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The Association between *Chlamydia trachomatis* and Ectopic Pregnancy in Lagos, Nigeria—A Case Control Study

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Abstract

Objectives: To determine the seropositivity of Chlamydia antibody in patients with ruptured ectopic pregnancy compared to normal pregnant women and the risk factors for ectopic pregnancy. Study Design: This was a prospective case-control study of 85 cases of ruptured ectopic pregnancy and 100 cases of second trimester on-going intrauterine pregnant controls presenting in Lagos State University Teaching Hospital (LASUTH) between September 2009 and March 2010. Study Site: This was at the gynaecological emergency room and antenatal clinic in the Department of Obstetrics and Gynaecology. Ethical approval was sought and granted by the ethics review committee of LASUTH. Study Participants: Patients presenting with ruptured ectopic pregnancy were recruited as cases while the controls were made up of those with uncomplicated second trimester intrauterine pregnancy. A semi-structured questionnaire containing socio-demographic and clinical characteristics was administered following informed consent. Five milliliters of venous blood was taken from each participant and tested for Lymphogranuloma Venerum (LGV) type 2 broadly reacting antigen of *Chlamydia trachomatis*. Data Analysis: Data gathered from the case notes and laboratories were imputed into the computer and analyzed using the statistical package Epi-Info 3.51, Atlanta, USA. Frequency tables were generated for continuous variables and chi-square analysis used to determine association between variables, with p values <0.05 considered statistically significant. Results: There were 91 cases of ectopic pregnancy among a total of 2468 deliveries giving an incidence of 3.68% or 1 in 27 deliveries. Factors which significantly contributed to increased incidence of ectopic pregnancy in this study were: level of education (p = 0.001), socio-economic status (p = 0.001), parity (p = 0.005), early age of sexual debut (p = 0.001), multiple sexual partners (p = 0.001), previous pelvic inflammatory disease (p = 0.003), previous induced abortion (p = 0.013) and previous postabortal/puerperal sepsis (p = 0.013). The seropositivity of Chlamydia IgG (62.4%) in the cases was significantly higher than that of 29% in the control (p <

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0.0001). Conclusion: There was a high incidence of ectopic during the period of study and the seropositivity of Chlamydia IgG antibody was significantly higher amongst the cases. Risk factors identified were low level of education, low socio-economic status, low parity, early age of sexual debut, multiple sexual partners, previous history of pelvic inflammatory disease, previous induced abortion and previous postabortal/puerperal sepsis.

Keywords

Chlamydia trachomatis, **Ectopic Pregnancy, Seropositivity, Risk Factors**

1. Introduction

Infection with *Chlamydia trachomatis*, which is generally asymptomatic in approximately 80% of infected women and 50% of infected men, is the most preventable cause of pelvic inflammatory disease in young women [1]. Pelvic inflammatory disease may lead to ectopic pregnancy, tubal factor infertility and chronic pelvic pain [2].

Ectopic pregnancy remains a major public health problem and its incidence has been increasing all over the world in recent times [3]-[6]. Approximately 1% - 2% of all pregnancies in Europe and the USA are ectopic and in the Western world, tubal ectopic pregnancy remains the most common cause of maternal mortality in the first trimester of pregnancy [7] [8]. In the developing world, the incidence is much higher where 1 in 10 women admitted for ectopic pregnancy ultimately dies from the condition [9].

Risk factors identified in previous studies include primary level of education, two or more lifetime sexual partners, smoking, prior history of vaginal discharge, previous use of intrauterine contraceptive device, previous history of induced abortion, early age of sexual debut and inconsistent condom use [10]-[16].

The link between past *Chlamydia trachomatis* infection and ectopic pregnancy is based mainly on sero-epidemiological case-control studies [10]-[14]. These reports conclude that *Chlamydia trachomatis* infection is a major cause of fallopian tube damage which predisposes to ectopic pregnancy.

Recent studies however, have shown divergent results concerning the risk of ectopic pregnancy in patients with past Chlamydia infection. While some studies found a reduced risk of ectopic pregnancy following *Chlamydia trachomatis* infection [17], others have found no association [18] [19]. Although a recent study in Nigeria demonstrated that a significantly higher proportion of women with ectopic pregnancy had serological evidence of previous infection with Chlamydia, it failed to show a strong independent association between Chlamydia antibodies and risk of ectopic pregnancy [20]. These conflicting views underscore the need for new studies on *Chlamydia trachomatis* infection and risk of ectopic pregnancy.

Our working hypothesis is that a higher proportion of women with ectopic pregnancy have serological evidence of past Chlamydia infection when compared to women with intrauterine pregnancy without history of previous ectopic pregnancy.

We therefore aim to determine the risk factors for ectopic pregnancy and ascertain the association between serological evidence of prior Chlamydia infection and risk of ectopic pregnancy.

2. Methods

2.1. Study Design

Case-control study of 85 cases of ruptured ectopic pregnancy and 100 cases of second trimester on-going intrauterine pregnant controls presenting in Lagos State University Teaching Hospital (LASUTH) between September 2009 and March 2010.

2.2. Study Site

Gynaecological emergency room and antenatal clinic in the Department of Obstetrics and Gynaecology (Ayinke House) of Lagos State University Teaching Hospital (LASUTH).

2.3. Study Participants

Patients presenting with ruptured ectopic pregnancy confirmed by histological evaluation of the extirpated fallopian tube were recruited as cases following informed consent. Exclusion criteria were: those Using Intrauterine Contraceptive Device (IUCD) at the time of conception, who had blood transfusion within the last 6 months and those on any form of antibiotics in the last three months. The controls were made up of those with uncomplicated second trimester intrauterine pregnancy without a history of previous ectopic pregnancy or tubal surgery coming for booking following informed consent. Other exclusion criteria were similar to those found in the cases.

2.4. Ethical Approval

The study was approved by the ethical review committee of Lagos State University Teaching Hospital, Ikeja, Lagos.

2.5. Data Collection

Informed consent was obtained from the participants upon recruitment into the study. They were interviewed using a semi-structured interview-administered questionnaire by any member of the research team. Questions relating to age, marital status, socio-economic status, level of education, religion and tribe were asked. Other information solicited were age at menarche, coitarche, parity, number of sexual partners, previous induced abortion, previous pelvic inflammatory disease, previous puerperal/postabortal sepsis, abdomino/pelvic surgery and previous use of Intrauterine Contraceptive Device (IUCD).

2.6. Serological Assay

Five milliliters (5 ml) of venous blood was collected from the volar surface of the forearm of all the participants and emptied into clean, sterile plain specimen bottle. The specimen was collected from patients with ectopic pregnancy before blood transfusion and surgery. The blood was taken to the research laboratory where the laboratory scientist allowed the specimen to clot and sera obtained. The sera were frozen at −20˚C until analyzed in batches for Chlamydia antibodies. The assay was done using the Immunocomb *Chlamydia trachomatis* Immunoglobulin G kit (Diagnostic Automatic Inc.), an indirect solid-phase Enzyme Immunoassay (EIA) test. This test quantitatively measures 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. The tests were validated with the control samples. The results are presented using IgG index. An index of 0.99 is regarded as negative while index of 1.00 and above is regarded as positive.

2.7. Data Analysis

Data gathered from the case notes and the laboratory were imputed into the computer and analyzed using the statistical package Epi-Info 3.51, Atlanta, USA.

Frequency tables were generated for continuous variables and chi-square analysis used to determine association between variables with p value <0.05 considered statistically significant.

3. Results

A total of 85 women out of 91 (93.4%) with a diagnosis of ectopic pregnancy (cases) confirmed by histological evaluation of the extirpated fallopian tube gave consent and participated fully in the study. These were matched with 85 out of 100 pregnant controls who consented. Both groups were similar with regards to age: the mean age for cases was 29.8 ± 5.2 with a range of 20 - 42 years and 29.6 ± 3.7 with a range of 23 - 42 years for controls. The mean gestational age at presentation for the cases was seven weeks (range 6 - 11 weeks) while the mean gestational age for the controls was 19.1 weeks (range 16 - 25 weeks)

Table 1 shows the socio-demographic characteristics of the studied population. Majority (64.7%) of the cases were in the low socio-economic class, while majority (90%) of the controls were in the middle socio-economic class and none in the high socio-economic class. This was statistically significant with a p-value of 0.0001. Both

Table 1. Socio-demographic characteristics of cases and controls.

	Cases n = 85 (%)	Controls n = 85 (%)	p-value
Age (years)			
11 - 20	4 (4.7)	0 (0)	
21 - 30	45 (52.9)	63 (74)	
31 - 40	33 (38.8)	25 (26)	
41 - 50	3 (3)	0 (0)	p = 0.0032
	Mean = 29.89 ± 5.22.	Mean = 29.56 ± 3.68	
Socioeconomic status			
High	0 (0)	0 (0)	
Middle	30 (35.3)	77 (90)	
Low	55 (64.7)	8 (10)	p = 0.0001
Marital status			
Married	79 (92.9)	83 (98)	
Single	6 (7.1)	2 (2)	p = 0.0918
Educational level			
None	7 (8.2)	0 (0)	
Primary	24 (28.2)	0 (0)	
Secondary	33 (38.8)	17 (20)	
Tertiary	21 (24.7)	68 (80)	p = 0.0001
Religion			
Christian	58 (69.0)	67 (79)	
Islam	26 (31.0)	18 (21)	p = 0.1231
Tribe			
Yoruba	61 (71.8)	61 (72)	
Igbo	16 (18.8)	14 (16)	
Hausa	0 (0)	1 (1)	
Others	8 (9.4)	9 (11)	p = 0.7590

Socio-economic class. This was based on the profession of the spouse's husband and there was patient in high socioeconomic class.

groups were similar with respect to marital status: 92.9% of cases were married while 98% of the controls were married (p-value = 0.0918). With respect to educational status, 21 (24.7%) of the cases had tertiary education as against 68 (80%) of the controls. This was statistically significant with p value of 0.0001. Other socio-demographic variables like religion and tribe are as shown in the table.

Table 2 shows the sexual and reproductive characteristics of the studied population. More than half (60%) of the cases were multiparous, while 51 (51%) of the controls were multiparous. Majority of both cases and controls attained menarche at or above 12 years of age (97.7% vs 97.0%) respectively (p = 0.9397). The age of sexual debut was less than 19 years in 63 (74.1%) of the cases, while the age of sexual debut was less than 19 years in 31 controls constituting 37%, with a p-value of 0.0001 which was statistically significant. Majority of the cases (87.1%) had multiple sexual partners when compared to 48 (56%) of the controls, p = 0.0009. Sixty two cases, constituting 72.9% had at least one induced abortion compared to 42 (50%) of controls with a p-value of 0.0113 which was statistically significant. From Table 2, it is also observed that 16 (16.5%) cases had previous history of pelvic inflammatory disease, while 2 (2%) of the controls had previous history of pelvic inflammatory disease with a p-value of 0.0003 which was statistically significant. Fourteen (16.5%) of cases had previous history of puerperal/postabortal sepsis compared to 2 (2%) of the controls with a p-value of 0.0013 which was statistically significant.

Table 3 shows the relationship between Chlamydia IgG index in both cases and controls. As shown in this table, 53 (62.4%) of the cases had a positive IgG index (titre >/= 1.00) compared to 25 (29%) of the controls with a p-value of 0.0001 which was statistically significant.

Table 2. Clinical characteristics of cases and controls.

	Cases N = 85 (%)	Control N = 85 (%)	p-value
Parity			
Nullipara	28 (32.9)	42 (49)	
Multipara	51 (60.0)	43 (51)	
Grandmultipara	6 (7.1)	0 (0)	p = 0.0050
Menarche			
Less than 12 years	2 (2.4)	3 (3)	
12 - 14 years	47 (55.3)	45 (63)	
Above 14 years	36 (42.4)	37 (44)	p = 0.9397
Coitarche			
≤19 years	63 (74.1)	31 (37)	
>19 years	22 (25.9)	54 (63)	p = 0.0001
Multiple sexual partners			
Yes	74 (87.1)	48 (56)	
No	11 (12.9)	37 (44)	p = 0.0009
Induced abortion			
No	23 (27.1)	43 (50)	
1	31 (36.5)	18 (21)	
2 - 4	28 (32.9)	21 (25)	
Above 4	3 (3)	3 (4)	p = 0.0113
Previous PID			
Yes	16 (18.8)	2 (2)	
No	69 (81.2)	83 (98)	p = 0.0003
Puerperal/postabortal sepsis			
Yes	14 (16.5)	2 (2)	
No	71 (84.5)	83 (98)	p = 0.0013
Abdominal/Pelvic Surgeries			
Yes	11 (12.9)	14 (17)	
No	74 (87.1)	71 (83)	p = 0.5742
IUCD usage			
Never	60 (70.6)	73 (86)	
Within 6 months	11 (12.9)	3 (3)	
Greater than 6 months	14 (16.5)	9 (11)	p = 0.1499

PID----Pelvic inflammatory disease; IUCD---Intrauterine contraceptive device.

Table 3. Chlamydia IgG index in cases and controls.

	Cases N = 85 (%)	Controls N = 85 (%)	
Chlamydia IgG index			
0 - 0.99	32 (37.6)	60 (71)	
1 - 1.99	13 (15.3)	16 (19)	
2 - 2.99	30 (35.3)	6 (7)	
≥3	10 (11.8)	3 (3)	p = 0.0001

4. Discussion

The study was designed to determine the association between ectopic pregnancy and previous infection with

Chlamydia trachomatis in Lagos, Nigeria. Both groups were similar with respect to age which formed a good basis for comparison.

The incidence of ectopic pregnancy in this study was 3.7%. This is similar to 3.8% found in an earlier study in this centre by the same author [21], higher than 2.31% that was reported in a previous study in Lagos [15], 2.1% in south eastern part of Nigeria [22], 2.31% reported in Benin City [3] and 1.8% in Sweden [23]. This high figure is expected considering the peculiarity of the centre where the study was conducted.

This is the busiest gynaecological centre in Lagos and serves as the referral centre for most emergencies in gynaecology coupled with the advertised free health care of Lagos state government making the centre the most patronised among the tertiary health institutions in Lagos.

The high sero-prevalence of *Chlamydia trachomatis* in patients with ectopic pregnancy-68% is in keeping with results from other centres in Nigeria [11] [12] [20] and outside Nigeria [23] [24]. The result of this study also confirmed that patients with ectopic pregnancy are more likely to demonstrate evidence of past infection with *Chlamydia trachomatis* when compared with normal intrauterine pregnant controls. This is corroborated by various studies [11]-[13] [25] [26]. This however is at variance with reports from Denmark [17] which found a reduced risk of ectopic pregnancy, and those from Sweden [18] which found no association. A recent study from Nigeria failed to show a strong independent association between past Chlamydia infection and the risk of ectopic pregnancy as demonstrated by the absence of antibodies to *Chlamydia trachomatis* in 52% of ectopic pregnant patients [20]. Reasons for these discrepancies include non-uniformity in the method for detecting past *Chlamydia trachomatis*, presence of cofounders that have not always been accounted for, such as smoking, effects of other sexually transmitted infections like *Neisseria gonorrhea* and more recently *Mycoplasma genitalium* [27]. Methods for testing for Chlamydia include cell culture, direct fluorescent antibody, enzyme immunoassay and the newer nucleic acid amplification technique. There is more variation in the costs, sensitivities and specificities of these methods, with nucleic acid amplification tests having specificity close to 100% but very expensive. However the assay used in this study is in line with World Health Organisation guidelines for laboratory diagnosis of *Chlamydia trachomatis* infection with 73% - 83% sensitivity and 97% - 99% specificity [28].

The socio-demographic risk factors associated with ectopic pregnancy in this study which were low socio-economic status, being single and low level of education have been corroborated by findings in previous studies [3] [4] [15] [20]-[22]. This association will not be unconnected with non-affordability of health care even when it is obvious they need medical attention and the poor health-seeking behavior among this group of clients. Although the population studied was not specifically asked questions on their health-seeking behavior, previous reports have suggested that women from the low socio-economic status were more likely to seek ineffective treatment from chemists and unorthodox medical practitioners than obtain effective evidence-based treatment from qualified health practitioners [29].

The sexual and reproductive risk factors that were found to be significant in this study-nulliparity, early age of sexual debut, history of multiple sexual partners, previous pelvic inflammatory disease and puerperal/postabortal sepsis have been found to positively influence the risk of ectopic in previous studies [15] [20] [21]. All these factors increase the risk of infections generally, Chlamydia inclusive. One of the sequele of past infections especially when not well or fully treated is pelvic inflammatory disease with resultant pelvic adhesions that could affect ovum pickup or cause damage to the ciliary lining of the tube slowing down the movement of the zygote leading to implantation in the fallopian tube.

The limitations to this study are the following: being hospital-based study the result cannot be extrapolated to the general population, the use of serology which is less sensitive than culture and nucleic acid amplification tests in the, diagnosis of Chlamydia infections.

5. Conclusion

This study has demonstrated a significantly higher seroprevalence of Chlamydia antibody in patients with ectopic pregnancy when compared to those with intrauterine pregnancy. It also identified the following risk factors for ectopic pregnancy: low socio-economic status, being single, low level of education, nulliparity, early age of sexual debut, history of multiple sexual partners, previous pelvic inflammatory disease and puerperal/postabortal sepsis.

Conflict of Interest

The authors have nothing to declare.

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