Original Article

ASSOCIATION BETWEEN ANTI-CHLAMYDIA TRACHOMATIS ANTIBODY AND ECTOPIC PREGNANCY AT THE FEDERAL MEDICAL CENTRE, UMUAHIA, ABIA STATE

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Abstract

Background: The rise in the incidence of ectopic pregnancies is partly attributed to the increase in pelvic infection rates. Pelvic inflammatory disease (PID) is the most important cause of tubo-peritoneal damage and subsequent reproductive morbidity. Chlamydia trachomatis (CT) is the commonest bacterial sexually transmitted infection worldwide and has been found to be the commonest cause of salpingitis and PID.

Objective: To determine the association between prior Chlamydia trachoma s infection and the development of ectopic pregnancy.

Materials and method: This was a hospital-based case-control study in which 43 women with diagnosis of ectopic pregnancy confirmed at laparotomy and also with the histology of extirpated specimen (cases) and another 43 with uncomplicated intrauterine pregnancy matched for maternal age (controls) were recruited. Structured interviewer-guided questionnaire was utilized to extract relevant data. Venous blood sample was taken from each woman for quantitative anti-Chlamydia trachomatis IgG antibody titres using an automated photometer. Data was analysed using SPSS version 20.

Results: A positive IgG antibody index seen in 62.8% of the case arm was significantly higher than 23.3% in the controls ($p<0.000$). The mean titre in those who were positive was also higher in the case arm. The Population attributable risk percent (PAR%) was 46%. Furthermore, the association between CT infection and ectopic pregnancy was sustained when the socio-demographic characteristics and sexual and reproductive factors were controlled for. Significant risk factors for ectopic pregnancy include being single (OR 32.82 CI 2.97- 362.75), early age at sexual debut (OR 4.65; CI 1.28-16.11) and a positive history of induced abortion (OR 2.06; CI 0.78-5.38).

Conclusion: Chlamydia trachomatis causes tubo-peritoneal damage which can predispose to ectopic pregnancy. A protocol for screening and prevention should be drawn for reproductive age women.

Keywords: Chlamydia trachomatis, Pelvic inflammatory disease, Ectopic pregnancy.

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INTRODUCTION

Ectopic pregnancies often pose significant diagnostic and treatment dilemma and contributes to the increasing pregnancy-related morbidity and mortality especially in low-resource settings.[1] Although the aetiology is multifactorial, it is often a serious sequela of pelvic inflammatory disease.[2]

Pelvic inflammatory disease (PID) may lead to ectopic pregnancy, tubal factor infertility and chronic pelvic pain.[3,4] The cause of salpingitis and PID in majority of the cases is either *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* or both with the former contributing more than half of proven cases.[5]

Ectopic pregnancy (EP) remains a major public health problem and its incidence has been

rising.[6] Approximately 1-2% of pregnancies in Europe and the United States of America are ectopic.[6] In the developing world, the incidence is much higher accounting for 1.5% of all pregnancies and 4.1% of gynaecological admissions in a review in Sokoto.[7] It also accounted for 1.1% of all pregnancies and 5.2% of gynaecological admissions in Nnewi[1] in the same geopolitical zone as Umuahia.

Risk factors for EP include PID, intrauterine contraceptive devices, progesterone only pills, previous tubal ectopic pregnancy, previous tubal surgeries, previous abortions, and assisted reproductive technologies.[6,7]

In our environment, majority of cases present late as usually after rupture and intraperitoneal haemorrhage.[1] However, with early diagnosis due to widespread use of ultrasound and other modern diagnostic tools, the prognosis has improved.

Infection with CT is generally asymptomatic in approximately 80% of women and 50% of men.[3] Many patients are completely ignorant of the existence of CT and consequently do not know of its associated complications. Some researchers have observed that men constitute a large reservoir of chlamydial infection and could repeatedly re-infect their partners inadvertently.[8]

The importance of sub-clinical PID became apparent with observations that most of the women with tubal factor infertility or ectopic pregnancy who had serologic evidence of CT infection apparently had no history of PID.[5,8] *Chlamydia trachomatis* infection is the most prevalent sexually transmitted bacterial infection recognized throughout the World.[9]

An estimated 340 million CT infections occur annually among sexually active adolescents and young adults worldwide with the largest proportion in the region of South and South-East Asia followed by sub-Saharan Africa.[10] The peak age is 20-24 years.[3] Reported incidence rates have been on the increase due mainly to initiation of screening programs, improvement in sensitivity of diagnostic tests and continued high infection rates.[2,9]

Since majority of infected individuals are asymptomatic, screening is therefore necessary to identify and treat this infection. Annual screening of all sexually active women is recommended.[3,9] In some studies, up to 30% of women with untreated infection develop PID.[11]

Regardless of symptom severity, the consequences of PID may be severe. Of those with PID, 20% will become infertile, 18% will experience debilitating chronic pelvic pain and 9% will have threatening tubal pregnancy.[8,12] Furthermore, some studies have shown an association between CT and precancerous lesions of the cervix.[13] It is thought that this infection causes epithelial injury that facilitates human papilloma virus (HPV) entry.[11]

Estimated direct cost of CT in the United States in 2008 alone was $516.7 million.[8] Even more important are the intangible costs including the psychological and emotional injury caused by infertility, chronic pelvic pain and ectopic pregnancy.

Risk factors that have been identified for CT infection include no or low educational attainment, two or more lifetime partners, multiple sexual partners, high risk male partner, smoking, prior history of vaginal discharge, previous user of intrauterine contraceptive device, previous history of induced abortion, early age at sexual debut and inconsistent use of condom.[3,14]

The link between past CT infection and ectopic pregnancy was initially based on sero-epidemiological case-control studies.[15,16,17,18] Agholor et al[14] demonstrated higher titres in women with ectopic pregnancy (48%) when compared to controls (16.3%). Ibe et al,[15] found

that 53.1% of cases (women with ectopic pregnancy) were positive for CT antibody as against 28.1% of controls with two-thirds of positive cases found to have pelvic adhesions at laparotomy. Higher titres have been shown to correlate with upper genital tract disease.[14,15,19] Complex arrays of technologies are now available for the diagnosis of CT infections. Serological tests are very useful because after an acute episode or after antibiotic use, the organism or it's antigens may no longer be detectable and chlamydia antibodies in the serum may be the only indication of previous chlamydia involvement.[5,20]

While some researchers found an association between prior CT infection and ectopic pregnancy,[2,15] other show contrasting findings[21,22,23] This underscores the need for further studies aimed at establishing the pattern of this relationship and its associations in our locality. Despite the need, no study was found that looked at the prevalence of CT in reproductive age women in Abia or any South-Eastern state, hence the need to document the prevalence of CT among various subgroups of pregnant women in this centre.

MATERIALS AND METHOD

The study was carried out at the Federal Medical Centre, Umuahia, Abia State, South-East Nigeria. This was a hospital-based case-control study. The study group (cases) was made up of 43 women who had laparotomy for ruptured ectopic pregnancy and subsequent confirmatory histology of the extirpated segment. Participants were counselled and informed consent obtained. A consecutive sampling method was employed in selecting the cases. The control group was made up of 43 age-matched pregnant women with uncomplicated intrauterine gestation in the second trimester of pregnancy who came for routine antenatal visit. A simple random sampling was employed. Every time a case was selected, age-matched women (± 3 years) that attended the antenatal clinic the next working day were assigned serial numbers in a piece of paper and the pieces of paper poured into a sample bag. A blind pick was performed to select a control sample. The participants will then complete the consent form. The study was carried out within a period of 7 months from first of August, 2017 to the twenty-eight of February, 2018. The appropriate sample size was calculated using the formula below which is suitable for sample size calculation for comparative study of two population proportions with binary outcome:[24]

$$\text{Sample size (n)} = \frac{2\bar{p}(1-\bar{p})(Z_{\alpha/2} + Z_{\beta})^2}{(p_1-p_2)^2}$$

Giving a response rate of 80% (0.8), for contingencies (including participants withdrawing from the study, sample processing and recording errors) and using attrition rate formula:

$n_2 = n_1/1\text{-NR}$. Thus, sample size will be approximately 43 patients.

Exclusion criteria include women who had had blood transfusion in the preceding 3 months, women who had intrauterine contraceptive device in situ or were on progesterone-containing contraceptives prior to the ectopic pregnancy, women on any antibiotic therapy in the preceding 3 months, women with history of tuboplasty or laparoscopic tubal cannulation prior to conception and women who conceived through assisted reproductive technique. Approval was obtained from the Health Research Ethics Committee (HREC) before recruitment of subjects was commenced. Five millilitres of blood sample were taken from prominent volar surface or forearm vein of participants while observing universal precautions into a plain container and left standing for 1 – 2 hours to allow for clot retraction and/or was centrifuged at 3000 revolutions per second for 5 minutes to obtain the serum. The labelled sera were stored in a

refrigerator at – 20 ^{o}C until analysed in batches. The specimen was collected from patients with ruptured ectopic pregnancy before blood transfusion or surgery. The assay was done using *Chlamydia trachomatis* immunoglobulin G (IgG) ELISA kit (CALBIOTECH Inc., 1935 Cordell Ct, El Cajon CA 92020 USA; www.calbiotech.com). Data obtained was analysed using the Statistical Package for Social Sciences (SPSS) version 20 (IBM incorporated, Illinois USA).

Descriptive statistics which include frequency, mean and standard deviation were used to summarise the variables. The associations between categorical variables were evaluated using logistic regression analysis. A P-value of < 0.05 was considered statistically significant.

RESULTS

Eighty-six women who fulfilled the inclusion criteria and consented to the study were recruited over a period of 7 months. Both groups were age-matched (± 3 years) and had similar age statistics and this formed a good basis for comparison.

The mean age in the cases arm was 29.9 years ± 5.9 (Range 18 – 44) while the mean age for the controls was 30.4 ± 6.2 years (range 19-43). Majority of the participants were Christians (91.9%) (Pentecostal and orthodox churches), of Ibo tribe (81.4%) and resided in the urban area (70.9%). Here, 55.8% of participants in the case arm had primary education when compared to 7% of the controls. (37.2% of controls had tertiary education).

There was also a preponderance of single women in the case arm (55.8%) than in the controls (9.3%) (p = 0.001) (Table 1).

Table 1; Socio-demographic characteristics of respondents

Characteristic	Control; n (%)	Case arm; n; (%)	*P* - value
Age in years			
16-20	1 (2.3)	2 (4.7)	0.992
21-25	7 (16.3)	7 (16.3)	
26-30	15 (34.9)	14 (32.6)	
31-35	12 (27.9)	13 (30.2)	
36-40	5 (11.6)	5 (11.6)	
>40	3 (7.0)	2 (4.7)	
Residence			
Urban	31 (72.1)	30 (69.8)	0.682
Rural	12 (27.9)	13 (30.2)	
Level of education			
No formal	4 (9.3)	3 (7.0)	0.001
Primary	3 (7.0)	24 (55.8)	
Secondary	20 (46.5)	10 (23.3)	
Tertiary	16 (37.2)	6 (14.0)	
Marital status			
Married	34 (79.1)	19 (44.2)	
Single	4 (9.3)	24 (55.8)	0.001
Divorced	2 (4.7)	0 (0.0)	
Widowed	3 (7.0)	0 (0.0)	
Religion			
Pentecostal	17 (27.9)	23 (53.5)	0.463
Orthodox	22 (36.1)	17 (39.5)	
Muslim	3 (4.9)	3 (7.0)	
Atheist	1 (1.6)	0 (0.0)	
Tribe			
Ibo	33 (76.7)	38 (88.4)	0.128
Non-Ibo	10 (23.3)	5 (11.6)	
Social class			
1	15 (34.9)	11 (25.6)	0.247
2	23 (53.5)	10 (23.3)	
3	5 (11.6)	22 (51.2)	

The mean gestational age at recruitment for the control arm was 16.7 ± 4.1 weeks (range 12 weeks and 4 days-23 weeks and 6 days) while the mean gestational age at presentation for the ectopic arm was 8.6 ± 1.6 weeks (range 5 weeks and 6 days-13 weeks and 3 days) (Table2).

Table 2: Gestational age distribution of respondents

Case arm		Control arm	
GA at presentation	**n; (%)**	**GA at Presentation**	**n; (%)**
≤6 weeks	1 (2.3)	≤16 weeks	17 (39.5)
6.1-8 weeks	10 (23.3)	16.1-20 weeks	16 (37.2)
8.1-10 weeks	15 (34.9)	20.1-24 weeks	10 (23.3)
≥10.1 weeks	1 (2.3)	>24 weeks	0 (0.0)
Total 43 (100)		**Total 43 (100)**	

The sexual and reproductive behaviour of the participants are shown in Table 3. More women began their sexual debut early (less than 18 years) in both case arm (67.4%) and control arm (62.8%). This was not statistically significant (p< 0.411). In the case arm, 34.9% were nulliparous when compared to 20.9% in the case arm. Furthermore, 46.7%% of the nulliparous women were positive in the case arm as against 44.5% in the control arm. This was not significant. (p < 0.544). More women in the case arm had multiple sexual partners (60.5%) when compared to the control group (41.9%) (OR 13.24; CI 2.97-58.62). The mean number of induced abortions for the case arm was 2.0 ± 0.62 with over 64.3% having had at least one induced abortion. Interestingly, 53.5% of the control group are primigravidae. Fourteen women (32.6%) in the case arm have had a history of PID when compared to 9.3% in the control group. This was statistically significant (p< 0.008). Many of the women who had EP (52.5%; n=23) reported abnormal vaginal discharge. The cases arm had more prior abdomino-pelvic surgeries 16.3% (n=7) when compared to only 6.6% (n=4) among the control group (p<0.260). More patients in the case arm have had post-abortion or puerperal sepsis (23.3%, n=10) as against 4.7% (n=2) in the control arm (p< 0.013).

Table 3: Sexual and reproductive history of participants

Characteristic	Control; n (%)	Cases; n (%)	*P* value
Parity			
0	9 (20.9)	15 (34.9)	0.0149
1	18 (41.9)	0 (0.0)	
2-4	11 (25.6)	25 (58.1)	
≥ 5	5 (11.6)	3 (7.0)	
Coitarche			
<18	27 (62.8)	29 (67.4)	0.411
≥18	16 (37.2)	14 (32.6)	
MSP			
Yes	18 (41.9)	26 (60.5)	0.065
No	25 (58.1)	17 (39.5)	
Induced abortion			
None	23 (53.5)	16 (37.2)	0.034
1	15 (34.9)	14 (32.6)	
≥2	5 (11.6)	13 (30.2)	
History of PID			
Yes	4 (9.3)	14 (32.6)	0.236
No	39 (90.7)	29 (67.4)	
Post abortion/Puerperal sepsis			

Yes	2 (4.7)	10 (23.3)	0.013
No	41 (95.3)	33 (76.7)	
Previous abdomino-pelvic surgeries			
Yes	4 (6.6)	7 (16.3)	0.260
No	39 (90.7)	35 (81.4)	

*MSP=multiple sexual partner

Table 4 showed that the IgG antibody index was positive in 62.8% of case arm and 23.3% in the control group (p<0.000). The difference was statistically significant. The presence of *Chlamydia trachomatis* IgG antibody is associated with a 6-fold increased risk of ectopic pregnancy (OR=5.70; CI 3.08-10.54). There was also higher mean titre in those who had ectopic pregnancy (1.20 ± 0.58) when compared to controls (0.63 ± 0.36) (Figure 3 and 4).

Table 4: IgG antibody indices of the participants

	Positive; n (%)	Negative; n (%)	P-value
Control	10 (23.3)	33 (76.7)	0.000
Case	27 (62.8)	16 (37.2)	

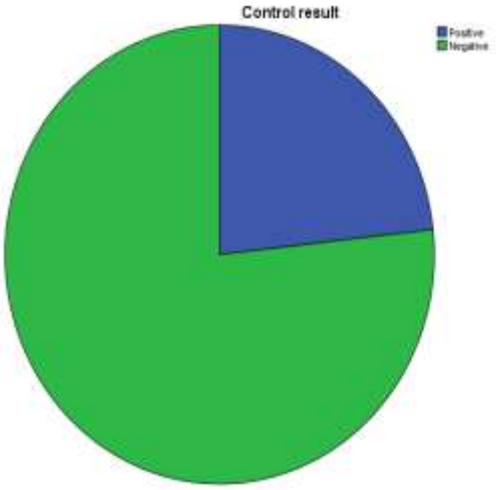

Figure 1: Pie chart showing antibody result in the control arm

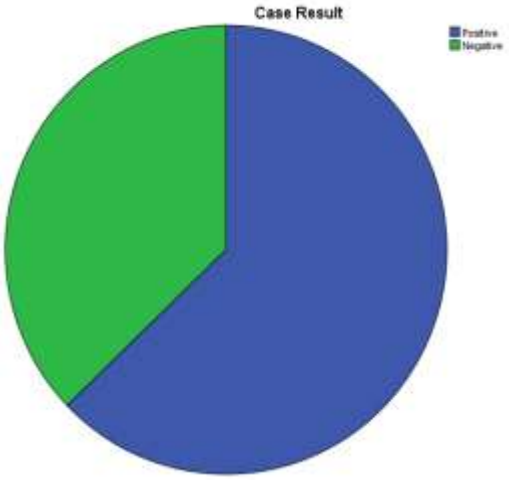

Figure 2: Pie chart showing antibody result in the case arm

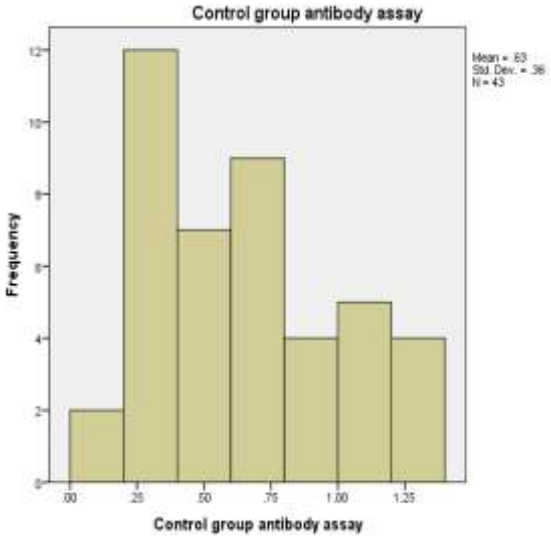

Figure 3: Histogram showing IgG antibody index distribution in the control arm

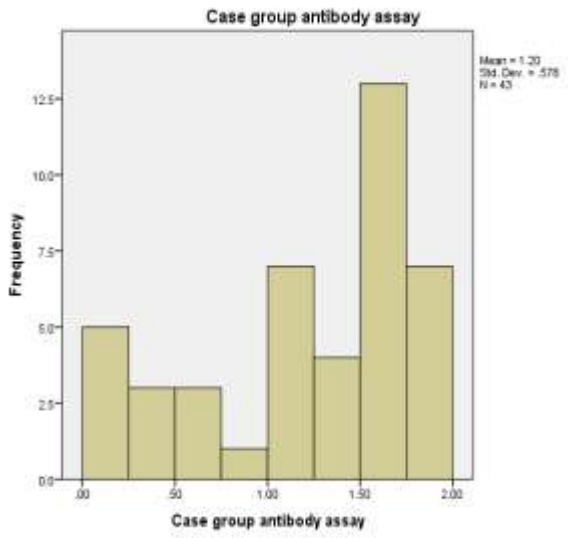

Figure 4: Histogram showing IgG antibody index distribution in the case arm

The attributable risk (AR%) is calculated using the formula:[25]
AR%= $I_e - I_u / I_e$ x 100. Where:

AR% = percent of incidence of the disease in the exposed that can be attributed to the exposure
I_u = Incidence in the unexposed =23.3, I_e = Incidence in the exposed = 62.8. Therefore; AR% = 62.8-23.3/62.8 x 100 = 62.9%. The population attributable risk (PAR %) is the incidence of the disease in the total population (exposed and unexposed) that is due to the exposure. It is the proportion of the incidence of the disease in the population that would be eliminated if the exposure were eliminated.[25] PAR % = $I_p - I_U / I_p$ X 100 where, I_p = the incidence in the total population = 43.0%, I_u = the incidence in the unexposed = 23.3%. Therefore; PAR % = 43.0-23.3/43.0 X 100 = 46.0%.

Multivariate analysis of the associated risk factors

Table 5 shows that being single, having had an early age at sexual debut, a positive history of puerperal/post-abortion sepsis, a positive history of PID and middle socio-economic class are independently associated with ectopic pregnancy at the FMC, Umuahia. The effects of these risk factors for ectopic pregnancy were increased when a positive anti-chlamydial antibody titre was added to the variables.

Table 5: Multivariate analysis of the risk factors

Variable	IgG antibody assay		*P*-value	OR (95% CI)
	Positive, n (%)	**Negative, n (%)**		
Marital Status				
Single	21 (48.8)	3 (7.0)	0.001	5.47 (1.82-16.47)
Married	6 (14.0)	13 (30.2)		
HLE				
No formal	1 (2.3)	2 (4.7)	0.4610	
Primary	14 (32.6)	10 (23.3)		
Secondary	8 (18.6)	2 (4.7)		
Tertiary	4 (9.3)	2 (4.7)		
Residence				
Urban	16 937.2)	7 (16.3)	0.137	2.72(0.71-10.41)
Rural	11 (25.6)	9 (21.0)		
Socio-econ class				
High	4 (9.3)	7 (16.3)		
Middle	10 (23.6)	0 (0)	0.006	
Low	13 (30.2)	9 (21.0)		
MSPs				

Yes	19 (44.2)	7 (16.3)	0.081	3.05 (0.84-11.07)
No	8 (18.6)	9 (21.0)		
Abortion				
None	11 (25.6)	5 (11.60	0.058	1.49 (0.42-5.39)
1	10 (23.3)	4 (9.3)		
≥2	6 (14.0)	7 (16.3)		
Coitarche				
<18	23 (53.5)	6 (14.0)	0.004	9.58 (2.21-41.56)
≥18	4 (9.30	10 (23.3)		
Post abortion/Puerperal				
Yes	10 (23.3)	0 (0)	0.005	1.94(1.39-2.40)
No	17 (39.6)	16 (37.2)		
Hx of PID				
Yes	19 (44.2)	8 (18.6)	0.018	2.36(0.66-8.56)
No	8 (18.6)	8 (18.6)		
Vaginal discharge				
Yes	16 (37.2)	7 (16.3)	0.324	1.87(0.55-6.53)
No	11(25.6)	9 (21.0)		
Prior A-P surgery				
Yes	5	2	0.605	1.59(0.27-9.35)
No	22	14		

*HLE = Highest level of education; MSPs = Multiple sexual partners; Socio-econ=socioeconomic status; Postab = post abortion sepsis; Hx of PID = History of pelvic inflammatory disease: A-P = abdominopelvic.

Logistic regression analysis;

Table 6 is a summary of output tables of binary logistic regression of the risk factors for ectopic pregnancy. Being single, having had an induced abortion and early coitarche are independently associated with ectopic pregnancy at the Federal Medical Centre, Umuahia. Single women were 32-fold more likely to have ruptured ectopic pregnancy than married women (*p* 0.000; CI 2.98-362.45). Those who had their first sexual intercourse before the age of 18 years were 4 times more likely to have ectopic pregnancy than those who delayed sexual debut ($p<0.001$; CI 1.28-16.11). Those who self-reported at least one induced abortion were 2 times more likely to present with ectopic pregnancy than those who had not. (OR 2.06; CI 0.79-5.38).

Table 6: Summary of the output of logistic regression analysis of the variables

Variable	Df	Significance	Exp B (OR)	95% CI
Marital status (Single)	1	0.000	32.82	2.97-362.45
MSPs	1	0.084	2.198	0.87-55.17
Induced abortion	1	0.001	2.05	0.79-5.38
Early Coitarche	1	0.001	4.65	1.28-16.11
Vaginal discharge	1	0.324	1.05	0.16-6.97
History of PID	1	0.182	1.61	0.21-1.25
Puerperal/Post abortion sepsis	1	0.005	2.66	1.98-6.67

*df = Degree of freedom

When multinomial logistic regression was applied to dichotomous and polychotomous variables while controlling for the effects of socio-demographic characteristics, early coitarche (OR 15.23; CI 2.32-66.11), a positive history of post abortion/puerperal sepsis (OR 13.52; CI .1.31-139.91) and multiparity (OR 2.13; CI 1.08-6.74) were more likely to be associated with ectopic pregnancy in the middle socio-economic class.

DISCUSSION

Serum immunoglobulin G (IgG) anti-*Chlamydia trachomatis* antibody has been associated with tubo-peritoneal damage and increased risk of ectopic pregnancy.[3,26,27] The prevalence of chlamydial antibody in women with ectopic pregnancy in this study is 62.8% while that for women with normal pregnancy is 23.3%. Therefore, the prevalence of serum antibodies to *Chlamydia trachomatis* (CT) was significantly higher in women with ectopic pregnancy (EP) when compared to women with normal second trimester pregnancy ($p< 0.000$). Similar findings were established in studies form Benin (48% versus 16.3%),[14] Lagos (62.4% versus 29%)[2] and Ilorin (70.8% versus 37.5%)[16] for the EP and normal pregnancy arms respectively.

Similar findings were also recorded in Uganda (60% versus 26.3%).[18] However, in India, a prevalence of 25% was seen in the case arm and 11.3% in the control group.[26] These differences may be accounted for by the different methodologies employed in various studies, presence of unaccounted risk factors like smoking and immune status, genetic factors that may prevent infectivity by CT and co-infection by other pathogenic organisms like *Neisseria gonorrhoeae* and *Mycoplasma genitalium*.[29] Lower figures are quoted from Europe; Turkey (25% versus 9.6%)[28] and Finland (21% versus 14.6%)[28] Lower figures from developed countries may be explained by increased awareness to CT, good health-seeking behaviour permitting early treatment of infections and screening protocols.[3,28] In this study, there is a 6-fold increase in the risk of EP in women with anti-chlamydial IgG antibodies. Similarly, Mpiima and colleagues found a 4-fold increase in Uganda.[18] while a 2-fold increased risk was found in UK.[27]

There is also a higher mean titre level of IgG antibody in those who were IgG index positive in the case arm (1.20 ± 0.58) when compared normal pregnancy arm (0.63 ± 0.36). This is explained by the repeated infections with CT with severe tubo-peritoneal damage which cause an exaggerated antibody response.[5,14] While examining the risk factor for CT infection among the participants, the sexual and reproductive characteristics of both groups were analysed. The established risk factors for CT infection in this study include single status (OR 32.82; CI 2.97-32.45), early age at first intercourse (OR 4.65; CI 1.28-16.11), having had an induced abortion (OR 2.06; CI 0.79-5.38) and middle socio-economic class ($p< 0.006$). This study suggests that more women with EP are likely to be single as found in some Nigerian studies.[14,15]

Single women are more likely to have multiple sexual partners, engage in unsafe sex and procure induced abortions. Those who had their sexual debut before the age of 18 years are more likely to have multiple sexual partners. Contraceptive uptake including consistent use of Condoms has been found to be low amongst this group of reproductive age women.[14,15] They are also more at risk because the columnar epithelium of the endocervix extends to the ectocervix making it more prone to pathogens.[5,15,18] Previous study found a higher incidence in the low socioeconomic class in Nigeria.[14] This is at variance with the finding here where middle class was a risk factor. This may be because of the recent upward review of hospital bills which has discouraged the low-income population from assessing the services at the FMC, Umuahia. Bivariate analysis showed a parallel pattern in terms of risk factors for Chlamydia infection and EP. This has been

reported by previous researchers.[14,26,31]

While analysing the risk factors for Chlamydia infection among both groups, this study assessed their sexual and reproductive attributes. The results of univariate analysis showed that women with EP had an earlier age at sexual debut. This has been found in other studies which has linked early coitarche to the risk of STDs.[2,14,15,26] Those who have had prior abdominopelvic surgeries were more likely to have ectopic pregnancy. This was also found in similar studies.[14,15] Prior abdomino-pelvic surgery may increase the risk of pelvic adhesions which may distort fallopian tubal anatomy.

This study strongly suggests that possession of serum antibodies to chlamydia is important in the aetiology of tubal disease and subsequent development of EP. The attributable risk percent (AR%; the proportion of the incidence of a disease in the exposed that would be eliminated if the exposure were eliminated)[32,33,34] was used to estimate the proportion of women with ectopic pregnancy associated with Chlamydia infection. This was found to be 62.9%.

The population attributable risk (proportional reduction in population with a disease or mortality that would occur if exposure to a risk factor were reduced to an alternative ideal exposure scenario) [32,33,34] measures the proportion of all ectopic pregnancies in both the exposed and unexposed associated with Chlamydia infection and this was found to be 46%. A PAR% value of 30.9% was found in a study from Benin.[14] Lower population-attributable fraction (synonymous with PAR%) of 15% was also seen in the USA.[33] This implies that there are other aetiologic factors in the causation of ectopic pregnancy.[6,32,33] Indeed, the cause of EP is unclear and many associated factors have been documented. The risk factors for ectopic pregnancy are many and varied.[6] The absence of anti-CT antibody in 36.2% of women with ectopic pregnancy in this study implies that there are other causative factors. Other infections such as *Neisseria gonorrhoea*, gram-negative organisms and *Mycoplasma genitalium* that cause PID may damage the fallopian tube.[35] This is supported by recent studies that showed antibodies against bacterial proteins such as the 60 kDa heat shock protein 60 (CHSP60) in the immunopathogenesis leading to fibrosis and luminal occlusion.[5,36] Also smoking, genetic factors, immune status, previous pelvic surgeries, the use of progesterone containing contraceptives and assisted reproductive technologies have all been found to be associated with ectopic pregnancy in many studies.[6,36]

When the risk factors in this study were subjected further to logistic regression analysis, the association between ectopic pregnancy and chlamydia infection was upheld when the effects of predictors such as being single, early age at sexual debut, prior history of PID and having had an induced abortion were combined. This was at variance with a study from Port Harcourt where the effect of these risk factors was attenuated when logistic regression was applied.[15] In Ilorin, Moses and colleagues found higher titres in women who did not have any significant risk factors for EP.[16] The difference was probably because more women in this study had early age at sexual debut and reported having had induced abortions.

Furthermore, the effects of these risk factors were rather more pronounced in the middle-income group when multinomial logistic model was applied to polychotomous variables. The FMC, Umuahia is patronized more by the middle socio-economic class in this study. The middle socio-economic class as loosely used here refers to women who belong to the working class, often graduates or professionals and/or those whose husbands are working class graduates or professionals. They have variously been described in literature as those above the lower 20% but below the upper 20%.[37]

The middle socio-economic class is made up of women who are 'elitist', more exposed and desirous to compete with the high-income class in material acquisition. Therefore, they are more likely to have more sexual partners, acquire more infections, indulge in termination of unwanted pregnancy and put their reproductive career in jeopardy. However, this was at variance with some reports which found that women with lower educational attainment were more at risk.[2,14] The reason for the variance was probably because the hospital is located in the state capital and had a recent upward review of hospital bills as well as the harsh economic situation in Nigeria. This became relatively unaffordable for the lower income class. Some studies in low-resource settings have shown nulliparity to be a risk factor for EP.[2,14] This was not found in this study as nulliparity was not a significant risk factor for EP in this study ($p < 0.892$).

Diagnostic tests for chlamydia and gonorrhoea traditionally, are based on laboratory cultures of the microorganisms. These laboratory culture tests are less sensitive for detection of chlamydia and gonorrhoea. NAATs are highly sensitive and specific for chlamydia and gonorrhoea testing on urine, cervical swab and urethral swab specimens. However, they are expensive and not readily available in our sub-region.[38]

CONCLUSION

The study found that a greater proportion of women with ectopic pregnancy have serological evidence of prior *Chlamydia trachomatis* infection than women with normal pregnancy. We also found a 6-fold increased risk in the risk of developing EP when there has been a prior CT infection. The statistically significant risk factors for ectopic pregnancy were single marital status, induced abortions and early age at sexual debut.

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AUTHOR'S CONTRIBUTION

This research was conceptualised and designed by author 1. All the authors participated in the laboratory assays, collated the results, analysed data and read through the manuscript for publication.

CONSENT

All participants were educated on the research and informed consent obtained.

ETHICAL APPROVAL

This was obtained from the health research ethics committee (HREC) of the Federal Medical Centre, Umuahia, Abia State, Nigeria.

RECOMMENDATIONS

Health research funding in developing nations should be reviewed upwards and foreign grants assessed for medical research so that the more sensitive NAATs will be used. There is need for further studies or the role of smoking, genetic factors and male partners and fallopian tubal segment polymerase chain reaction for CT. There is need to include CT screening in the periodic medical evaluation of reproductive age people and for infertility work-up.

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