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Association of malaria parasitaemia and cytomegalovirus infection with serum interferon-γ levels among women with spontaneous abortion: a hospital-based cross-sectional study in Kano, North-West Nigeria

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Abstract

Background: Malaria and cytomegalovirus (CMV) infections have been implicated in adverse pregnancy outcomes, but their relationship with immune activation during spontaneous abortion remains incompletely understood. This study investigated the association of malaria parasitaemia and CMV infection with serum interferon-gamma (IFN-γ) concentrations among women with spontaneous abortion in Kano, North-West Nigeria.

Methods: A hospital-based cross-sectional study was conducted among 91 women with spontaneous abortion at two tertiary hospitals in Kano, Nigeria. Malaria parasitaemia was detected by microscopy, anti-CMV IgM antibodies and serum IFN-γ concentrations were measured by ELISA, and CMV DNA was detected by qPCR in IgM-positive samples. Associations were assessed using chi-square tests, Mann–Whitney U and multivariable logistic regression ($p<0.05$).

Results: Malaria parasitaemia was detected in 19/91 (20.9%) participants, while 11/91 (12.1%) were CMV IgM-seropositive, of whom 3/11 (27.3%) had detectable CMV DNA. Women from monogamous households had lower odds of malaria parasitaemia than those from polygamous households (AOR = 0.28, 95% CI: 0.10–0.82; p = 0.021). The median serum IFN-γ concentration was 0.969 pg/mL (IQR: 0.803–1.095). Serum IFN-γ concentrations were higher in women with malaria parasitaemia (median: 1.067 vs. 0.898 pg/mL; p = 0.007) and CMV IgM seropositivity (median: 1.067 vs. 0.942 pg/mL; p = 0.014).

Conclusion: Malaria parasitaemia and CMV IgM seropositivity were associated with higher serum IFN-γ concentrations among women with spontaneous abortion. These findings highlight

the importance of strengthening infection prevention, early diagnosis, and appropriate clinical management during pregnancy in malaria-endemic settings.

Clinical trial number: Not applicable

Keywords: Malaria parasitaemia; Cytomegalovirus; Interferon-gamma (IFN-γ); Spontaneous abortion; Pregnancy; Nigeria

1. Introduction

Spontaneous abortion is one of the most common adverse pregnancy outcomes, affecting approximately 10–20% of clinically recognized pregnancies worldwide and imposing substantial physical, psychological, and social burdens on affected women and their families [1]. Although chromosomal abnormalities account for many cases, infectious diseases remain important and potentially preventable contributors to pregnancy loss, especially in low- and middle-income countries where maternal exposure to multiple pathogens is common [2,3].

Human cytomegalovirus (CMV), also known as human herpesvirus 5, is a ubiquitous herpesvirus that establishes lifelong latency following primary infection and may reactivate when host immunity is altered, including during pregnancy [4,5]. CMV seroprevalence is particularly high in developing countries and among women of reproductive age [6,7]. CMV has an estimated global seroprevalence of approximately 83% [8]. In Nigeria, studies have also demonstrated widespread CMV exposure among pregnant women, with reported CMV IgG seroprevalence ranging from 86.8% in Kano [9] to 87.9% in Ido-Ekiti [10]. Evidence of recent infection has also been reported, with CMV IgM seroprevalence ranging from 7.8% in Ido-Ekiti [10] to 32.6% in Kaduna [11] and 39.3% in Bauchi [12]. CMV infection has been implicated in adverse pregnancy

outcomes through placental infection, impaired trophoblast function, and dysregulation of maternal immune responses [13,14].

Malaria remains another major threat to maternal and fetal health in sub-Saharan Africa. Infection during pregnancy has been associated with miscarriage, maternal anaemia, fetal growth restriction, stillbirth, and other adverse obstetric outcomes [15]. In addition to its direct pathological effects, malaria induces marked inflammatory and immune responses and has been reported to promote the reactivation of several latent herpesviruses, suggesting that coexisting infections may further influence pregnancy outcomes [16].

Interferon-gamma (IFN-γ) is a key cytokine produced primarily by activated T lymphocytes and natural killer cells that plays an essential role in antiviral and antiparasitic immunity [17-19]. During normal pregnancy, tightly regulated IFN-γ responses contribute to immune defence while maintaining maternal-fetal immune tolerance [20]. However, excessive or dysregulated IFN-γ production may promote inflammatory responses at the maternal-fetal interface, impair placental function, and increase the risk of pregnancy loss [19,21]. Both CMV infection and malaria are capable of stimulating IFN-γ production [22,23], making this cytokine a biologically plausible marker of infection-associated immune activation during pregnancy.

Despite the high burden of both malaria and CMV in Nigeria, limited data are available on their relationship with systemic IFN-γ responses among women experiencing spontaneous abortion, particularly in northern Nigeria where both infections are endemic. Improved understanding of these associations may provide insights into infection-related immune responses accompanying spontaneous abortion and inform future studies investigating the immunopathogenesis of

adverse pregnancy outcomes. Therefore, this study investigated the association of malaria parasitaemia and cytomegalovirus infection with serum interferon-gamma levels among women with spontaneous abortion in Kano, North-West Nigeria.

2. Methods

2.1 Study design and setting

The study was a hospital-based cross-sectional study conducted among women who experienced spontaneous abortion and sought medical care at Murtala Muhammad Specialist Hospital (MMSH) and Bamalli Nuhu Maternity Hospital (BNMH) in Kano, North-West Nigeria. Both hospitals are tertiary healthcare facilities that provide comprehensive obstetric and gynaecological services and serve as major referral centres for maternal healthcare within Kano State. Participant recruitment was undertaken through the obstetrics and gynaecology units of the two hospitals during the study period.

2.2 Study population

The study population comprised women who presented with spontaneous abortion at the participating hospitals during the study period. Women who provided written informed consent were eligible for inclusion irrespective of age. Women with threatened abortion and those who were critically ill were excluded.

2.3 Sample size and sampling

The minimum sample size was determined using the single population proportion formula described by Lwanga and Lemeshow [24]:

$$n = \frac{Z^2 p(1-p)}{d^2}$$

where n is the minimum required sample size, Z is the standard normal deviate corresponding to a 95% confidence level (1.96), p is the estimated prevalence of cytomegalovirus (CMV) infection among women with recurrent miscarriage, and d is the desired margin of error (0.05). An estimated CMV prevalence of 4.8%, reported among women with recurrent miscarriage in Nigeria [25], was used for the calculation, yielding a minimum sample size of 70 participants. To improve the precision of the study estimates and increase statistical power, 91 participants were recruited.

Participants were enrolled using a convenience sampling technique from women who presented with spontaneous abortion at Murtala Muhammad Specialist Hospital (MMSH) and Bamalli Nuhu Maternity Hospital (BNMH) during the study period. Sample allocation between the two hospitals was proportional to their average patient attendance, with 63 participants recruited from MMSH and 28 participants from BNMH.

2.4 Data collection and specimen processing

A structured questionnaire was administered to collect participants' sociodemographic and clinical information, including age, educational attainment, occupation, family type, obstetric history, gestational age, medical history, and presenting symptoms.

Venous blood samples were collected from all eligible participants using standard aseptic procedures. Whole blood was used for malaria microscopy, while blood intended for serological and cytokine analyses was collected into plain collection tubes and allowed to clot at room

temperature for approximately 30 min. The samples were then centrifuged at 1,000–2,000 × *g* for 10–15 min to separate the serum. The serum was carefully aliquoted into sterile cryovials and stored at 2–8°C for up to 72 h before long-term storage at −20°C until analysis for anti-CMV IgM antibodies, serum interferon-γ concentrations, and CMV DNA detection where applicable.

2.5 Laboratory procedures

2.5.1 Malaria microscopy

Malaria parasitaemia was determined by examination of Giemsa-stained thick and thin blood films according to World Health Organization (WHO) guidelines. Thick blood films were used for parasite detection, while thin films were used for species identification. All slides were independently examined by two experienced microscopists, with discrepant results resolved by a third reader.

2.5.2 Detection of anti-CMV IgM antibodies

Serum anti-CMV IgM antibodies were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Ideal Medical Technology Co., Ltd., China) according to the manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader, and results were interpreted according to the manufacturer's recommended cut-off values.

2.5.3 Quantification of serum IFN-γ

Serum interferon-γ concentrations were quantified using a commercially available human IFN-γ ELISA kit following the manufacturer's instructions. Absorbance was read at 450 nm, and

cytokine concentrations (pg/mL) were determined from a standard calibration curve generated using recombinant IFN-γ standards.

The primer and probe sequences, qPCR reaction mixture, and thermocycling conditions are provided in Supplementary File 1 (Supplementary Tables S1–S3).

2.5.4 Detection of CMV DNA

Only serum samples positive for anti-CMV IgM antibodies underwent molecular testing. Viral DNA was extracted using a commercial extraction kit according to the manufacturer's protocol. Quantitative real-time polymerase chain reaction (qPCR) targeting the CMV UL83 (pp65) gene was performed using sequence-specific primers and hydrolysis probes. Positive and negative controls were included in each amplification run to ensure assay validity.

2.6 Statistical analysis

Data were analysed using IBM SPSS Statistics version 26. Continuous variables were assessed for normality using the Shapiro–Wilk test. Because serum IFN-γ concentrations were not normally distributed, continuous data are presented as median and interquartile range (IQR), and between-group comparisons were performed using the Mann–Whitney U test. Effect size for the Mann–Whitney U test was calculated as $r = Z/\sqrt{N}$.

Categorical variables are presented as frequencies and percentages. Associations between categorical variables were assessed using Pearson's chi-square test or Fisher's exact test where appropriate. Variables significantly associated with malaria parasitaemia in the bivariate analysis were entered into a multivariable binary logistic regression model to identify

independent predictors. Model performance was evaluated using the Omnibus test of model coefficients, the Hosmer–Lemeshow goodness-of-fit test, and Nagelkerke's R^2. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were reported. Because only 11 participants were positive for CMV IgM antibodies, multivariable logistic regression was not performed for CMV infection due to insufficient outcome events. All statistical tests were two-tailed, and statistical significance was set at $p < 0.05$.

3. Results

3.1 Characteristics of the study participants

The sociodemographic and clinical characteristics of the study participants are presented in Table 1. A total of 91 women who experienced spontaneous abortion were enrolled in the study. Nearly half of the participants (49.5%) were aged 18–26 years, while most were married (90.1%), engaged in business (69.2%), had attained tertiary education (59.3%), and resided in urban areas (59.3%). Most participants were multigravida (79.1%), and 63.7% reported a previous history of miscarriage. Fever was the most frequently reported presenting symptom (78.0%), and 76.9% of participants were receiving medical care at the time of enrolment. Participants were relatively evenly distributed across the first trimester of pregnancy, with 28.6%, 36.3%, and 35.2% recruited at 1–4, 5–8, and 9–12 weeks of gestation, respectively.

Table 1. Sociodemographic and clinical characteristics of the study participants (N = 91)

Variable	Category	n (%)
Age (years)	18–26	45 (49.5)
	27–34	31 (34.1)
	≥35	15 (16.5)
Occupation	Businesswoman	63 (69.2)
	Full housewife	28 (30.8)
Educational level	Islamic education	14 (15.4)

	Primary	1 (1.1)
	Secondary	22 (24.2)
	Tertiary	54 (59.3)
Place of residence	Rural	37 (40.7)
	Urban	54 (59.3)
Marital status	Married	82 (90.1)
	Single	6 (6.6)
	Divorced	3 (3.3)
Family type	Monogamous	51 (56.0)
	Polygamous	40 (44.0)
Gravidity	Primigravida	19 (20.9)
	Multigravida	72 (79.1)
Number of pregnancies	0–2	44 (48.4)
	3–5	35 (38.5)
	≥6	12 (13.2)
History of miscarriage	Yes	58 (63.7)
	No	33 (36.3)
History of stillbirth	Yes	17 (18.7)
	No	74 (81.3)
Physical disability	Yes	9 (9.9)
	No	82 (90.1)
Diagnosed illness	Yes	31 (34.1)
	No	60 (65.9)
Presenting symptoms	Fever	71 (78.0)
	Abdominal pain only	11 (12.1)
	Other/combined symptoms	9 (9.9)
Gestational age (weeks)	1–4	26 (28.6)
	5–8	33 (36.3)
	9–12	32 (35.2)
Receiving medical care	Yes	70 (76.9)
	No	21 (23.1)
Currently on antenatal care	Yes	9 (9.9)
	No	82 (90.1)

3.2 Prevalence of malaria parasitaemia and cytomegalovirus infection

Malaria parasitaemia was detected in 19 of the 91 participants (20.9%), while 11 participants (12.1%) tested positive for anti-CMV IgM antibodies. Among the 11 participants who tested positive for CMV IgM antibodies and subsequently underwent qPCR testing, 3 (27.3%) had detectable CMV DNA. The prevalence of malaria parasitaemia and CMV infection among the study participants is presented in Table 2.

Table 2. Prevalence of malaria parasitaemia and cytomegalovirus infection among women with spontaneous abortion (N = 91)

Marker	Number tested	Positive, n (%)	Negative, n (%)
Malaria parasitaemia	91	19 (20.9)	72 (79.1)
CMV IgM	91	11 (12.1)	80 (87.9)
CMV qPCR*	11	3 (27.3)	8 (72.7)

*CMV qPCR was performed only on samples that tested positive for anti-CMV IgM antibodies.

3.3 Factors associated with malaria parasitaemia and cytomegalovirus infection

Bivariate analysis showed that malaria parasitaemia was significantly associated with participant age ($p = 0.002$) and family type ($p = 0.020$). The prevalence of malaria parasitaemia increased with age, from 11.1% among women aged 18–26 years to 53.3% among those aged ≥35 years, and was higher among women from polygamous households (32.5%) than those from monogamous households (11.8%). No sociodemographic or clinical characteristic was significantly associated with CMV IgM seropositivity, although primigravidity ($p = 0.064$), immunosuppressive drug use ($p = 0.062$), and gestational age ($p = 0.076$) showed borderline associations.

In the multivariable logistic regression model, women from monogamous households had significantly lower odds of malaria parasitaemia than those from polygamous households (AOR = 0.28, 95% CI: 0.10–0.82; $p = 0.021$), whereas age group was not independently associated with malaria parasitaemia after adjustment. The model demonstrated good calibration based on the Hosmer–Lemeshow goodness-of-fit test ($\chi^2 = 0.342$, $p = 0.987$), although the overall model did not reach statistical significance (Omnibus $\chi^2 = 7.122$, $p = 0.068$). The model accounted for approximately 7.6%–11.8% of the variability in malaria parasitaemia (Cox & Snell

R^2 = 0.076; Nagelkerke R^2 = 0.118). The detailed results of the bivariate and multivariable analyses are presented in Table 3.

Table 3. Factors associated with malaria parasitaemia and CMV IgM seropositivity among women with spontaneous abortion

Variable	Category	Malaria positive n (%)	Bivariate p-value	Adjusted OR (95% CI)	Adjusted p-value	CMV IgM positive n (%)	p-value
Age group (years)	18–26	5 (11.1)	**0.002**	0.41 (0.11–1.62)	0.204	1 (2.2)	0.718
	27–34	6 (19.4)		0.46 (0.12–1.72)	0.245	3 (9.7)	
	≥35 (Ref)	8 (53.3)		Reference	—	7 (46.7)	
Family type	Monogamy	6 (11.8)	**0.02**	0.28 (0.10–0.82)	**0.021**	4 (7.8)	0.391
	Polygamy (Ref)	13 (32.5)		Reference	—	7 (17.5)	

AOR: Adjusted odds ratio; CI: Confidence interval; Ref = Reference category. Logistic regression was performed for malaria parasitaemia only because the number of CMV IgM-positive participants (n = 11) was insufficient for reliable multivariable logistic regression.

3.4 Serum IFN-γ concentrations among the study participants

Serum IFN-γ concentrations were measured in all 91 study participants (Table 4). The median serum IFN-γ concentration was 0.969 pg/mL (interquartile range [IQR]: 0.803–1.095 pg/mL), with values ranging from 0.133 to 3.807 pg/mL. The Shapiro–Wilk test indicated that serum IFN-γ concentrations were not normally distributed ($p < 0.001$); therefore, comparisons of IFN-γ concentrations between study groups were performed using non-parametric statistical tests.

Table 4. Distribution of serum IFN-γ concentrations among the study participants (N = 91)

Parameter	Value
Number of participants	91

Median (IQR), pg/mL	0.969 (0.803–1.095)
Range, pg/mL	0.133–3.807
Shapiro–Wilk test	$p < 0.001$

Abbreviations: IFN-γ, interferon-gamma; IQR, interquartile range.

3.5 Association between malaria parasitaemia, CMV IgM serostatus and serum IFN-γ concentrations

Serum interferon-γ (IFN-γ) concentrations were compared according to malaria parasitaemia and CMV IgM serostatus using the Mann–Whitney U test because IFN-γ concentrations were not normally distributed. Both malaria parasitaemia and CMV IgM seropositivity were associated with significantly higher circulating serum IFN-γ concentrations among women who experienced spontaneous abortion (Table 5).

Women with malaria parasitaemia had significantly higher serum IFN-γ concentrations than those without malaria parasitaemia. The median IFN-γ concentration was 1.067 pg/mL (IQR: 0.985–1.165 pg/mL) among malaria-positive participants compared with 0.898 pg/mL (IQR: 0.768–1.039 pg/mL) among malaria-negative participants. Consistent with this finding, malaria-positive participants had a significantly higher mean rank than malaria-negative participants (60.47 vs. 42.18). The difference was statistically significant (Mann–Whitney $U = 409.0$, $Z = -2.685$, $p = 0.007$), with a small-to-moderate effect size ($r = 0.28$).

Similarly, serum IFN-γ concentrations were significantly higher among participants with evidence of recent CMV infection. CMV IgM-positive participants had a median IFN-γ concentration of 1.067 pg/mL (IQR: 0.985–1.219 pg/mL) compared with 0.942 pg/mL (IQR:

0.772–1.071 pg/mL) among CMV IgM-negative participants. The corresponding mean ranks were 64.27 and 43.49, respectively. This difference was statistically significant (Mann–Whitney U = 239.0, Z = −2.447, p = 0.014), with a small-to-moderate effect size (r = 0.26).

These differences in serum IFN-γ concentrations according to malaria parasitaemia and CMV IgM serostatus are illustrated in Fig. 1 (Fig. 1a and Fig. 1b), respectively.

Table 5. Comparison of serum interferon-γ concentrations according to malaria parasitaemia and cytomegalovirus IgM serostatus among women with spontaneous abortion

Variable	Category	n	Median (IQR), pg/mL	Mean Rank	Mann–Whitney U	Z	*p*-value	Effect size (r)
Malaria parasitaemia	Negative	72	0.898 (0.768–1.039)	42.18				
	Positive	19	1.067 (0.985–1.165)	60.47	**409**	**−2.685**	**0.007**	**0.28**
CMV IgM serostatus	Negative	80	0.942 (0.772–1.071)	43.49				
	Positive	11	1.067 (0.985–1.219)	64.27	**239**	**−2.447**	**0.014**	**0.26**

Abbreviations: CMV, cytomegalovirus; IFN-γ, interferon-gamma; IQR, interquartile range.

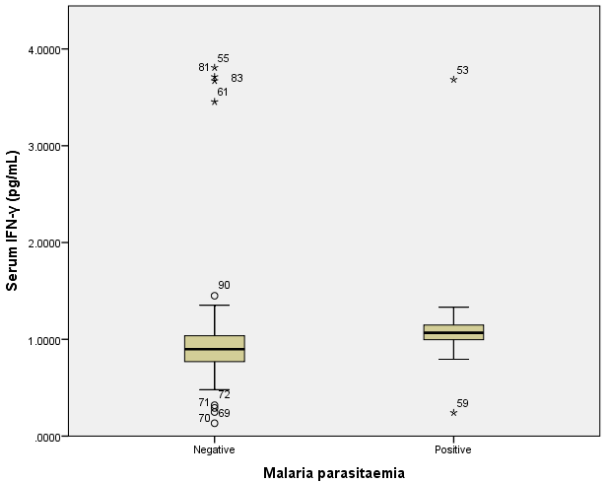


Fig. 1a Serum IFN-γ concentrations according to malaria parasitaemia status

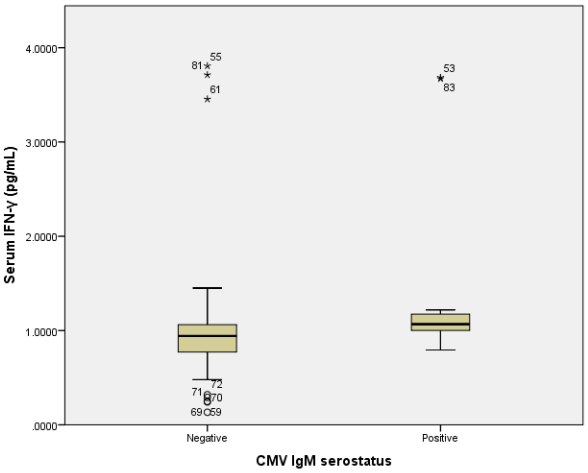


Fig. 1b Serum IFN-γ concentrations according to CMV IgM serostatus

Fig. 1. Distribution of serum interferon-γ concentrations according to infection status among women with spontaneous abortion. (a) Serum IFN-γ concentrations according to malaria parasitaemia status. (b) Serum IFN-γ concentrations according to CMV IgM serostatus. The central line represents the median, the box indicates the interquartile range (IQR), and asterisks denote outlying observations.

4. Discussion

This study investigated the association of malaria parasitaemia and cytomegalovirus infection with serum interferon-γ concentrations among women with spontaneous abortion in Kano, North-West Nigeria. Malaria parasitaemia and anti-CMV IgM antibodies were detected in 20.9% and 12.1% of participants, respectively, while CMV DNA was identified in approximately one-quarter of IgM-seropositive women. Family type was the only independent predictor of malaria parasitaemia, with women from monogamous households having lower odds of infection than those from polygamous households. Both malaria parasitaemia and recent CMV infection were associated with significantly higher serum IFN-γ concentrations among women with spontaneous abortion. These findings contribute to the limited evidence on infection-related

immune responses among women experiencing spontaneous abortion in malaria-endemic settings.

Malaria parasitaemia was identified in approximately one-fifth of the study participants, indicating that malaria remains an important infectious burden among women presenting with spontaneous abortion in North-West Nigeria. The prevalence observed in this study was higher than reports from a similar study conducted in Mali [26]. These differences may reflect variations in malaria transmission intensity, seasonality, implementation and uptake of malaria prevention interventions during pregnancy, as well as differences in study populations and diagnostic approaches. Given that Nigeria continues to account for a substantial proportion of the global malaria burden [27], our findings underscore the persistent risk of malaria during pregnancy and the need to sustain effective prevention and control measures in endemic settings.

Increasing age was associated with malaria parasitaemia in the bivariate analysis, although this relationship was no longer statistically significant after adjustment for other variables. In contrast, family type remained an independent predictor, with women from monogamous households having significantly lower odds of malaria parasitaemia than those from polygamous households. The higher malaria burden observed among women from polygamous households may reflect larger household sizes, greater crowding, and socioeconomic factors that could increase exposure to mosquito vectors and reduce access to preventive measures, as previously reported in Ethiopia [28]. The attenuation of the association between age and malaria parasitaemia after multivariable adjustment suggests that the observed age differences may have been influenced by other participant characteristics rather than age alone.

The prevalence of anti-CMV IgM antibodies observed in this study was higher than the pooled estimate of 6% reported among pregnant women in Africa [29] and the 9.5% prevalence reported among women with miscarriage in Jos, Nigeria [25], but lower than the 38.3% reported among women with pregnancy loss in Sudan [30] and the 40.6% reported among women with miscarriage in Bauchi, Nigeria [12]. These variations may reflect differences in study populations, geographical and epidemiological settings, socioeconomic conditions, timing of specimen collection, and diagnostic methods. Furthermore, CMV DNA was detected in only 3 of the 11 participants who were positive for anti-CMV IgM antibodies, consistent with the understanding that CMV IgM seropositivity does not necessarily indicate active viraemia, as IgM antibodies may persist following primary infection or occur during viral reactivation [31]. This finding highlights the importance of molecular testing for confirming active CMV infection among individuals with positive CMV IgM serology.

No sociodemographic or clinical characteristic was significantly associated with anti-CMV IgM seropositivity in this study. This finding may reflect the high endemicity of CMV in Nigeria, where infection is commonly acquired early in life, resulting in widespread exposure across demographic groups [32]. In addition, the relatively small number of participants with positive anti-CMV IgM antibodies may have limited the statistical power to detect modest associations with potential risk factors. Larger studies are therefore needed to better characterize determinants of recent CMV infection among pregnant women in this setting.

One of the principal findings of this study was that women with malaria parasitaemia had significantly higher circulating serum IFN-γ concentrations than those without malaria infection. This observation is consistent with the established role of IFN-γ as a key pro-inflammatory

cytokine produced during the cellular immune response to *Plasmodium* infection. IFN-γ contributes to parasite control through activation of macrophages, enhancement of antigen presentation, and promotion of cell-mediated immunity [33]. Elevated circulating IFN-γ concentrations among malaria-infected participants therefore likely reflect activation of the host immune response to infection rather than a process specific to spontaneous abortion.

Women with anti-CMV IgM seropositivity also demonstrated significantly higher serum IFN-γ concentrations than seronegative participants. This finding is biologically plausible because CMV infection elicits a strong T-cell- and natural killer cell-mediated immune response, with IFN-γ playing a central role in antiviral defence [34,35]. The observed association is consistent with systemic immune activation accompanying recent CMV infection or viral reactivation. However, because of the cross-sectional design and the inability of CMV IgM alone to distinguish primary infection from reactivation, these findings should be interpreted as an association rather than evidence of a causal relationship.

The findings of this study indicate that both malaria parasitaemia and recent CMV infection were associated with higher circulating serum IFN-γ concentrations among women presenting with spontaneous abortion. Although the cross-sectional design precludes conclusions about temporal or causal relationships, this study adds to the limited evidence on the immunological profile of infection-associated spontaneous abortion in malaria-endemic settings. The findings also provide a basis for future prospective studies exploring the temporal dynamics of malaria, CMV infection, immune activation, and pregnancy outcomes.

5. Policy and practice implications

The findings of this study have implications for the clinical management of women presenting with spontaneous abortion in malaria-endemic settings. The significant association between malaria parasitaemia and elevated serum IFN-γ concentrations highlights the importance of strengthening malaria prevention, early diagnosis, and prompt treatment among women of reproductive age and during early pregnancy. Although no independent association was demonstrated between CMV infection and spontaneous abortion, the observed elevation in IFN-γ concentrations among CMV IgM-seropositive women suggests that further investigation of CMV-related immune responses in pregnancy is warranted. Larger prospective studies incorporating comprehensive molecular diagnostics and serial cytokine measurements are needed to clarify the temporal relationships between infection, immune activation, and adverse pregnancy outcomes. The present findings may also contribute to the evidence base for developing integrated infection surveillance and maternal health strategies in malaria-endemic regions.

6. Limitations of the study

This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design precludes establishing temporal or causal relationships between malaria parasitaemia, CMV infection, and serum IFN-γ concentrations. Second, participants were recruited through convenience sampling from two tertiary healthcare facilities in Kano, which may limit the generalizability of the findings to women with spontaneous abortion in other settings or at the community level. Third, although the overall sample size was adequate for the primary analyses, the small number of participants with CMV IgM seropositivity (n = 11) and particularly CMV DNA positivity (n = 3) limited statistical power

for CMV-related analyses and precluded multivariable modelling of CMV infection. Consequently, findings relating to CMV should be interpreted with caution. Fourth, only serum IFN-γ was evaluated as a marker of immune activation. Measurement of additional pro-inflammatory and anti-inflammatory cytokines would have provided a more comprehensive assessment of the immunological response associated with spontaneous abortion. Finally, residual confounding cannot be excluded because some potential determinants of immune response and infection, including HIV status, nutritional status, parasite density, and other concurrent infections, were not evaluated.

Despite these limitations, the study has several strengths. It combined parasitological, serological, molecular, and immunological methods to investigate malaria parasitaemia, CMV infection, and serum IFN-γ concentrations within the same cohort of women with spontaneous abortion. Furthermore, this is among the few studies from northwestern Nigeria to examine these relationships concurrently, providing important baseline evidence for future, larger prospective investigations.

7. Conclusion

This study demonstrated that malaria parasitaemia and cytomegalovirus infection remain detectable among women with spontaneous abortion in Kano, North-West Nigeria. Malaria parasitaemia was independently associated with family type, with women from monogamous households having significantly lower odds of infection than those from polygamous households. Importantly, women with malaria parasitaemia and those with CMV IgM

seropositivity exhibited significantly higher serum interferon-γ concentrations, which may suggest heightened systemic immune activation in the presence of these infections.

These findings highlight the potential contribution of malaria and CMV infection to the inflammatory milieu observed among women with spontaneous abortion and underscore the importance of strengthening infection prevention, timely diagnosis, and appropriate management during pregnancy. Larger prospective studies incorporating broader immunological profiling and longitudinal follow-up are warranted to clarify the temporal relationships and underlying mechanisms linking these infections, immune responses, and adverse pregnancy outcomes.

List of abbreviations

AOR – Adjusted Odds Ratio

CI – Confidence Interval

CMV – Cytomegalovirus

DNA – Deoxyribonucleic Acid

ELISA – Enzyme-Linked Immunosorbent Assay

IFN-γ – Interferon-gamma

IgM – Immunoglobulin M

IQR – Interquartile Range

qPCR – Quantitative Polymerase Chain Reaction

WHO – World Health Organization

Declarations

Ethical approval

This study was conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the Health Research Ethics Committee, Ministry of Health, Kano State, Nigeria, with reference number NHREC/17/03/2018.

Consent to participate

Written informed consent was obtained from all participants, ensuring they understand the study's purpose, procedures, potential risks, and benefits, and their right to withdraw at any time without any repercussions.

Consent to publish

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Code availability

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SMN contributed to the conceptualization, study design, data collection, data management, sample collection and analysis, provision of resources, and critical revision of the manuscript. DTP, RAB, and BA were responsible for data collection, analysis and management. LOP participated in data analysis, drafted the manuscript, and participated in its revision. MA and AOA supervised the study, contributed to the conceptualization and design, validated the study instruments, and critically revised the manuscript. All authors have read and approved the final manuscript.

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