Kanem J Med Sci 2026; 20(1): 71-76
Open Access article distribution in the terms of the Creative Commons Attribution License (CC by 4.0)

ISSN 2006-4772
https://doi.org/10.36020/kjms.2026.2001.010

Original Article

Association of anti-chlamydial antibodies with spontaneous miscarriage

Emmanuel. O. Agbogo,[1] Hassan T. Olayinka,[1] Sunday E. Achanya,[1] Ikechukwu C. Ugwu,[1] Chama Calvin.[2]

[1]Department of Obstetrics and Gynaecology, Federal University of Health Sciences, Azare, Bauchi State, Nigeria. [2]Department of Obstetrics and Gynaecology, Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria

Correspondence to: Dr Emmanuel Onyilo Agbogo, Department of Obstetrics and Gynaecology, Federal University of Health Sciences, Azare, Bauchi State, Nigeria. Email: **emmanuel.agbogo@fuhsa.edu.ng, agbogo14@gmail.com**. Tel: +2348035392616.

Abstract

Background: Miscarriage is a common adverse pregnancy outcome with significant impact on the woman's health, her family and the community at large. Chlamydia trachomatis is the commonest Sexually Transmitted Infection worldwide and has been implicated in the aetiology of miscarriage. However, its role in spontaneous miscarriage is still unclear, as reports are conflicting. **Objective**: This study was to determine the association between Chlamydia trachomatis infection and spontaneous miscarriage. **Methodology**: This case-control study was conducted at the Gynaecological Emergency Clinic and Antenatal Clinic of the Abubakar Tafawa Balewa University Teaching Hospital, Bauchi. Eighty-five women with spontaneous miscarriage (case group) were compared with eighty-five women with ongoing pregnancy beyond twenty-eighth weeks of gestation and no prior miscarriage (control group) matched for age and parity. Sera were obtained from both groups and tested for Chlamydia IgG antibodies using ELISA. The data obtained, including their socio-demographic characteristics, were recorded and analysed using IBM SPSS version 26. **Results:** The overall sero-prevalence of Chlamydia trachomatis IgG among the participants in this study was 7.1%, while the prevalence for the case group was 10.6% and that for the control group was 3.5%. This sero-prevalence of Chlamydia trachomatis was not statistically significant between the cases and controls. (p=0.132, OR=3.237, CI=0.845-12.407). **Conclusion**: Demonstration of association of chlamydia with miscarriage was not established in this study despite Chlamydia trachomatis IgG being detected more in women with miscarriage than those without miscarriage, suggesting an increased risk for miscarriage from exposure to the organism. ELISA technique alone may not be sufficient if diagnosis of pregnant women is to be undertaken. A multi-centred prospective cohort study, employing the combination of ELISA and PCR, may yield further clarity in the future.

Keywords: *Chlamydia trachomatis, ELISA, IgG, Miscarriage*.

Introduction

Miscarriage, the spontaneous termination of pregnancy before the age of foetal viability, occurs worldwide and results in significant morbidity and mortality.[1] Recognisably, up to 30% of pregnancies suffer this outcome, but some pass unnoticed.[1,2] It is of health concern, being responsible for most gynaecological emergency consultations and directly impacting the woman, her family and community at large.[1-3]

Although the aetiology in some instances remains unknown, reports have indicated an association between reproductive tract infections and risk of

Cite this article as: Emmanuel. O. Agbogo, Hassan T. Olayinka, Sunday E. Achanya, Ikechukwu C. Ugwu, Chama Calvin. Association of anti-chlamydial antibodies with spontaneous miscarriage. Kanem J Med Sci 2026; 20(1): 71-76.

Received: 25th March 2025 **Accepted: 19th May 2026** **Published: 20th June 2026**

spontaneous pregnancy loss, just like other pregnancy complications such as Ectopic pregnancy, Preterm delivery and Stillbirth.[4,5] Generally, it has been suggested that 10-66% of Miscarriages are attributed to infection as a cause, and this is more pronounced in low and middle-income countries.[4,5] Chlamydia trachomatis is among the leading causes, being also the most reported.[4-7] A previous study in Zaria,[8] Nigeria showed Chlamydia seroprevalence of 8.4% in women with spontaneous miscarriage and another from Sokoto, 9 Nigeria showed prevalence of 11%, but surprisingly, a UK study,[10] showed far greater seroprevalence of 28% in women with spontaneous miscarriage. These studies, including others,[11-13] have established conflicting associations between chlamydia infection and miscarriages.

Chlamydia trachomatis, a Gram-negative obligate intracellular bacterium of the family Chlamydiaceae with humans as its only natural host, primarily infects epithelial cells, with serovars D–K being responsible for most sexually transmitted infections.[14] The organism exists as an infectious elementary body and a replicative reticulate body; its association with miscarriage is thought to involve inflammatory and immunological mechanisms which may activate inflammatory pathways that stimulate uterine contractions and contribute to miscarriage or preterm birth.[14,15] Persistent infection can also alter endometrial stromal cell function, impair decidualization, and disrupt trophoblastic invasion necessary for successful pregnancy maintenance.[15] Its frequently asymptomatic nature contributes to delayed diagnosis and increases the risk of adverse reproductive outcomes.[14-17]

Some of the common diagnostic methods include culture, antigen detection methods and serology. Serology employs the determination of antibodies against the organism, and of the available methods, ELISA has been useful in sero-epidemiological settings with comparable sensitivity.[18] The control and prevention measures against Chlamydia trachomatis infection involve screening for those at risk, prompt diagnosis and treatment.[19]

Chlamydia genital infection occurs as silent epidemics among blacks and more so, in their women for years because of poor awareness, screening and monitoring.[20] As no data exist in our setting regarding the prevalence of Chlamydia infection in our women and, more importantly, in pregnancy, this study therefore became compelling, and the association between Chlamydia infection and miscarriage could also eventually be established.

This study aimed to determine the association of anti-chlamydial antibodies among women experiencing spontaneous miscarriage.

Materials and methods

This case-control study was conducted between March 2023 and September 2023 at the Department of Obstetrics and Gynaecology, Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Bauchi. Bauchi State, Nigeria. Sample size was estimated from the study by Bagderi et al,[21] with P1=11%, P2=0%; using a 95% confidence level, 80% power and a case-control ratio of 1:1, a minimum sample size of approximately 80 participants per group was obtained. It was conducted among 85 women presenting with spontaneous miscarriages at the Gynaecological emergency and Gynaecological out-patient clinic (cases) and 85 women with ongoing pregnancy beyond 28 weeks' gestation at Ante-natal clinic (controls). The cases were consenting women with threatened, inevitable, incomplete, complete and missed miscarriage, while the control group were consenting women with ongoing pregnancy beyond 28 weeks without prior miscarriage, which ensured they were truly free of the outcome. Women with induced miscarriage, cervical incompetence, poorly controlled diabetes, obesity, uncontrolled hypertension, preceding febrile illness, women who smoke or take alcohol were excluded. All the eligible enrollees were recruited to the case group at presentation. For each participant in the case group, a pregnant woman with an ongoing pregnancy at least 28 weeks was matched with a similar age group and same parity as the control group. The ethical clearance for the study was obtained from the Health Research Ethics Committee of the Abubakar Tafawa Balewa University Teaching Hospital. Written consent was obtained from participants, and their sociodemographic characteristics were entered into a proforma.

Procedure

Samples were collected by trained research assistants. About 5millilitres of venous blood was collected from the peripheral vein on the upper limb using standard venipuncture phlebotomy under aseptic conditions for both cases and controls. The blood was carefully and gently dispensed into a plain bottle container which was appropriately labelled with the participant's identification number. The blood was allowed to clot at room temperature and centrifuged to get the serum. The serum obtained was immediately stored at -20ºc (frozen) in the chemical laboratory until all the study samples were pooled for analysis according to the manufacturer's instructions.

Immunoglobulin detection was done using Chlamydia trachomatis IgG ELISA catalogue number 202308001 obtained from IVD Diagnostics Reagents, Pharmacopia of the People's Republic of China (diagnostic sensitivity 95% and specificity 99%).

Serology

All reagents were allowed to equilibrate to room temperature (20–25°C) for 10 minutes before use. The wash buffer concentrate was diluted 1:40 with distilled water according to the manufacturer's instructions. A 1:10 dilution of each serum sample was prepared by adding 10 µL of serum to 1.0 mL of sample diluent. Subsequently, 100 µL of sample diluent was dispensed into the designated wells, excluding the blank, negative control, and positive control wells. Thereafter, 5 µL of each diluted serum sample was added to the corresponding wells and mixed thoroughly using a micropipette. The negative and positive controls were then added to their respective wells. The microplate was gently shaken for 30 seconds to ensure proper mixing and incubated at 37°C for 30 minutes with the plate sealed using the adhesive membrane provided. After incubation, the sealing membrane was removed, and the wells were washed five times with diluted wash buffer. Residual wash buffer was removed by gently tapping the inverted plate on absorbent paper. Next, 100 µL of enzyme conjugate was added to each well, and the plate was incubated at 37°C for 30 minutes. Following incubation, the wells were washed five times with wash buffer to remove unbound conjugate. Substrate solutions A and B (50 µL each) were then added to each well, and the plate was incubated at 37°C for 15 minutes. The enzymatic reaction was terminated by adding 50 µL of stop solution to each well. The optical density (OD) was measured at 450 nm using a microplate ELISA reader within 15 minutes of adding the stop solution. The assay was considered valid when the following criteria were met: Mean OD value of the negative controls ≤ 0.10, while Mean OD value of the positive controls ≥ 0.80.

Calculation and Interpretation of Results

The cut-off value was calculated as three times the mean OD value of the negative controls. When the mean OD of the negative controls was less than 0.10, a value of 0.10 was used for the calculation. When the mean negative control OD exceeded 0.10, the actual mean value was used. **Cut-off value =** Mean negative control OD × 3. For this assay: Mean negative control OD = 0.20, Cut-off value = 0.20 × 3 = 0.60.

Samples with OD values greater than or equal to the cut-off value (≥ 0.60) were considered positive for *Chlamydia trachomatis* IgG antibodies, while samples with OD values below the cut-off value (< 0.60) were considered negative.

Data Analysis

The data were analysed using computer-based IBM SPSS version 26 software. Categorical variables were expressed in the form of frequency and percentages, and significant differences between groups were determined using Chi-square or Fisher's test where appropriate. The continuous variables were presented as mean with standard deviations. In all cases involving data analysis, a p-value of < 0.05 was considered statistically significant.

Results

There was no case of attrition during the study, so the participation rate was 100%. The overall age range for all the participants was between 17-44 years. The majority of the women were aged 20-29 years (52.9% and 54.1% for cases and controls, respectively). They had an overall mean age of 27.9 ± 6.8 years, while the mean ages for cases and controls were 27.8± 7.057 and 27.99 ±6.652 respectively, with a p-value of 0.893, which was not statistically significant. All the participants in this study were married, and the majority (77.6%) of them were Muslims, and others were Christians. Only 30% of the subjects were gainfully employed, 77.7% had at least senior secondary education, and 91.1% of their partners also had at least senior secondary education. The least parity was nulliparity, while the highest parity was eleven.

The IgG seropositivity of Chlamydia trachomatis for cases was 10.6% (9/85) and for controls 3.5% (3/85), as indicated in Table 2. The p-value was noted to be 0.132, which was found not to be statistically significant (>0.05). The odds ratio of 3.237 implies that those with miscarriage were three times more likely to have been infected with Chlamydia trachomatis.

Table 1: Sociodemographic characteristics of case and control group in a study of Chlamydia Trachomatis IgG seropositivity and spontaneous miscarriage.

CHARACTERISTICS	CASES (%)	CONTROLS (%)	P-VALUE
AGE(Years) Mean	27.85 ±7.057	27.99 ±6.652	0.893ŧ
Age (years)			
15-19	7(8.2)	7 (8.2)	
20-24	27 (31.7)	26 (30.6)	0.99*
25-29	18 (21.2)	20 (23.5)	
30-34	10 (11.7)	10 (11.7)	
35-39	17 (20)	17 (20)	
40-44	6 (7)	5 (5.9)	
TRIBE			
Hausa/Fulani	64 (75.3)	55 (64.7)	
Igbo	2 (2.4)	6 (7)	0.082†
Yoruba	0 (0)	4 (4.7)	
Others	19 (22.4)	20 (23.5)	
MARITAL STATUS			
Married	85 (100)	85 (100)	NA
Single	0 (0)	0 (0)	
OCCUPATION			
Unemployed	47 (55.3)	40 (47.1)	
Study	12 (14.1)	20 (23.5)	
Employed	12 (14.1)	18 (21.2)	
Trading	7 (8.2)	4 (4.7)	0.187†
Others	7 (8.2)	3 (3.5)	
WOMEN EDUCATION			
Primary	13 (15.3)	4 (4.7)	
Secondary	27 (31.8)	24 (28.2)	
Tertiary	36 (42.4)	45 (52.9	0.105†
Qur'anic	6 (7.1)	5 (5.9)	
None	3 (3.5)	7 (8.2)	
PARTNER'S EDUCATION			
Primary	3 (3.5)	0 (0)	
Secondary	20 (23.5)	21 (24.7)	0.273†
Tertiary	54 (63.5)	60 (70.6)	
Qur'anic	4 (4.7)	3 (3.5)	
None	5 (5.9)	1 (1.2)	

† Fisher's Exact * Chi-squared ŧ student t-test NA not applicable

Table 2: Association of IgG sero-positivity and Spontaneous miscarriage in a study of genital Chlamydia Trachomatis

Chlamydia IgG	Cases (%)	Control (%)	Odds ratio	95% ci	p-value
Positive	9 (10.6)	3 (3.5)	3.237	0.845- 12.404	0.132
Negative	76 (89.4)	82 (96.5)			
Total	85 (100)	85 (100)			

Discussion

The socio-demographic characteristics for both groups were found to be similar or closely related, with no statistically significant difference. This outcome implies that the subjects were sampled from the same population and thus comparison can be made reliably and reasonable inferences can be drawn.

The overall prevalence of infection was found to be 7.1%; the sero-positivity was 10.6% and 3.5% for cases and controls, respectively. This is like observations in other studies in other parts of Northern Nigeria by Aliyu et. al,[8] in Zaria (8.4% versus 3.6%) and Spencer et al. [9] in Sokoto (11.0% versus 4.4%). Horne et. Al[10] in the United Kingdom observed much higher but equal sero-positivity rates for cases and controls (28% vs 25.9%), which may be attributed to the possibility of a higher prevalence of Chlamydial infection in that population and a significantly larger sample size compared to ours. Although the sero-prevalence obtained in this study and others in Northern Nigeria using ELISA technique, was higher among women with miscarriage, no statistically significant association was established (with p-values of 0.132, 0.192,[8] and 0.4312[9] respectively) and obviously Horne et. al,[10] using the same technique, with closely similar prevalence rates for both cases and controls could not have established any statistically significant association between Chlamydia infection and miscarriage (p=0.71).

Bagheri et. al,[21] also didn't find any significant association between Chlamydia and miscarriage using ELISA IgG (p=0.64), but using PCR (p=0.03) demonstrated a positive association, and this was corroborated in the reports by Gutierrez et al. [22,] and Ahmadi et.al.[23] Shaheen et. al,[24,] however, demonstrated significant association using both ELISA and PCR in their study.

Although expensive, PCR is the recommended diagnostic method for Chlamydia trachomatis, being very sensitive with high specificity and can detect the organism even in very low levels,[18,25] but literature has reported no significant difference exists between the use of culture, molecular methods and serology in the determination of Chlamydia trachomatis in association with miscarriage.[26] It is also a fact that some upper genital tract infections are not amenable to PCR, and the assessment of Chlamydia trachomatis sequelae is best done using serology owing to the fact that the organism is an obligate intracellular bacterium affecting the genital tract, with most of its infection being asymptomatic, thus occurring unnoticed[27]. ELISA is cost-effective and known to help evaluate disease conditions, differentiating between ongoing and past damage due to Chlamydia.[18] There is a possibility that acute or recent infections by Chlamydia trachomatis, which can cause miscarriage, were not picked up or detected by ELISA IgG. This could be due to the inability of the kit to detect IgM antibodies, which are first elaborated.

Conclusion: Demonstration of association of chlamydia with miscarriage was not established in this study despite Chlamydia trachomatis IgG being detected more in women with miscarriage than those without miscarriage, suggesting an increased risk for miscarriage from exposure to the organism. ELISA technique alone may not be sufficient if diagnosis of pregnant women is to be undertaken. A multi-centred prospective cohort study, employing the combination of ELISA and PCR, may yield further clarity in the future.

References

1. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. Miscarriage matters: The epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet. 2021;397(10285):1658-1667.
2. World Health Organisation. Improving maternal and newborn health and survival and reducing stillbirth: progress report 2024. Geneva: World Health Organisation; 2024.
3. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Håberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register-based study. BMJ. 2019;364: l869.
4. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SEM, Horne AW. The role of infection in miscarriage. Hum Reprod Update. 2016;22(1):116-133.
5. Wastnedge EAN, Reynolds RM, van Boeckel SR, et al. Pregnancy and fertility-related adverse outcomes associated with *Chlamydia trachomatis* infection: a global systematic review and meta-analysis. Sex Transm Infect. 2021;97(5):322-329.
6. Heumann CL, Quilter LAS, Eastment MC, Heffron R, Hawes SE. Adverse birth outcomes and maternal *Chlamydia trachomatis* infection:

7. systematic review and meta-analysis. Sex Transm Dis. 2017;44(11):679-690.
8. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted infections treatment guidelines, 2021. MMWR Recomm Rep. 2021;70(4):1-187.
9. Aliyu RM, Adesiyun AG, Aliyu S. Does genital chlamydia trachomatis cause spontaneous miscarriage in black women? Trop J Obstet Gynaecol. 2019; 36(3):455-458.
10. Spencer TH, Yahaya U, Mohammed K, Argbonlahor DE, Okwori AE, Garba MK, et al. Chlamydiasis and Human Heat Shock Protein 60-KDa Expression among Women with Miscarriages in Sokoto Metropolis, North-Western Nigeria. Microbiol Res J Intl. 2017:1-2.
11. Horne AW, Wheelhouse N, Horner PJ, Duncan WC. Association of past Chlamydia trachomatis infection with miscarriage. JAMA Network Open. 2020; 3(10):1-3.
12. Wilkowska-Trojniel M, Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Redzko S, Przepiesc J, Zdrodowski M. The influence of chlamydia trachomatis infection on spontaneous abortions. AdvMed Sci. 2009;54(1):86-90.
13. Avasthi K, Garg T, Gupta S, Grewal RK, Ram S. A study of prevalence of chlamydia trachomatis infection in women with first trimester pregnancy losses. Ind J Pathol Microbiol 2003; 46:133-136.
14. Baud D, Goy G, Jaton K, Osterheld MC, Blumer S, Borel N, et al. Role of Chlamydia trachomatis in miscarriage. Emerg. Infect Dis. 2011; 17(9):1630-1635.
15. Bambani T, Srivastava A, Saif S, Kedia N. A comprehensive review on prevalence and its adverse outcome of *Chlamydia trachomatis* on female genital tract. Int J Reprod Contracept Obstet Gynecol. 2024;13(3):768-775.
16. World Health Organisation. Chlamydia: Fact Sheet. Geneva: World Health Organisation; 2025.
17. Peuchant O, Touati A, Sperandio C, Hénin N, Laurier-Nadalié C, Bébéar C. Changing pattern of *Chlamydia trachomatis* infections and implications for diagnosis and screening. Clin Microbiol Rev. 2023;36(4): e00019-22.
18. Giakoumelou S, Wheelhouse N, Brown J, Wade J, Simitsidellis I, Gibson D, et al. Chlamydia trachomatis infection of human endometrial stromal cells induces defective decidualisation and chemokine release. Sci. Rep. 2017; 7(1):1-9.
19. Meyer T. Diagnostic procedures to detect Chlamydia trachomatis infections. Microorganisms. 2016; (3):25, 1-10.
20. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted infections treatment guidelines, 2021. MMWR Recomm Rep. 2021;70(4):1-187.
21. Diesel J, Peterson A, Peterman T. Reported chlamydia and gonorrhoea are decreasing among young Black women: good news or bad news? A narrative review. *Sex Transm Dis*. 2021;48(12): e228-e235. doi:10.1097/OLQ.0000000000001491.
22. Bagheri S, Roghanian R, Golbang N, Golbang P, Nasr Esfahani MH. Molecular evidence of *Chlamydia trachomatis* infection and its relation to miscarriage. *Int J Fertil Steril*. 2018;12(2):152-156. doi:10.22074/ijfs.2018.5184.
23. Gutiérrez-Campos R, Gutiérrez-Santillán EA, Bravo-Aguirre DE, Robles-Martínez MDC, Cumplido-Mier CD, Rosas-Cabral A. Association between early miscarriage and Chlamydia trachomatis infection in Aguascalientes, Mexico, Rev Med Inst Mex Seguiro SOC. 2020; 58(1)21-27.
24. Ahmadi A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Roshani D, Ghaderi E, et. All. The relationship between chlamydia trachomatis genital infection and spontaneous abortion. J Reprod Infertil. 2016; (2):110-116.
25. Shaheen, UpmaSaxena. Association between Chlamydia trachomatis infection and recurrent pregnancy loss; Int J Reprod Contracept Obstet Gynecol. 2022; 11(2):557-562.
26. Centres for Disease Control and Prevention (CDC) – Chlamydial Infections: STI Treatment Guidelines. Atlanta (GA): CDC; 2021
27. Chernesky MA. The laboratory diagnosis of Chlamydia trachomatis infections. Can J Infect Dis Med Microbiol. 2005; 16(1):39-44.
28. Persson K. The role of serology, antibiotic susceptibility testing and serovar determination in genital chlamydia infections. Best Pract Res Clin. Obstet. Gynaecol 2002; 16(6): 801-814.