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First Serological Evidence of Cytomegalovirus Infection Among Eligible Blood Donors in Ilorin, Nigeria and Its Implications for Transfusion Safety

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Abstract

This study investigated the prevalence of CMV immunoglobulins and associated risk factors among eligible blood donors in Ilorin, Nigeria. A cross-sectional study was conducted among 235 eligible voluntary blood donors aged 18–53 years at the blood bank of Sobi Specialist hospital, Ilorin. Serum samples were screened for anti-CMV IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA) based kit. Demographic data, risk factors, and clinical symptoms were collected through structured interviews while statistical correlation was via chi-square/p value, and Estimated Marginal Means (EMM). Seroprevalence for CMV-IgG and IgM were 92.3% and 22.1% respectively. A significant association was found between overall CMV serostatus and IgM positivity (χ^2 = 5.634, p = 0.018). IgG seropositivity was significantly associated with age (p = 0.001) religion (p = 0.003), education level (p = 0.047), occupation (p = 0.001), and residential area (p = 0.049). IgM status showed significant associations with education (p = 0.042) and tattooing (p < 0.001). Symptoms such as sore throat (p = 0.028), muscle ache (p = 0.040), and tiredness (p = 0.016) were more common in IgM-positive individuals. EMM analysis revealed that tattooing significantly influenced CMV IgM means after adjusting for other variables. The high CMV burden in Ilorin highlights the need for routine screening of blood donors and women in antenatal care. Public health interventions targeting risk awareness, especially among women with tattoos and limited education, are strongly recommended.

Keywords: Blood donors, seroprevalence, IgG, IgM, women, Ilorin, EMM, Nigeria

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1. INTRODUCTION

Cytomegalovirus (CMV) is a double stranded DNA virus belonging to the herpesvirus family (HHV-5). Over the years, the virus has assumed an important public health issue as it has been shown to be a major cause of morbidity and mortality, particularly in congenitally infected neonates, pregnant women, transplant recipients, and immunocompromised individuals.[1] It can be transmitted through bodily fluids such as saliva,

urine, breast milk, and blood. Like most other herpes viruses, CMV remains latent in the host after primary infection and persists for life. Latent CMV is associated with white blood cells which facilitates its transmission by transfusion of cellular blood components making blood transfusion a significant route of transmission of infection.[2] Despite its public health significance, routine CMV screening of blood donors is not practiced in many developing countries, including Nigeria thereby further exacerbating this threat.[3] Other viruses transmissible via blood transfusion are Hepatitis B and C; and human immunodeficiency virus (HIV).[4] The risk of transmitting these viruses has been reduced drastically by screening for their antibodies before transfusion.[5]

CMV infections can be primary or due to reactivation. The presence of CMV-specific IgM antibodies indicates acute or recent infection, while IgG antibodies reflect past exposure and possible immunity.[6] In clinical practice, especially among vulnerable populations, the distinction between these markers is crucial for risk assessment and management.[7] Studies have revealed that most adults across the globe are seropositive for CMV with many being asymptomatic.[8] Depending on the socioeconomic status, seropositivity for adult population above forty years of age ranges between sixty to hundred percent probably due to breastfeeding, intrauterine transmission (or at parturition), sexual contact and blood transfusion.[9]

In developed countries, transfusion-transmitted CMV is controlled through implementation of stringent donor eligibility criteria and the use of more sensitive methods of Viral genome detection. However, limited resources in developing countries hinder such preventive measures, increasing the risk of CMV transmission via transfusion.[3,7] This study presents the first documented investigation into the seroprevalence of CMV in this region, aimed at evaluating the necessity of routine screening among blood donors particularly to enhance transfusion safety for immunocompromised individuals and pregnant women.

2. MATERIALS AND METHODS

2.1 Place of work

This study was conducted at the Blood Bank of Sobi Specialist Hospital, Ilorin, Kwara State, Nigeria. Sobi Specialist Hospital is a major tertiary healthcare facility and serves as a primary blood collection center for the region. The laboratory analyses were performed in the hospital's associated immunology/serology laboratory.

2.2 Experimental set-up

The was a purposive hospital-based study conducted at Sobi Specialist Hospital, Ilorin, Kwara State, Nigeria. Ethical approval was obtained from the Kwara State Ministry of Health, and all participants provided written informed consent.

2.3 Procedure of experiment and data collection

The study population consisted of 235 consecutive eligible blood donors aged 18–53 years. Sociodemographic data were collected using structured questionnaires after which Approximately 5 mL of venous blood was aseptically collected from each participant into a sterile plain serum tube. The blood samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 5 minutes to separate the serum. The separated serum was aliquoted into cryovials and stored at –20°C until it was analyzed for CMV antibodies.

The presence of anti-CMV IgG and IgM antibodies in the serum samples was detected using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (Diagnostic Automation, Inc., USA), following the manufacturer's instructions. The test principle is based on the indirect ELISA method. Briefly, diluted serum samples and controls were added to wells coated with CMV antigen. After incubation and washing, enzyme-conjugated anti-human IgG or IgM was added. Following a second incubation and wash cycle, a

tetramethylbenzidine (TMB) substrate solution was added. The enzyme-substrate reaction was stopped with a stop solution, and the Optical Density (OD) was measured at 450 nm using a microplate reader.

The results were interpreted as follows: A sample was considered positive if the OD Ratio (Sample OD/Calibrator OD) was ≥ 1.1, negative if the ratio was ≤ 0.9, and equivocal if the ratio was between 0.91 and 1.09. Any sample yielding an equivocal result was re-tested; if it remained equivocal, it was excluded from the final analysis.

2.4 Statistical analysis

Data were entered into Microsoft Excel 2016 and analyzed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were used to summarize sociodemographic characteristics and seroprevalence, presented as frequencies and percentages. The Chi-square (χ^2) test was employed to determine associations between categorical variables (e.g., sociodemographic factors, risk factors, symptoms) and CMV IgG/IgM serostatus. A p-value of less than 0.05 was considered statistically significant.

To further explore the influence of significant risk factors identified in the bivariate analysis (specifically tattooing, history of blood transfusion, and number of sexual partners) on recent CMV infection (IgM status), an Analysis of Covariance (ANCOVA) model was fitted. From this model, Estimated Marginal Means (EMM) were generated to compare the mean IgM positivity across different risk factor groups while statistically adjusting for the effects of the other factors.

3. RESULTS AND DISCUSSIONS

A total of 235 voluntary blood donors partook in the study, comprising 124 (53.4%) males and 112 (47.7%) females. The mean age was 30.17 years with a range of 18-53 years. Seropositivity for CMV-IgG antibodies were found in 217 (92.3%) respondents while 52 (22.1%) were IgM positive. Figure 1 presents the cross relation of the serostatus of respondent alongside the exponential decay function at significant statistical correlation of p=0.018.

Majority of the donors (62 of 235) were within the age bracket of 28-32 years (26.4%). The highest IgM prevalence of 7.7% was noticed at 23-27years (p=0.103) while for IgG (24.7%) was at 28-32years (p=0.001). There was no significant statistical difference in the CMV IgM status but was significant for CMV IgG across the different age brackets (Table 1). Furthermore, the result on Table 2 shows that some of the evaluated demographic data of respondent such as religion, education, occupation and residential area to the CMV immunological status had significant statistical association while gender, marital status and the size of household did not show any significant correlation (p>0.05). Table 3 presents the association between antibody status and exposure to selected CMV risk factors such as transplants, blood intake, number of sexual partners, and tattooing. The IgM status had no correlation with blood transfusion but for past infection as represented by the recorded IgG positivity which was significantly higher in respondent that had history of blood transfusion. No statistical correlation was recorded for CMV IgG and IgM based on the number of sexual partners in this study (P= 0.331 and 0.958 respectively). However, participants with tattoo had higher significance for CMV IgM (P= 0.000) while tattoo did not predict past exposure or immunity of respondent to CMV.

To further analyse the impact of specific significant risk factors of CMV recorded in this study, figure 2 presents the Estimated Marginal Means of IgM status under specific factors namely, tattoos, blood transfusion, and number of sexual partners. For respondent with tattoo, the EMM of IgM status decreased when moving from "Yes" to "No" on the blood transfusion variable in both group of number of sexual partners. This suggests that individuals who have taken in blood (Yes) have a higher IgM positive rate than those who have not (No). The single-partner group has a slightly higher IgM Positivity rate than the multiple-partner group across both conditions.

The statistical correlation of the participants' clinical symptoms in relation to the CMV serostatus is explored in Table 5. No significant correlation was notice for fever, stomach upset and rashes in relation to

the immunological status in this study (P>0.05). Tiredness, muscle ache and headache correlated either IgG or IgM while only sore throat was recorded to be statistically significant at both immunological states.

Table 1: Blood donors age in relation to CMV sero status

		IgM Status X^2=10.559; p=0.103		IgG Status X^2=22.131; p=0.001		
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Total (%)
Age (years)	18-22	6 (2.6)	38 (16.2)	38 (16.2)	6 (2.6)	44 (18.7)
	23-27	18 (7.7)	41 (17.4)	57 (24.3)	2 (0.9)	59 (25.1)
	28-32	17 (7.2)	45 (19.1)	58 (24.7)	4 (1.7)	62 (26.4)
	33-37	2 (0.9)	19 (8.1)	21 (8.9)	0 (0.0)	21 (8.9)
	38-42	3 (1.3)	17 (7.2)	14 (6.0)	6 (2.6)	20 (8.5)
	43-47	6 (2.6)	15 (6.4)	21 (8.9)	0 (0.0)	21 (8.9)
	48-52	0 (0.0)	8 (3.4)	8 (3.4)	0 (0.0)	8 (3.4)
Total (%)		52 (22.1)	183 (77.9)	217 (92.3)	18 (7.7)	235 (100)

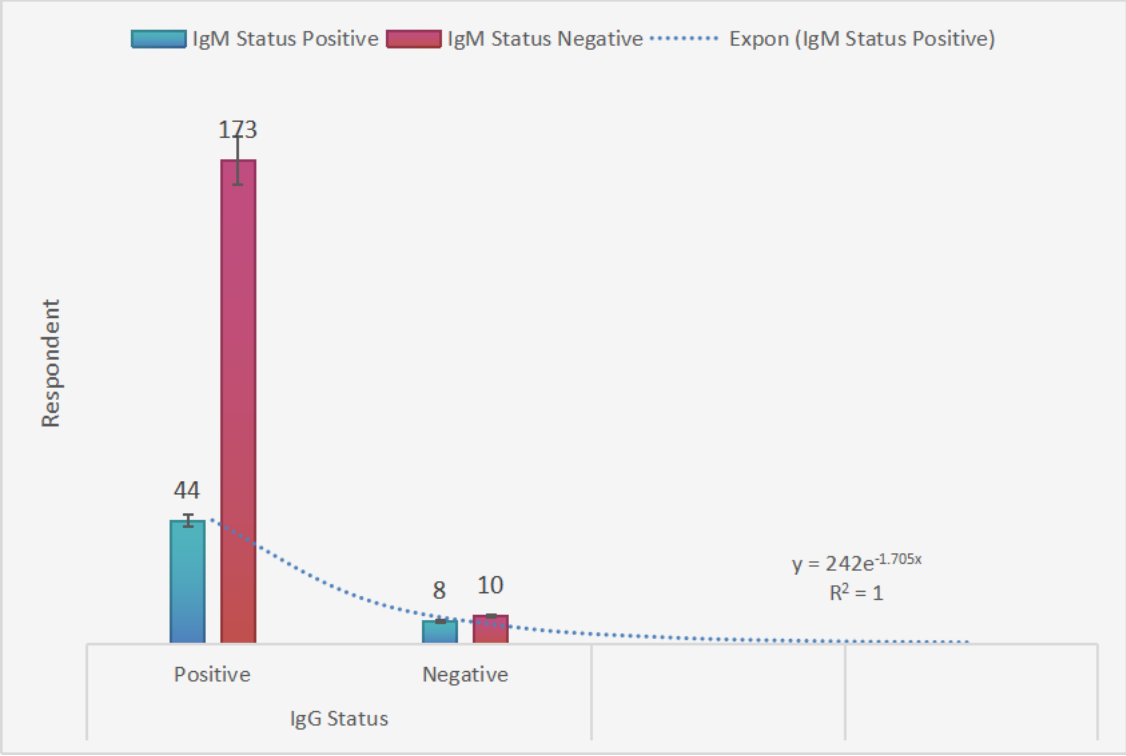

Figure 1: CMV serostatus cross evaluation among respondents **(**X^2=5.634, P= 0.018**)**

Table 2: CMV prevalence based on respondents' demographic data

		IgM Status			IgG Status		
		Positive	Negative	Statistics	Positive	Negative	Statistics
Gender	Male	25	98	X^2=0.487 P= 0.485	113	10	X^2=0.081 P= 0.776
	Female	27	85		104	8	
Religion	Islam	32	105	X^2=1.462 P= 0.481	133	4	X^2=11.955 P= 0.003
	Christianity	18	75		79	14	
	Other	2	3		5	0	
Level of education	Nursery	2	2	X^2=8.203 P= 0.042	4	0	X^2=7.933 P= 0.047
	Primary	9	11		16	4	
	Secondary	29	105		128	6	
	Tertiary	12	55		59	8	
Occupation	Artisan	4	12	X^2=11.721 P= 0.110	10	6	X^2=24.163 P= 0.001
	Civil servant	5	26		29	2	
	Farmer	4	9		13	0	
	Housewife	14	28		38	4	
	Other	18	47		61	4	
	Self employed	4	45		47	2	
	Student	1	8		9	0	
	Unemployed	2	8		10	0	
Marital status	Married	20	92	X^2=2.265 P= 0.132	102	10	X^2=0.487 P= 0.485
	Unmarried	32	91		115	8	
Residential area	Urban	41	155	X^2=1.002 P= 0.317	178	18	X^2=3.879 P= 0.049
	Rural	11	28		39	0	
Number of persons living in the house	One	3	18	X^2=6.056 P= 0.109	19	2	X^2=3.214 P= 0.360
	Two	17	40		51	6	
	Three	10	33		41	2	
	More than three	8	55		61	2	

Table 3: CMV prevalence in relation to Risk factors of infection

		IgM Status			IgG Status		
		positive	negative	Statistics	positive	negative	Statistics
Organ transplant	Yes	0	0	NA	0	0	NA
	No	52	183		217	18	
Bone marrow transplant	Yes	0	0	NA	0	0	NA
	No	52	183		217	18	
Stem cell transplant	yes	0	0	NA	0	0	NA
	No	52	183		217	18	
Intake of blood	Yes	5	8	X^2=2.131 P= 0.144	9	4	X^2=10.391 P= 0.001
	No	47	175		208	14	
Number of sexual partners	Single	48	160	X^2=0.947 P= 0.331	192	16	X^2=0.003 P= 0.958
	Multiple	4	23		25	2	
Tattoo	Yes	4	0	X^2=14.321 P= 0.000	4	0	X^2=0.338 P= 0.561
	No	48	183		213	18	
Total		**52**	**183**		**217**	**18**	

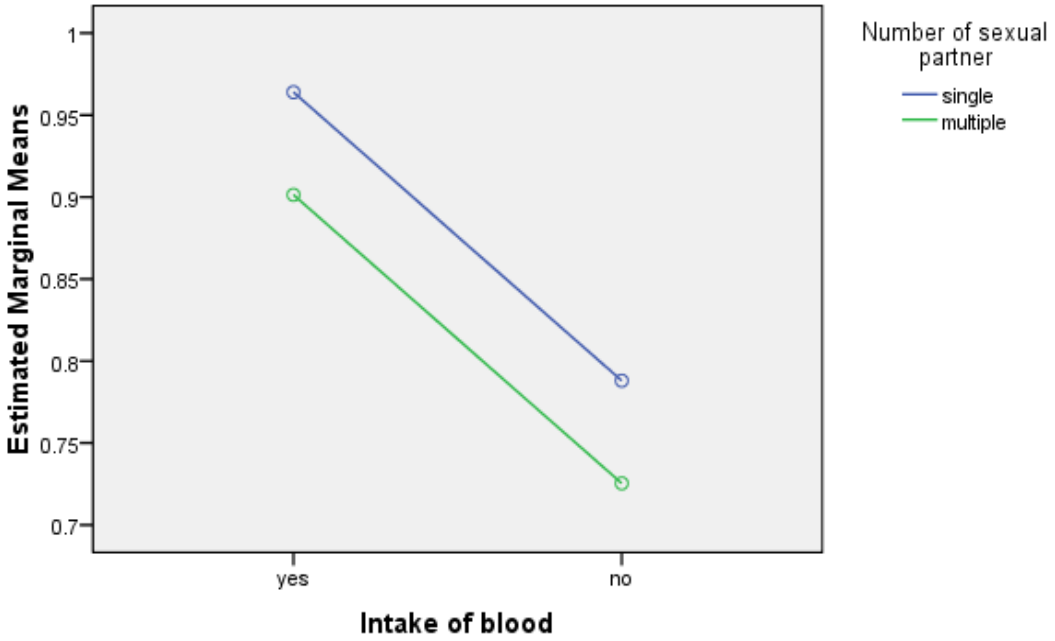

Figure 2: Estimated Marginal Means of CMV-IgM status based on selected risk factors for respondent with tattoo.

Table 4: CMV prevalence based on related signs and symptoms of infection

		IgM Status			IgG Status		
		positive	negative	Statistics	positive	negative	Statistics
Fever	yes	13	49	X^2=0.066	56	6	X^2=0.485
	no	39	134	P= 0.708	161	12	P= 0.486
Tiredness	yes	17	37	X^2=3.560	54	0	X^2=5.816
	no	35	146	P= 0.059	163	18	P= 0.016
Stomach upset	yes	10	19	X^2=2.931	27	2	X^2=0.027
	no	42	164	P= 0.087	190	16	P= 0.869
Muscle ache	yes	11	19	X^2=4.219	30	0	X^2=2.853
	no	41	164	P= 0.040	187	18	P= 0.091
Sore throat	yes	16	31	X^2=4.840	47	0	X^2=4.873
	no	36	152	P= 0.028	170	18	P= 0.027
Rashes	yes	9	19	X^2=1.850	28	0	X^2=2.637
	no	43	164	P= 0.174	189	18	P= 0.104
Headache	yes	18	41	X^2=3.211	51	8	X^2=3.877
	no	34	142	P= 0.073	166	10	P= 0.049
Total		52	183		217	18	

This study evaluated the CMV sero-epidemiological status of eligible blood donors in Ilorin, Nigeria. The study also assessed selected risk factors to CMV infection while using estimated marginal means for statistically significant factors. The analysis suggests that most individuals with past exposure i.e. IgG positive do not have an active infection (IgM negative). In the IgG negative group, the number of IgM Positive (8) and IgM Negative (10) respondents is very small indicating that among those without prior exposure (IgG negative), IgM presence is not significantly different from IgM absence (Figure 1). The figure further reveals the exponential decay function of the IgM positivity where the dotted trend line follows an exponential decay function of $y=242e^{-1.705x}$, indicating that as IgG positivity increases, IgM positivity

declines rapidly. This aligns with immunological response, as IgM is an early response antibody that wanes as IgG develops. Additionally, the R^2 value of 1 suggests a perfect exponential relationship, meaning that the decline in IgM positivity as IgG increases follows a strict mathematical pattern. Furthermore, it also suggests reactivation of infection which is common with CMV ability to be latent and reactivate in condition of immunosuppression. The result of CMV seroprevalence amongst the respondent showed that IgM positive individuals are more likely to be in the early phase of infection. There is a limited new infection in the sampled group as depicted by the small number of CMV-IgG negative and IgM positive cases. A strong inverse relationship is posited by the exponential decline relationship between IgG and IgM, aligning with how the immune system shifts from an early to a long-term immune response.

The IgM positivity recorded in this study is lower to a study by Garba *et al*. [11] as well as that of Nalha *et al*.[12] which had 94.3% among women of reproductive or childbearing age and neonates respectively. However, it is higher that the report of 3.1% among blood donors in Edo state.[13] The variance in serological report can be adduced to the sample target and difference of study locations in Nigeria and Africa in general, but this posits the strong evidence of circulation of CMV in Nigeria. The link of CMV to congenital infection makes it an important virus of public health concern that needs to be properly surveyed and controlled to avoid complication in foetus. The trend of positivity in females aligns with this study where the female gender (Table 2) was noticed to have higher prevalence than the male. In another cross-sectional study by Zenebe *et al*.[14], a prevalence of 8.2% and 88.7% IgM and IgG was reported respectively amongst pregnant women further supporting the presence of the virus in Africa. The positivity was reported to be significant with women that were unmarried which also correlates with the outcome presented in Table 2. In contrary to reported IgM from this study, a study in Iran presented 85% anti-CMV IgM among healthy blood donors.[15] The seropositivity of CMV IgG in this study aligns with continental CMV review for Africa which was posited between the range of 60% to 100% [16] alongside the significant trend in CMV-related congenital cases.[17] Previous IgG and IgM seropositivity rates of 77.3% and 8.1% was reported in Kenya[18], 93% and 11.1% in Nigeria [19], and 94% and 8.5% in Tanzania [20] which were all comparable to the findings from this study.

On the other hand, the reported rate of CMV IgM in this study was noticed to be higher than report by Chibwe *et al*.[21] (0.4%), Hamdan *et al*. [22] (2.5%) and Kamel *et al*. [23] (7%) in Tanzania, Sudan, and Egypt respectively. Similar lower prevalence of CMV IgM has been reported in Kano (8.9%), and Kaduna (11.8%) while higher rates was reported in Zaria (29.8%) and Abakaliki (43.6%) respectively.[24,25,26]. In a study in Minna,[27] prevalence of 96.2% was reported for IgG prevalence which was higher than the report from this study while lower IgM of 2.6% where this study presents a higher prevalence. The variation in the prevalence report can be due to the difference in study location. The presence of anti-CMV antibodies (IgM and IgG) among blood donors is a sign of potentially infectious virus in transfused blood products.[28] The global prevalence of anti-CMV IgG and IgM among blood donors was 83.16% and 13.77% reflecting the endemic nature of CMV infection worldwide where 82.6% was posited in Africa, 82.7% in Asia, and 99.2% in South America depicting that CMV exposure is nearly universal in developing regions, particularly in populations with low socioeconomic indices.[29]

Based on the gradual increase in CMV rate among other reasons, in most of the developed countries, pregnant women are screened for CMV due to the risk it poses to foetus development. In Nigeria, such screening is usually via independent research leading to advocacy for inclusion of selected virus of public concern in the nations routine schedule for pregnant women.[28] Furthermore, the reported significant seropositivity among eligible blood donors in this study points to the existing negligence and the need to adopt screening of volunteers to limit transmission of CMV which can pose risk to pregnant women and foetus.

Gender, marital status and size of household did not influence the status of respondent CMV status. In a similar study by Ojide *et al.*[13], 100% IgG positivity was recorded among the female respondent which is in contrary to this study. However, the recorded female participants were low (n=16) while this study recorded higher female participants (n=112). Based on religion, the IgM had no statistical significance, but lower past exposure was noticed among Christian respondent. The level of education has effects on the IgM and IgG status of the respondent where higher levels of education had lower prevalence of IgM

positivity which can be adduced to better awareness or protective behaviour of the respondent. The CMV IgG was noticed to have statistical correlation where civil servants had higher prevalence compared to self-employed or unemployed, which can be adduced to level of exposure as a potential risk factor (P=0.001). The risk of level of exposure and respondent behaviour based on demographic data also aligns with residential area where higher immune status of IgG positivity was recorded at borderline significant correlation (P=0.049). These findings suggest that socioeconomic and behavioural factors (such as education, religion, and occupation) may influence past exposure/immunity more than they influence recent infection. This aligns with the report by Zenebe *et al*.[14] that evaluated similar predictors for seropositivity to CMV. Similarly, the Minna study noted that blood donors earning ₦18,000 or more monthly were significantly more likely to be CMV-positive than those earning less, suggesting that higher economic activity might increase social and occupational exposure risk.[27] This finding contrasts reports from California, where lower-income individuals were more affected, implying that CMV epidemiology may vary contextually across socio-economic strata.[30]

Transmission via blood is one of the viable ways by which virus can spread. This aligns with the higher statistical correlation (p=0.001) for participants with blood transfusion having higher IgG positivity and to the 100% positivity recorded by Ojide *et al.*[13]. Sexual transmission of disease amongst other route has been implicated in a number of diseases spread, however, CMV transmission in this study had no significant statistical correlation either with single or multiple sexual partners despite increased positivity suggesting viable transmission. This deduction relates to report by Staras *et al*.[31], that confirmed the positive influence of sexual activity on CMV prevalence. Although this study did not evaluate how recent the tattoo was on respondents, all the participants with tattoo were noticed to have significant statistical correlation with IgM positivity. This can be adduced to exposure to body fluid during or after the process.[30] CMV has been posited to possibly transmit through blood amongst other body fluid.[31] These findings underline the importance of monitoring tattoo practices and blood transfusion safety, while also noting that sexual behaviour and transplants despite not statistically significant in this dataset are important predictors to CMV positivity. This is further supported by the estimated marginal means (EMM) analysis. It is also important to note that CMV seropositive blood donors has been reported to exhibit significantly lower packed cell volume (PCV) and haemoglobin levels ($P < 0.05$), suggesting that CMV infection may predispose individuals to anaemia through bone marrow suppression. This observation further supports the argument for improved blood donor screening and hemovigilance systems in Nigeria to enhance transfusion safety.[27]

The result on EMM of IgM status shows that IgM positivity is higher in individuals who have tattoos and have taken in blood or had blood transfusion, indicating a risk factor for exposure to CMV infection. The number of sexual partners seem to slightly influence IgM status, with single partners having slightly higher levels than multiple partners. This depicts that the number of sexual partners didn't impact the outcome of CMV IgM status in this study. Although, the frequency of exposure to different partners would practically increase the risk of contracting the virus however, it takes exposure to an infected partner for transmission of the virus. The correlation between the evaluated variables i.e. tattooing, blood intake, and sexual partners in relation to CMV-positivity aligns with transmission route if viral diseases.[33,34,35] The findings of this study contribute valuable regional data to the global CMV sero-epidemiological landscape, affirming that CMV is widespread among Nigerian blood donors, and supporting global evidence that donor screening, improved assay sensitivity, and targeted preventive strategies are essential to safeguard transfusion safety.

Most of the viral infections usually presents with similar symptoms and thus the need for proper screening to determine the cause of the symptoms.[34] Fever was not a predictor in this study for CMV positivity, likewise for stomach upset and rashes. The symptoms that were statistically linked to recent infection include muscle pain or ache and sore throat while tiredness and headache were significant for past infection. Although only sore throat was revealed to be a strong predictor of infection and immunological status, headache and tiredness were also linked at borderline statistical correlation (p=0.049) suggesting valid symptoms of CMV infection. This further reinforce the need for specific screening to avoid misdiagnosis that occurs with infections presenting similar clinical signs and symptoms.

4. CONCLUSIONS AND RECOMMENDATIONS

This study presents a comprehensive sero-epidemiological assessment of cytomegalovirus (CMV) among eligible blood donors in Ilorin, revealing a strikingly high IgG seropositivity rate of 92.3%. The data strongly demonstrate an inverse immunological relationship between IgG and IgM antibodies, with IgM positivity declining exponentially as IgG levels rise as evident in the fitted curve ($R^2 = 1$). Crucially, the Estimated Marginal Means (EMM) analysis offers further granularity into exposure patterns and risk associations. Respondents with a history of blood transfusion and tattoos exhibited significantly elevated EMM values for IgM positivity, reinforcing these factors as statistically robust predictors of recent CMV infection. These underscore the epidemiological relevance of parenteral and dermal exposure routes in CMV transmission, warranting stricter safety oversight in transfusion services and informal body modification practices. While the number of sexual partners showed only a modest influence on IgM status, the data suggest that transmission is more plausibly linked to direct exposure to infected bodily fluids rather than partner multiplicity. This reflects the complex interplay between behaviour, viral shedding patterns, and immune response timing.

Additionally, the integration of EMM analytics into this epidemiological framework enhances our understanding of CMV transmission dynamics, immune response trajectory, and socio-behavioural risk determinants. These insights provide critical evidence for public health planning, advocating for routine CMV screening of blood donors, pregnant women in antenatal programs and stringent regulation of blood and tattoo practices with targeted health education initiatives.

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LIMITATIONS

The study limitations include sample size which could not cover for several populations in the state due to limited resources. Also, the study is limited to one state without being able to assess the prevalence of the virus among blood donors from other regions in the county. Last, we could not carry out the molecular confirmation of the virus among the positive subjects.

NOVELTY STATEMENT

This manuscript reports the first comprehensive sero-epidemiological study of CMV in Ilorin's blood donors. Its novelty lies not only in documenting a high community burden but also in employing Estimated Marginal Means analysis to statistically validate specific factors as key independent risk factors for recent CMV exposure, a finding with direct implications for transfusion safety protocols.

AUTHOR'S CONTRIBUTION

M.B. Odebisi-Omokanye and A.G. Olayiwola regarded the main research idea, sample collection and laboratory analysis, M.B. Odebisi-Omokanye, M.M. Suleiman and A.O. Udeze did data management, analysis, interpretation and manuscript preparation, M.B. Odebisi-Omokanye, M.M. Suleiman and A.O. Udeze helped in experimental layout and designing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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