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# Prior Chlamydia Trachomatis Infection and First Trimester Miscarriage in a Tertiary Health Institution in Jos, Plateau State, Nigeria

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#### **Abstract**

**Background:** Chlamydia trachomatis infection is the most common sexually transmitted disease, and it runs subtle course. This organism can cause spontaneous first trimester miscarriage even with past or latent infection by directly affecting embryonal trophoblastic tissues, or indirectly through immunoglobulin-G (IgG) antibody raised against Chlamydia heat shock protein-60 (HSP-60) cross-reacting against human HSP-60 of embryonal cells, and affectation of endometrial lining thereby causing first trimester miscarriage. The HSP-60 of Chlamydia trachomatis has amazing analogy with that of human embryo. IgG anti-Chlamydia antibody can be measured quantitatively in women with past or latent Chlamydia trachomatis infection. This study aimed to determine the association between prior Chlamydia trachomatis infection and first trimester miscarriage. Methodology: A case control study carried out in Jos University Teaching Hospital, Plateau State, Nigeria within 8-month period. The study group comprised 51 women aged 20 - 45 years with current spontaneous first trimester miscarriage while control group comprised 51 uncomplicated second-trimester women attending antenatal care at same facility matched for age with cases. A convenience sampling was used for both cases and controls. 5 ml of venous blood was collected from subjects to determine proportions and serum levels of IgG anti-Chlamydia antibody in both cases and controls using BIOS Chlamydia T. IgG ELISA kits. The subjects' socio-

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demographic factors, obstetric characteristics, sexual and reproductive risk factors were obtained using study pro forma. The data obtained were compiled and analyzed using IBM-SPSS version 26. **Result:** This study revealed a strong association between prior Chlamydia trachomatis infection and first trimester miscarriage (OR =15.9; 3.9-66.7; P-value <0.001). **Conclusion:** Our study revealed strong association between prior Chlamydia trachomatis infection and spontaneous first trimester miscarriage. We recommend Chlamydia trachomatis testing and treatment for preconception care prior to subsequent conception in women with first trimester miscarriage.

# **Keywords**

Chlamydia Infection, First-Trimester Miscarriage, JUTH

## 1. Introduction

Chlamydia trachomatis infection is the most prevalent and damaging of all sexually transmitted diseases worldwide [1]-[3]. The prevalence of Chlamydia trachomatis (Ct) in Jos, Nigeria was reported as 56.1% among women aged 14 - 45 years [3], and two-third of the infection remains undetected because most infected women are asymptomatic and do not seek medical attention. It is an obligate intracellular gram-negative bacterium with 15 serotypes. Serotypes A, B and C causes 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 cause Lymphogranuloma venereum (LGV) associated with genital ulcers [2] [3].

This infection may ascend to the upper reproductive tract resulting in pelvic inflammatory disease, and consequently, leading to chronic pelvic pain, tubal ectopic pregnancy, miscarriage and tubal infertility [4]-[6].

Positive Chlamydia trachomatis IgG serology reflects either a past or latent asymptomatic infection [7]. The exact mechanism by which Chlamydia trachomatis infection causes first trimester miscarriage is not yet fully understood. However, the antibody to Chlamydia trachomatis heat shock protein (particularly cHSP60-kDa) reacting with human heat shock protein (hHSP) in the trophoblastic tissue of the embryo is said to be an important factor in the immune-pathogenesis of spontaneous first trimester miscarriage [8]-[10]. The Chlamydia heat shock protein shows an amazing analogy to human proteins. Thus, there is a cross-reactivity between the human HSP60 and the Chlamydial HSP60, which leads to the formation of antibodies against the human HSP60 in serum and follicular fluid of women exposed to Chlamydia trachomatis [7]. These antibodies have a negative impact on embryonic growth and increase adverse pregnancy outcomes. Besides, Chlamydia heat shock protein (cHSP) has been found to directly induce trophoblast apoptosis by stimulating the toll-like receptor 4, which naturally mediates immune responses in the placenta [8] [9] [11]. Endometrial damage by past Chlamydia trachomatis infection has also been implicated in causing first

trimester miscarriage [7] [8].

Miscarriage occurs in about 15% of pregnancies and 25% of women who become pregnant will experience at least one miscarriage, and this can be a frustrating experience for the patient and her family with considerable physiological, psychological and cost implications aside hemorrhage and maternal mortality in some cases [7] [12]. First trimester miscarriage can be defined as spontaneous loss of pregnancy within the first 13 weeks of gestation. This could be due to chromosomal aberrations, such as structural alteration or abnormal chromosomal numbers in about 50% especially those above 35 years of age [13]-[15]. Other causes of first trimester miscarriage include endocrine disorders, anatomical anomalies, systemic diseases, immunological disorders and infective causes; potentially preventable infections such as Chlamydia trachomatis infection may account for up to 15% of miscarriages [7] [12] [16]. Only 30 to 50% of conceptions progress beyond the first trimester [8] [16]. The vast majority of those that do not progress are spontaneously aborted before the woman is aware of the conception, and many pregnancies are lost before medical practitioners could detect the presence of an embryo [16]. Between 15% and 30% of known pregnancies end in clinically apparent spontaneous miscarriage [8] [14] [15]. About 0.5% - 1.0% of women wishing to have children may experience three or more successive miscarriages (recurrent miscarriage) [12] [15] [16]. First trimester miscarriage remains a major public health problem especially in many developing nations where it is a significant contributor to pregnancy related morbidity and mortality especially when complicated by haemorrhage and sepsis [13].

Spontaneous or recurrent first trimester miscarriage could be induced by persistent asymptomatic Chlamydia trachomatis infection spreading to fetal tissue or endometrium [7] [8] [15]. This study aimed to determine the association between prior Chlamydia trachomatis infection and first trimester miscarriage in Jos University Teaching Hospital (JUTH).

#### 2. Methods

# 2.1. Study Design

This was a matched case-control study of 51 women with first trimester miscarriage and 51 uncomplicated second trimester pregnant women as control in Jos University Teaching Hospital (JUTH) over 8 months period.

# 2.2. Study Site

Gynaecological emergency unit (GEU) and antenatal care unit of the Department of Obstetrics and Gynaecology JUTH, Jos, Plateau State, Nigeria.

## 2.3. Study Participants

The study participants comprised consented 51 women with spontaneous first trimester miscarriage confirmed by histology who had presented at the GEU of JUTH compared with 51 uncomplicated second trimester pregnant women matched for

age. These second trimester pregnant women are non-primigravidae who never had a history of miscarriage, infertility or ectopic pregnancy. Past miscarriage and infertility may be due to prior Chlamydia trachomatis infection, thereby affecting comparability of study groups.

#### 2.3.1. Inclusion Criteria

Cases: Consenting women with spontaneous first trimester miscarriage confirmed by histology.

Control: Consenting uncomplicated second trimester pregnant women attending antenatal clinic without history of previous miscarriage, ectopic pregnancy, infertility or tubal surgery.

#### 2.3.2. Exclusion Criteria

Cases: Patients who declined consent, had blood transfusions within the last six months or have been on any form of antibiotics in the last three months.

Controls: Second trimester pregnant women with history of ectopic pregnancy, miscarriage, infertility, tubal surgery or a primigravida.

## 2.4. Sample Size Estimation

The sample size was estimated using the formula

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

where:

*n*: number of sample size in each of the group

 $P_1$  = proportion of positive IgG anti chamydial antibody among cases (0.26 in Iran)

 $P_2$  = proportion of positive IgG anti chamydial antibody among controls (0.05 in Iran)

 $Z_{-\alpha/2}$  = value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at 0.05)

 $Z_{-\beta/2}$  = value of standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%)

$$n = \frac{\left\{ \left( 0.26 \times 0.74 + 0.05 \times 0.95 \right) \right\} \times \left( 1.96 + 0.84 \right)^2}{\left( 0.21 \right)^2}$$
= 42.65

The sample size was approximated to be 51 for the cases; and 51 for the controls considering an attrition rate of 20%.

# 2.5. Ethical Approval

This study was approved by the ethical committees of Jos University Teaching Hospital with approval reference—JUTH/DCS/ADM/127/X/X/6502.

### 2.6. Data Collection

The participants were recruited by convenience sampling, a non-probability sampling technique which is biased by non-applicability to the wider population. Informed consents of participants were also obtained. The participants' history included socio-demographic factors, obstetric history, sexual and reproductive risk factors were obtained using a study pro-forma.

# 2.7. Serological Assay

Five milliliters (5 ml) of venous blood was collected from the antecubital fossa of all the participants and emptied into clean, sterile plain specimen bottles. The specimen was collected from participants before any form of blood transfusion. Blood samples were transferred to the chemical pathology laboratory in JUTH, allowed to clot and sera obtained. The sera were frozen at −20°C until analyzed in batches for Chlamydia IgG antibodies, the assay was done using the BIOS Chlamydia T. IgG ELISA kits, Chlam-T-G-326 (Chemux BioScience, INC. USA) and an indirect solid-phase Enzyme Immunoassay (EIA) test was carried out by a consultant chemical pathologist. 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 × 10. The titre levels refer to the denominator of the dilution. Positive result was defined as  $\ge 10\%$  more than calibrator titre level (i.e. 10) = 11. Therefore, positive result was defined as  $\geq 11$  while < 11 was classified as negative.

## 2.8. Data Analysis

The data collected 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 spontaneous first trimester miscarriage and logistic regression performed to determine association.

## 3. Results

A total of 102 women were enrolled in this study. This comprised of 51 women who had spontaneous first trimester miscarriage (cases) matched for age with 51 uncomplicated parous second trimester pregnant women (control).

The mean age of study participants is  $29.85 \pm 5.4$  years. The participants were largely (80.4%) below age 35 years. Over 90% of the participants have at least secondary education. All the participants were married, and 30% of these women are housewives (**Table 1**).

The prevalence of IgG anti Chlamydia antibody in cases was 68.6% and 17.6% in controls, the difference is significant (p < 0.001). The overall prevalence of IgG anti Chlamydial antibody is 43.1%. Average anti-chlamydia antibody titre in cases

and control were 12.35  $\pm$  3.7 and 8.65  $\pm$  2.46 respectively, the difference is statistically significant (IgG antibody level  $\geq$  11 was said to be positive) (**Table 2**).

After controlling for confounding variables Chlamydia IgG antibody was significantly associated with miscarriage (Odd Ratio =  $15.9\ 3.9\ -\ 66.7,\ 95\%$  CI, p-value <0.001) (Table 3).

Table 1. Socio-demographic characteristics of study participants.

Ch ana stanisti sa	Study group			
Characteristics	Case n = 51 (%)	Control n = 51 (%)		
Age (years)				
<35	35 (68.6)	47 (92.2)		
35+	16 (31.4)	4 (7.8)		
Education				
Nonformal	3 (5.9)	0 (0.0)		
Primary	4 (7.8)	3 (5.9)		
secondary	18 (35.3)	31 (60.8)		
Tertiary	26 (51.0)	17 (33.3)		
Marital Status				
Married	51 (100.0)	51 (100.0)		
Occupation				
Housewife	15 (29.4)	26 (35.4)		
Civil servant	16 (31.4)	6 (11.8)		
Seamstress	4 (7.8)	4 (7.8)		
Students	2 (3.9)	1 (2.0)		
Teaching	4 (7.8)	4 (7.8)		
Business/Trading	9 (17.6)	8 (15.7)		
Others	1 (2.0)	2 (3.9)		
Religion				
Christianity	35 (68.6)	21 (41.2)		
Islam	16 (31.4)	30 (58.8)		
Parity				
0	7 (13.8)	0 (0.0)		
1 - 4	40 (78.4)	42 (82.4)		
≥5	4 (7.8)	9 (17.6)		
GA				
<10	22 (43.1)	0 (0.0)		
≥10	29 (56.9)	55 (100.0)		

**Table 2.** Chlamydial antibody titre in cases and controls.

Chlamydial ant	ibody	Case	Control	Total	$X^2$	p-value
Yes	:	35 (68.6)	9 (17.6)	44 (43.1)		
No		16 (31.4)	42 (82.4)	58 (56.9)		
Total	5	1 (100.0)	51 (100.0)	102 (100.0)	27.019	< 0.001
Antibody Tit (Mean ± SD	12	2.35 ± 3.76	$8.65 \pm 2.46$	11.46 ± 4.71	t-test = 5.880	<0.001

**Table 3.** Logistic regression analysis of factors associated with miscarriage.

Characteristics	Odds ratio	95%CI	P-value
Chlamydia antibody:			
Positive	15.9	3.9 - 66.7	< 0.001
Negative	1		
Age			
<35	7.230	1.263 - 41.378	0.026
35+	1		
Religion			
Christianity	11.774	2.258 - 61.396	0.003
Islam	1		
Education			
Nonformal	0.000	-	0.999
Primary	0.343	0.009 - 12.640	0.561
Secondary	1.320	0.311 - 5.604	0.707
Tertiary	1		
Gestational age (weeks)			
<10	0.000	-	0.997
≥10	1		
Parity			
0	0.000	-	0.999
1 - 4	0.143	0.02 - 10.210	0.372
≥5	1		

The positive predictive value of this test was 80% while negative value was 72% (Table 4).

# 4. Discussion

This study showed a strong association between prior Chlamydia trachomatis infection and spontaneous first trimester miscarriage. The sero-prevalence of IgG

**Table 4.** Sensitivity, specificity and predictive values.

Anti Chlamardia antihada	Miscarriage		– Total
Anti-Chlamydia antibody -	Yes	No	- Total
Yes	35	9	44
No	16	42	58
Total	51	51	102
PPV			79.55%
NPV			72.41%
Sensitivity			68.63%
Specificity			82.35%

anti-Chlamydial antibody was significantly higher (68.6%) in women with spontaneous first trimester miscarriage (cases) than uncomplicated second trimester pregnant women (control) with sero-prevalence of 17.6%, p-value = 0.001. Chlamydial IgG seropositivity was significantly found to have 16-fold risk association for spontaneous first trimester miscarriage, (OR = 15.9, 3.9 - 66.7; 95% CI, P-value = 0.001). Our finding is in keeping with that of Baud et al. in Swizaland 15.2% in the miscarriage group and 7.3% in the controls (p-value = 0.018), [7] Vismovsky et al. in Slovakia 67.3% versus 36.0% (p < 0.05) [11], Jahromi et al. in Iran 26.0% versus 5.0% (p < 0.05) [17], Rastogi et al. in New Delhi 15.6% versus 4.0% (p < 0.05) [18]. Gutierrez-Campos et al. in Mexico analyzed Chlamydia trachomatis in early abortion product samples using polymerase chain reaction (PCR) and the proportions for cases and controls were 34% versus 4.7% (p = 0.002) [19]. Though we could not do PCR and other confirmatory methods in this study due financial constraint. The Gutierrez-Campos et al.'s findings suggest an association between prior Chlamydia trachomatis infection and first trimester miscarriage. However, Sozio et al.'s nested case-control study in Pennsylvania did not support an association between miscarriage and Chlamydia trachomatis infection using ligase chain reaction DNA amplification (3.8% in cases versus 8.5% in controls) [20]. In a similar study Horne et al. in Scotland could not establish an association between Chlamydia infection and first trimester miscarriage, this study detects Chlamydia infection in cases and control by assaying antibodies to Chlamydia plasmid-encoded protein (Pgp3) which may not be fully established as a risk for miscarriage like Chlamydial IgG [21]. Rantsi et al. in Finnish study did not establish association between chlamydia seropositivity and miscarriage with 16.3% for miscarriage group versus 16.8% for the control group (p-value > 0.05) [22].

We found the difference in average IgG anti-Chlamydial antibody for cases and controls to be statistically significant (12.35  $\pm$  3.76 versus 8.65  $\pm$  2.46, p-value < 0.05); and this finding is at par with other reports [7] [11] [18].

The sensitivity and specificity of the test were 68.63% and 82.35% respectively, while the positive predictive value (PPV) and negative predictive value (NPV)

were 79.55% and 72.40% respectively.

## 5. Conclusion

This study demonstrated an association between past Chlamydia trachomatis infection and spontaneous first trimester miscarriage, so treatment of women who had first trimester miscarriage for latent infection, or for pre-conception care is advocated to reduce this adverse pregnancy outcome.

#### 6. Limitations

Polymerase chain reaction, or ligase chain reaction was not assayed alongside IgG anti-Chlamydia antibody. Chromosomal anomalies, anti-phospholipid syndrome, and other infective causes of first trimester miscarriage were not ruled out in this study.

## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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