}$$

Where; n: number of sample size in each of the group, P_1= proportion of positive IgG anti Chamydial antibody among cases (0.62 in Lagos)[19], P_2= proportion of positive IgG anti Chamydial antibody among controls (0.29 in Lagos)[19], $Z_{-\alpha/2}$= value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at the 0.05), $Z_{-\beta/2}$=value of standard normal distribution corresponding to the desired level of power (o.84 for a power of 80%)

$$n = \frac{\{(0.62\times0.38 + 0.29 \times 0.71)\} \times (1.96 + 0.84)^2}{(0.33)^2}$$

n= 31.78

The sample size was approximated to be 40 for the cases; and 40 for the controls considering an attrition rate of 20%.

Data collection

The participants were recruited based on convenience sampling technique, and informed consent was obtained. The subject's history included socio-demographic factors, obstetric history, sexual and reproductive risk factors were obtained using a study pro forma which was used to determine if they met the criteria for inclusion as 'case' (consented tubal ectopic pregnant patients), or 'control' (consented uncomplicated second trimester pregnant women).

Serological assay

Five milliliter (5ml) of venous blood was collected from the antecubital fossa of all the participants and emptied into clean, sterile plain specimen bottle. The specimen was collected from participants with tubal ectopic pregnancy before blood transfusion and surgery. The blood was taken to the laboratory where the laboratory scientist allowed the specimen to clot and sera obtained. The sera were frozen at -20^{0}C until analyzed in batches for Chlamydia IgG antibodies by a consultant chemical pathologist. The assay was done using the BIOS Chlamydia T. IgG ELISA kits, Chlam-T-G-326 (Chemux BioScience, INC. USA); an indirect solid-phase Enzyme Immunoassay (EIA) test. This test quantitatively measured IgG antibodies to Chlamydia trachomatis in human serum. The reagent test strip was brought to room temperature and 10 microlitre pippetted serum was assayed with reagent control samples for Chlamydia trachomatis antibodies as specified by the manufacturer. Absorbance value of Calibrator was calculated and the results (titre levels) of the samples were calculated thus: Sample absorbance value/Calibrator absorbance value x 10. The titre levels refer to the denominator of the dilution. Positive result was defined as ≥10% in excess of calibrator titre level (i.e. 10) = 11. Therefore, positive result was defined as ≥11 while <11 was classified as negative.

Data analysis

The collected data were compiled using Microsoft excel and analysed using IBM-SPSS 26. Odds Ratio (OR) was calculated to determine the effect of previous exposure to Chlamydia trachomatis infection on tubal ectopic pregnancy and logistic regression performed to determine association.

RESULTS

A total of 80 women were enrolled in this study. This comprised of 40 women who had tubal ectopic pregnancy (cases) matched for age with 40 uncomplicated parous second trimester pregnant women (control).

Mean ages for cases and controls were 29.45±5.66 years and 29.03±4.41 years respectively. The participants were largely (83.8%) below age 35 years; 34 (85%) of cases were married and 6 (15%) were single while the control were all married. Majority of cases 24 (60.0%) and controls 33 (82.5%) have had between 1-4 children. Approximately 63% of tubal ectopic pregnancy occurred at gestational age <10 weeks with average gestational age of 8.83±1.57 weeks; average gestational age for control was 20.88±3.78 weeks (Table 1).

Table 1: Socio-demographic characteristics of study participants.

Characteristics	Study group	
	Case, n=40 (%)	Control, n=40 (%)
Age (years)		
<35	31 (77.5)	36 (90.0)
≥35	9 (22.5)	4 (10.0)
Education		
Primary	4 (10.0)	2 (5.0)
Secondary	20 (50.0)	23 (57.5)
Tertiary	16 (40.0)	15 (37.5)
Marital status		
Married	34 (85.0)	40 (100.0)
Single	6 (15.0)	0 (0.0)
Occupation		
Housewife	14 (35.0)	21 (52.5)
Business	15 (37.5)	6 (15.0)
Civil servant	4 (10.0)	10 (25.0)
Others	7 (17.5)	3 (7.5)
Religion		
Christianity	32 (80.0)	18 (45.0)
Islam	8 (20.0)	22 (55.0)
Parity		
0	14 (35.0)	0 (0.0)
1-4	24 (60.0)	33 (82.5)
≥5	2 (5.0)	7 (17.5)
EGA		
<10	25 (62.5)	0 (0.0)
≥10	15 (37.5)	40 (100.0)
Alcohol intake		
Yes	8 (20.0)	0 (0.0)
No	32 (80.0)	40 (100.0)
Smoking		
Yes	0 (0.0)	0 (0.0)
No	40 (100.0)	40 (100.0)

Table 2: Chlamydia IgG titre in cases and control (positive chlamydia antibody titre ≥11.0).

Chlamydia IgG titre levels	Cases, n=40 (%)	Control, n=40 (%)	χ^2	P value
0-10.9	22 (55.0)	34 (85.0)	11.771	0.008
11-11.9	2(5.0)	3 (7.5)		
12-12.9	1 (2.5)	0 (0.0)		
≥13	15 (37.5)	3 (7.5)		
Positive chlamydial antibody				
Yes	18 (45.0)	6 (15.0)	8.571	0.003
No	22 (55.0)	34 (85.0)		
Mean antibody titre (mean±SD)	11.07±2.91	8.75±2.45	t-test=3.880	0.001

Prevalence of IgG anti-chlamydia antibody in cases was 45.0% and 15.0% in controls. The difference was statistically significant (p value=0.003). The mean serum level of IgG anti-chlamydia antibody titre for cases was 11.07±2.91 and 8.75±2.43 for controls. The difference was statistically significant (p value =0.001). The overall prevalence of IgG anti-chlamydial antibody was 30.0% (Table 2).

An 57.5% of the cases gave history suggestive of past PID while 42.5% did not, none of the controls gave history suggestive of past PID (p<0.05). There was no significant difference in age at coitarche in both cases and controls.

57.5% of cases gave history of infertility; while 37.5% had induced abortion. 6 (15.0%) of the cases had recurrent ectopic pregnancy. Significant number of women with ectopic pregnancy had multiple sex partners (67.5%) than the controls (42.5%), p value=0.025 (Table 3).

Table 3: Relationship between clinical/risk factors for pelvic infection and chlamydia trachomatis among study participants.

Characteristics	Case, n=40 (%)	Control, n=40 (%)	χ2	P value
STI				
Yes	23 (57.5)	0 (0.0)	32.281	0.001
No	17 (42.5)	40 (100.0)		
Lower abdominal pain				
Yes	18 (45.0)	0 (0.0)	23.226	0.001
No	22 (55.0)	40 (100.0)		
Fever				
Yes	9 (22.5)	0 (0.0)	-	0.002
No	31 (77.5)	40 (100.0)		
Dyspareunia				
Yes	13 (32.5)	0 (0.0)	15.522	0.001
No	27 (67.5)	40 (100.0)		
Sexual partner				
1	13 (32.5)	23 (57.5)	5.051	0.025*
≥2	27 (67.5)	17 (42.5)		
Previous history of PID				
Yes	23 (57.5)	0 (0.0)	32.281	0.001
No	17 (42.5)	40 (100.0)		
Age at coitarche (years)				
<18	20 (50.0)	18 (45.0)	0.201	0.654
≥18	20 (50.0)	22 (55.0)		
Infertility				
Yes	23 (57.5)	0 (0.0)	32.281	0.001
No	17 (42.5)	40 (100.0)		
Used condom				
No	24 (60.0)	29 (72.5)	1.398	0.237
Occasionally	16 (40.0)	11 (27.5)		
Induced abortion				
0	25 (62.5)	40 (100.0)	18.462	0.001
≥1	15 (37.5)	0 (0.0)		
Previous ectopic pregnancy				
Yes	6 (15.0)	0 (0.0)	-	0.026
No	34 (85.0)	40 (100.0)		

*; Having multiple sexual partner is associated with tubal ectopic pregnancy

Table 4: Sensitivity, specificity and predictive values of Chlamydial IgG for tubal ectopic pregnancy.

Chlamydial antibody	Tubal ectopic pregnancy		Total (%)
	Yes	**No**	
Yes	18	6	24
No	22	34	56
Total	40	40	80
PPV			75.0
NPV			60.7
Sensitivity			45.0
Specificity			85.0

Positive Likelihood Ratio=Sensitivity/1-specificity=0.45/0.15=3.0
Negative Likelihood ratio=1-sensitivity/specificity=0.55/ 0.85=0.6

The positive predictive value (PPV) of tubal ectopic pregnancy using IgG anti-chlamydia antibody was 75.0%. The negative predictive value (NPV) of IgG for tubal ectopic pregnancy was 60.7%. The sensitivity was 45% while the specificity was 85.0%. The positive likelihood ratio was 3.0, and negative likelihood ratio was 0.6 (Table 4).

Odds ratio for chlamydia infection was 4.636; 95% CI=1.593-13.494, p value=0.005, and the Odds ratio for multiple sex partners was 2.658; 95% CI=1.045-6.762, p value=0.040 (Table 5).

Table 5: Logistic regression of factors associated with tubal ectopic gestation.

Factors	Odds ratio	95%CI	P value
Chlamydia antibody			
Yes	4.636	1.593-13.494	0.005
No	1		
Sexual partner			
≥2	2.658	1.045-6.762	0.040
1	1		

DISCUSSION

This study showed significant association between prior Chlamydia trachomatis infection and tubal ectopic pregnancy. It also revealed IgG anti-chlamydial antibody to have a high predictive value for tubal ectopic pregnancy.

The sero-prevalence of IgG anti-chlamydial antibody was significantly higher (45.0%) in women with tubal ectopic pregnancy (cases) than uncomplicated second trimester pregnant women (control) with sero-prevalence of 15.0%, P-value=0.003. This is in keeping with the findings of Adewumi et al in Lagos (62.4% versus 29.0%), Agholor et al in Benin (48.0% versus 16.3%) and Ibe et al in Port Harcourt (53.1% versus 28.1%).[16,19,21]

The average IgG anti-Chlamydial antibody titre for women with tubal ectopic pregnancy was 11.07±2.91 and that of uncomplicated second trimester pregnant women was 8.75±2.43. This is statistically significant (p-value=0.001), and it's in keeping with other studies on IgG anti-Chlamydial antibody and tubal ectopic pregnancy.[16,22,23]

However, this is divergent with the reports by Anderson et al in Northern Jutland Denmark and Low et al in Uppsala Denmark.[24,25] Both studies showed no association between IgG anti-chlamydial antibody and tubal ectopic pregnancy, there submission was that tubal damage following chlamydia infection causes tubal blockage thereby preventing ectopic pregnancy.

The overall prevalence of IgG anti-chlamydia antibody in this study was 30.0%. This is comparable with prevalence of 30.0% in UK and 38.5% in Zaria Nigeria.[26,27] However, it is lower than 56.1% reported in Jos, Lagos 51.0%, and South Eastern Nigeria 40.7%, but higher than the prevalence in Benin 13.3%.[3,9,28,29]

There was no significant difference in anti-chlamydia IgG antibody level between those with history suggestive of PID and those without such history. This may be explained by genital Chlamydia infection being asymptomatic in 80% of cases.[3]

The factors found to be associated with tubal ectopic pregnancy in this study were chlamydia antibody positivity and having two or more sex partners. These factors were subjected to logistic regression and chlamydia antibody positivity was found to have approximately 4.6-fold risk association for tubal ectopic pregnancy (OR 4.636; 95% CI 1.593-13.494, p=0.005). This is in keeping with the findings in other reports.[16,19,21-23] In this study having two or more sex partners has approximately 2.7-fold risk association for tubal ectopic pregnancy (OR 2.658; CI 1.045-6.762, p value=0.040), this finding was corroborated by the Benin study.[16]

Chlamydia antibody level could be of predictive value in detection of tubal damage and is quantitatively related to the severity of damage.[30-32] The sensitivity and specificity of Chlamydia antibody testing (CAT) in this study were 45.0% and 85.0% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 75.0% and 60.7% respectively. A positive predictive value of tubal ectopic pregnancy of 75.0% means that for women with suspected tubal ectopic pregnancy who tested positive to IgG anti Chlamydia antibody has 75% chance of having tubal ectopic pregnancy due to tubal damage, and a negative predictive (NPV) of 60.7% means that women with suspected tubal ectopic pregnancy who tested negative to IgG anti Chlamydia antibody have 60.7% chance of not developing tubal ectopic pregnancy due to tubal damage.[33]

The positive predictive value of Chlamydia antibody testing for tubal ectopic pregnancy of 75.0% in this study is higher than 66% noted by Olaleye et al for tubal damage, but lower than 80.7% by Gharajeh et al, and 100% by Singh et al.[30-32] The negative predictive value of 60.7% is lower than 89% by Olaleye et al, 86.7% by Gharajeh et al, and 100% by Singh et al.[30-32] The positive likelihood ratio of 3.0 was noted with a negative likelihood ratio of 0.6. A positive likelihood ratio greater than 1 indicates the test is associated with the disease.[33] Hence; in situation of diagnostic dilemma for un-ruptured tubal ectopic pregnancy, where no facility for diagnostic laparoscopy, Chlamydia IgG antibody determination could be helpful in making diagnosis of tubal ectopic pregnancy.

This study has few limitations. Neisseria gonorrhea and other microorganisms such as Mycoplasma genitalium that can cause tubal ectopic pregnancy were not ruled out. The result would have been more representative if polymerase

chain reaction (PCR) or ligase chain reaction (LCR) were assayed alongside anti Chlamydia trachomatis antibody.

CONCLUSION

This study has shown that there is an association between prior Chlamydia trachomatis infection and tubal ectopic pregnancy, and Chlamydia IgG positivity could be predictive of tubal ectopic pregnancy. Having multiple sexual partners was associated with tubal ectopic pregnancy. Hence, the need to re-address safe sex practices through sex education; and prompt and effective treatment of Chlamydia trachomatis infection will likely reduce the burden of tubal ectopic pregnancy.

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