Does genital *Chlamydia trachomatis* cause spontaneous miscarriage in black women?

**Aliyu RM, Adesiyun AG, Aliyu S**

Departments of Obstetrics and Gynaecology and 1Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

**ABSTRACT**

**Background:** *Chlamydia trachomatis* (Ct) is the commonest bacterial sexually transmitted disease worldwide and is now being considered as an emerging “obstetric pathogen”. It is an enemy to the human reproductive system causing infertility, adverse pregnancy and perinatal outcomes but its role in causing spontaneous miscarriage is still unclear.

**Objective:** To assess the association between genital *Chlamydia trachomatis* and spontaneous miscarriage in black women.

**Materials and Methods:** Eighty three women with spontaneous miscarriage (case group) were compared with 83 women with on-going pregnancy beyond 28 weeks’ gestation (control group). Sera of both groups were tested for the presence of *Chlamydia trachomatis* Immunoglobulin G (IgG) antibody using ELISA.

**Results:** Seroprevalence of IgG to Ct was 8.4% and 3.6% among women with spontaneous miscarriage and on-going pregnancy, respectively. Ct IgG seropositivity was not significantly different between the two groups ($P = 0.192; OR = 0.41$, CI 0.10 – 1.63).

**Conclusion:** *Chlamydia trachomatis* IgG seropositivity is not associated with spontaneous miscarriage in this study. However, multicentre study with larger sample size and using polymerase chain reaction as a diagnostic technique is recommended.

**Key words:** Association; black women; *Chlamydia trachomatis*; enzyme-linked immunoabsorbent assay; immunoglobulin G; miscarriage.

**Introduction**

Miscarriage represents the most frequent complication of pregnancy worldwide occurring in 15% of all clinically recognized pregnancies. It is a public health concern because of its impact on maternal morbidity and mortality. It not only undermines a woman’s physical health but is also a significant cause of psychological morbidity. In north-western Nigeria, miscarriage is the commonest indication for emergency gynecological consultations in the hospitals.

A recognized risk factor for miscarriage is infection accounting for about 15% of early miscarriages (<12 weeks’ gestation) and up to 66% of late miscarriages (>12 weeks’ gestation). Genital *Chlamydia trachomatis* (Ct) infection is the commonest bacterial sexually transmitted disease worldwide which causes a threat to human reproduction due to its potentially devastating reproductive consequences. Worldwide, it affects 105.7 million cases annually with about 8.3 million cases occurring in the WHO African region in women aged 15–49 years. The black race is said to be a risk marker for genital Ct infection as black women are known to...
Limited data exist worldwide on the prevalence of Ct in pregnant women. In sub-Saharan Africa, prevalence ranges from 0–31.1% with a pooled prevalence of 6.9% in West Africa.\[9\] Epidemiological studies have shown chlamydial infection to be associated with adverse pregnancy outcomes such as miscarriage, stillbirth, preterm birth, and perinatal morbidity and mortality.\[10-14\] However, conflicting evidence on the role of Ct in causing spontaneous miscarriage exists in the literature. Some studies have shown that chlamydial infection could lead to spontaneous miscarriage.\[10-14\] The mechanism by which chlamydial infection leads to spontaneous miscarriage is not well understood. Direct fetal infection triggering a harmful inflammatory response with cytokine release possibly causing a maternal inflammatory response which induces embryonic rejection (due to homology of the chlamydial and human 60 kDa heat shock proteins) and placental damage have been postulated.\[9\]

Screening for Ct allows for detection and prompt treatment of this largely asymptomatic infection thereby averting morbidity and has been recommended in well-resourced settings. However, in many sub-Saharan African countries characterized by poor-resourced settings, screening for Ct is not readily available due to lack of standard diagnostic techniques, high cost of chlamydial testing, and paucity of data on the role of Ct in causing adverse pregnancy outcomes. Establishing the role of genital Ct in causing spontaneous miscarriage in black women might influence recommendations for routine screening of women in the preconception and antenatal period to reduce morbidity and mortality associated with miscarriage. This would be enabled by the fact that safe, effective, and cheap drugs are readily available to treat this infection. The study aimed to assess the association between genital \textit{Chlamydia trachomatis} IgG seropositivity and spontaneous miscarriage in black women.

**Materials and Methods**

It was a case-control study that was conducted in the Gynecologic emergency unit/clinic and Antenatal clinic of Ahmadu Bello University Teaching Hospital, Zaria between January-March 2018. Eighty three served as the case group and another 83 women served as a control group. The case group were consenting black women presenting with spontaneous inevitable, incomplete, complete, and missed miscarriage before 28 weeks’ gestation while the control group were consenting black women with on-going pregnancy beyond 28 weeks’ gestation matched for age and parity to the case group. Women with induced miscarriage, cervical incompetence, poorly controlled diabetes mellitus, uncontrolled hypertension, and current febrile illness were excluded from the study. Written informed consent was obtained from all eligible participants and ethical approval was obtained from the Health Research Ethical Committee of Ahmadu Bello University Teaching Hospital, Zaria (ABUTHZ/HREC/B03/2017).

**Data collection**

Sociodemographic characteristics were obtained from each participant using a proforma and 5mL of venous blood was obtained from each participant.

**Serological assay**

The 5 mL of venous blood obtained was put into a plain bottle, allowed to clot and then centrifuged to obtain serum. The sera were frozen at -70°C till the sample size was attained. Immunoglobulin G detection was done using Ct IgG ELISA catalogue No: IB19202 obtained from Immuno-Biological Laboratories (IBL-America), Minneapolis. The assay was performed in the ART laboratory by a laboratory scientist. The reagent was prepared by adding the contents of the reagent bottle (25 mL, 20×) to 475 mL of distilled water and was stored at 18–26°C. All specimens and kit reagents were brought to room temperature (18–26°C) and gently mixed. The desired number of coated strips were placed into the holder. The test samples were prepared into a 1:21 dilution by adding 10 µL of sample and adding 200 µL of sample diluent and were mixed well. This was followed by addition of 100 µL of diluted sera, calibrator, and control into appropriate wells. For the reagent blank, 100 µL of the sample diluent was dispensed into the 1A well position. The holder was tapped to remove air bubble from the liquid and to ensure adequate mixing. These were incubated for 20 minutes at room temperature. The liquid was removed from all wells. The wells were washed three times with 300 µL of 1× wash buffer and were blotted on an absorbance paper; 100 µL of enzyme conjugate was dispensed into each well and was incubated for 20 minutes at room temperature. The enzyme conjugate was removed from all wells and the wells were washed three times with 300 µL of 1× wash buffer; were blotted on an absorbance paper, 100 µL of TMB substrate was dispensed and incubated for 10 minutes at room temperature. This was followed by addition of 100 µL of stop solution. The optical density (OD) at 450 nm was read using an ELISA reader within 15 minutes. The tests were validated with the control samples. The cut-off value was calculated as follows: Calibrator OD × calibrator factor. The antibody index was obtained by dividing the OD value of each sample by cut-off value. The test was considered positive if the antibody index >1.1 and negative if the antibody index was ≤1.1.

**Data analysis**

This was done using IBM Statistical Package for Social Sciences version 21. The level of significance was set at $P < 0.05$. The
sociodemographic characteristics of the two groups were compared using the Fisher exact test. Chi-square test was used to test for association between IgG seropositivity and spontaneous miscarriage.

**Results**

**Sociodemographic characteristics of participants**

A total of 166 women were studied. Majority of the women were aged between 15–29 years with a mean age of 28.9 ± 6.7 years. Majority of the participants were predominantly Hausa, married Muslim women. Over 40% were gainfully employed. Less than 50% of the women had tertiary level of education while the majority of their partners had tertiary level of education. The two groups were similar in all the sociodemographic characteristics (P > 0.05). These characteristics are shown in Table 1.

**Association of Ct IgG seropositivity with spontaneous miscarriage**

Prevalence of Ct IgG seropositivity was higher in women with spontaneous miscarriage than in women with on-going pregnancy but the difference was not significant (P = 0.192) as seen in Table 2.

**Discussion**

There is paucity of published reports in the literature on the role of genital Ct in causing miscarriage in black women despite some studies have shown black women are at least five times more likely to acquire genital Ct infection than white women.[12-14] The studies conducted with the aim of elucidating the association between Ct and spontaneous miscarriage were largely conducted among non-black women and revealed conflicting results. This study is one of the pioneer studies that have been carried out to elucidate the role of genital Ct infection in spontaneous miscarriage in Nigeria. This study found no association between Ct IgG seropositivity and spontaneous miscarriage. This is consistent with findings from other studies that did not find a significant association between genital Ct and spontaneous miscarriage.[15,16] However, this is in contrast to the findings of other studies that have established an association between IgG seropositivity and spontaneous miscarriage.[10-13] This finding of a lack of association between Ct infection and spontaneous miscarriage in this study may be due to the technique employed in the diagnosis of Ct in the study which is ELISA. The sensitivity of polymerase chain reaction (PCR) has been found to be very high and is capable of detecting <10 DNA copies of Ct while ELISA technique is inferior in Ct detection.[17] This is further supported by studies that demonstrated an association between Ct and spontaneous miscarriage using PCR but no association when antibody detection was used in the same subjects.[18] Despite this, use of ELISA in cases of past Ct infections or upper genital infections not amenable to sampling, remains an effective method to detect the infection and its importance.

| Characteristic                  | Case group n=83 | Control group n=83 | Odds ratio | Confidence interval |
|--------------------------------|-----------------|--------------------|------------|---------------------|
|                                |                 |                    |            | Lower               |
| **Age (years)**                |                 |                    |            | Upper               |
| 15-19                          | 6 (7.2)         | 5 (6.0)            | 1.00       |                     |
| 20-24                          | 18 (21.7)       | 19 (22.9)          |            |                     |
| 25-29                          | 24 (28.9)       | 24 (28.9)          |            |                     |
| 30-34                          | 13 (15.7)       | 13 (15.7)          |            |                     |
| 35-39                          | 14 (16.9)       | 15 (18.1)          |            |                     |
| 40-44                          | 8 (9.6)         | 7 (8.4)            |            |                     |
| **Tribe**                      |                 |                    |            |                     |
| Hausa                          | 61 (73.5)       | 59 (71.1)          | 0.18       |                     |
| Igbo                           | 3 (3.6)         | 0 (0.0)            |            |                     |
| Yoruba                         | 1 (1.2)         | 4 (4.8)            |            |                     |
| Others                         | 18 (21.7)       | 20 (24.0)          |            |                     |
| **Marital status**             |                 |                    |            |                     |
| Married                        | 81 (97.6)       | 83 (100)           | 0.15       |                     |
| Single                         | 2 (2.4)         | 0 (0.0)            |            |                     |
| **Religion**                   |                 |                    |            |                     |
| Islam                          | 72 (86.7)       | 72 (86.7)          | 1.00       |                     |
| Christianity                   | 11 (13.2)       | 11 (13.2)          |            |                     |
| **Occupation**                 |                 |                    |            |                     |
| Unemployed                     | 32 (38.6)       | 30 (26.1)          | 0.99       |                     |
| Study                          | 12 (14.5)       | 11 (13.2)          |            |                     |
| Employed                       | 17 (20.5)       | 17 (20.5)          |            |                     |
| Trading                        | 18 (21.7)       | 21 (25.3)          |            |                     |
| Others                         | 4 (4.9)         | 4 (4.8)            |            |                     |
| **Woman’s education**          |                 |                    |            |                     |
| Primary                        | 14 (16.9)       | 15 (18.1)          | 0.24       |                     |
| Secondary                      | 28 (33.7)       | 28 (33.7)          |            |                     |
| Tertiary                       | 32 (38.5)       | 37 (44.6)          |            |                     |
| Quranic                        | 9 (10.8)        | 3 (3.6)            |            |                     |
| None                           | 2 (2.4)         | 0 (0.0)            |            |                     |
| **Partner’s education**        |                 |                    |            |                     |
| Primary                        | 1 (1.2)         | 2 (2.4)            | 0.05       |                     |
| Secondary                      | 29 (34.9)       | 22 (26.5)          |            |                     |
| Tertiary                       | 44 (53.0)       | 58 (69.9)          |            |                     |
| Quranic                        | 8 (9.6)         | 1 (1.2)            |            |                     |
| None                           | 1 (1.2)         | 0 (0.0)            |            |                     |

| IgG       | Case group n=83 | Control group n=83 | Odds ratio | Confidence interval |
|-----------|-----------------|--------------------|------------|---------------------|
| Positive  | 7 (8.4)         | 3 (3.6)            | 0.41       | 0.10                | 1.63                 |
| Negative  | 76 (91.6)       | 80 (96.4)          |            |                     |
| Total     | 83 (100)        | 83 (100)           |            |                     |

Table 1: Sociodemographic characteristics of the case and control groups in a study of genital Chlamydia trachomatis IgG seropositivity and spontaneous miscarriage in ABUTH, 2018

Table 2: Association of IgG seropositivity and spontaneous miscarriage, in a study of genital Chlamydia trachomatis IgG seropositivity and spontaneous miscarriage in ABUTH, 2018
has not faded while PCR is the test of choice to detect current Ct infection at the earliest days of transmission and persistent Ct infection in arrested-immunity cases.[18] However, PCR is not readily available for clinical utility in many hospitals in poor-resourced settings.

Specific serovars of Ct are associated with particular racial groups.[7] Chlamydia trachomatis serovar Ia has been found to be more frequent in black women while serovar D is found less commonly and this may have epidemiologic significance.[17] This may suggest the possibility that not all Ct serovars are associated with spontaneous miscarriage thus possibly explains why no association was found between Ct seropositivity and spontaneous miscarriage in this study.

A limitation of this study was the lack of exhaustive investigations for other infectious causes of spontaneous miscarriages like Cytomegalovirus, Parvovirus, Brucella abortus, and Mycoplasma hominis that have also been known to cause spontaneous miscarriage.

**Conclusion**

Ct IgG seropositivity was not found to be associated with spontaneous miscarriage in black women. This finding does not support the use of ELISA as a diagnostic technique to screen women in the preconception and antenatal period with the aim of reducing maternal morbidity and mortality from miscarriage. However, multicentre study with larger sample size and using PCR as a diagnostic technique and serovar typing is recommended to completely elucidate the role of Ct in causing spontaneous miscarriage in black women.

**Acknowledgements**

Dr Olorukooba AA of Department of Community Medicine, Ahmadu Bello University, Zaria for contributing to the analysis of data.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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