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Prevalence of cytomegalovirus IgG antibodies among women in Buguma, Rivers State, Nigeria

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Abstract

Cytomegalovirus (CMV) is a ubiquitous virus that belongs to the family of herpes virus. CMV causes serious illness in immuno-compromised patients and congenitally infected neonates. This study was conducted to determine the prevalence of the immunoglobulin (IgG) antibodies to cytomegalovirus among women in Buguma-City, Rivers State, Nigeria. Blood samples were collected from 93 women from Buguma-City Health Centre between February and August 2022. Serum obtained from the blood samples was examined for CMV IgG antibodies by ELISA method. Among the 93 women investigated, 89 (95.7%) were seropositive for Cytomegalovirus IgG antibodies. A total prevalence rate of 100.0% was recorded in the age group of 20 to 30 years, with the highest prevalence, while 89.5% was found in the age group of 31 to 56 years. Among the 38 single women, all were found to be seropositive (100.0%) for Cytomegalovirus antibodies, while 51 (92.7%) of the 55 married women were seropositive. The women with a secondary level of education had a higher prevalence of 97.0% than those with a tertiary level of education, which had a prevalence of 95.0%. The unemployed women had a higher prevalence (95.8%) than the employed, which had a prevalence of 95.7%. The results were statistically significant with age ($p = 0.01$). There was no significant association ($p > 0.05$) between CMV infection, marital status, education and occupation. The high prevalence of CMV infection observed in this study indicates that the virus is prevalent in the study area. Therefore, routine screening for CMV infection is advisable for all antenatal women in the State.

Keywords: Anti-CMV; IgG Antibodies; Women; Buguma; Nigeria

1 Introduction

Cytomegalovirus is mainly a problem for certain high-risk groups, which include unborn babies whose mothers become infected with CMV during pregnancy (Yeroh et al., 2015) and children or adults whose immune systems have been weakened by diseases or drug treatment, such as organ transplant recipients or people infected with HIV (Schleiss, 2010; Yeroh et al., 2015). It is a common opportunistic infection among Human immunodeficiency virus (HIV) infected individuals, a significant source of viral complication among organ transplant recipients and a leading cause of hearing loss, vision loss and mental retardation among congenitally infected children (Yeroh et al., 2015).

CMV infection occurs in many parts of the world irrespective of geographical location and socioeconomic group (Redwan & Ahmedi, 2001; Akinbami et al., 2011; Umeh et al., 2015). The prevalence of the infection appears to be higher in developing nations than in developed ones (Arun et al., 2012), and Africa seems to have the highest prevalence (Umeh et al., 2015). Nonetheless, high seroprevalence has been reported in some developed nations of the world (De Paschale

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et al., 2009; Lübeck et al., 2010). CMV is frequently transmitted to the developing fetus during pregnancy (Umeh et al., 2015). Sexual activity and contact with the urine or saliva of young children are other means of transmitting the infection (Ogbaini-Emovon et al., 2013).

Detection of virus-specific IgG antibodies in the blood of a pregnant woman who was previously seronegative or specific IgM antibodies associated with low IgG avidity is diagnostic of primary maternal CMV infection (Sonoyama et al., 2012; Odebisi-Omokanye et al., 2017). The detection of CMV IgG antibodies shows that the pregnant women had previously been infected with CMV. After CMV infection, IgG antibodies persist in the body for life and confer considerable immunity the subsequent infections. This observation indicates that negative results of the CMV IgG antibodies test indicate that the women have not been infected with the virus and are at a high risk of CMV infection (Odebisi-Omokanye et al., 2017).

Serological surveys have shown CMV infections in virtually every population that has been tested (Okwori et al., 2008), with seropositivity ranging from 40 – 100% in different parts of the world (Seferi et al., 2009; Matos et al., 2010). For example, a serological survey of over 20,000 women in London found that 54.4% were seropositive for CMV (Tookey et al., 1992). It has been reported that African Continent has the highest prevalence of CMV IgG antibodies. Seroprevalence of 72.2% and 96.0% have been reported in Western Sudan and Egypt (Hamdan et al., 2011). In Asian countries such as Malaysia, the seroprevalence of CMV IgG antibodies among pregnant women was reported to be 84.0% (Saraswathy et al., 2011). In Nigeria, a serological survey conducted in Bida, Lagos and Sokoto among pregnant women found a seroprevalence of 84.2%, 97.2% and 98.7%, respectively (Okwori et al., 2008; Ahmad et al., 2011; Akinbami et al., 2011; Yeroh et al., 2015). These results show that CMV is rising in Nigeria (Yeroh et al., 2015).

Cytomegalovirus infection has been studied in relation to other infections in Nigeria. In a serological study in Ibadan, Nigeria, a seroprevalence of 86.6% among tuberculosis (Tb) patients, 50.6% among patients other than Tb patients and 54.6% among healthy blood donors were recorded (Olaleye et al., 1990; Yeroh et al., 2015). In general, a seroprevalence of 68.3% was obtained among all patients, with Tb patients having the highest rate (Olaleye et al., 1990; Yeroh et al., 2015). The Seroprevalence of CMV among blood donors in Nigeria is also high. Seroprevalence of 92.0% CMV IgG antibodies has been observed among blood donors in Jos (Alao et al., 2008). Similarly, the seroprevalence of CMV among paid and unpaid blood donors in Tirana, Albania, gave an overall prevalence of 83.0% (Seferi et al., 2009).

HCMV is considered the most common cause of congenital viral disease in developed countries, associated with hearing loss (Pati et al., 2016; Korver et al., 2017). The seropositivity rate of HCMV is low in developed countries and high in developing countries (Almaghrabi et al., 2019). There is a dearth of data on CMV prevalence among pregnant women in our environment, whereas studies from other regions have implicated age, parity and social class as associated factors (Gratacap-Cavallier et al., 1998; Galia et al., 2007;). The few available studies did not explore this relationship. There is also no available data to justify the need for routine antenatal screening (Ogbaini-Emovon et al., 2013). This study sought to determine the seroprevalence of Cytomegalovirus antibodies among women in Buguma, Rivers State, Nigeria.

2 Material and methods

2.1 Study Area

This study was carried out among Buguma Primary Health Centre (BHC) patients in Rivers State, Southern Nigeria. The new Buguma Primary Health Centre was established in 2010, and it is located at Lawson Compound Sandfield with coordinates; 4.7403°N Latitude and 6.8619°E Longitude. According to Nigeria Census on 21 March 2016, Buguma-City has a population of 308,800 (NPC, 2016)

2.2 Study Population

This study examined 100 women of different ages and socioeconomic statuses at the Buguma-City Primary Health Centre (BPHC), Rivers State. Blood samples were obtained from consenting women. This study was carried out between February to August 2022.

2.3 Serological analysis of CMV IgM Antibodies

Venipuncture collected blood samples (Mocha et al., 2003). The blood samples were collected into EDTA bottles and transported on a cold chain to the Virus Research Laboratory, University of Port Harcourt, for processing and analysis under standard laboratory procedures. ELISA Kit (IgG for Cytomegalovirus) manufactured by DIA.PRO Diagnostic Bioprobes, Milano-Italy, was used to screen for CMV Specific-IgG antibodies according to the manufacturer's instructions. Samples with a concentration lower than 0.5 WHO IU/ml were all considered negative for anti-

Cytomegalovirus IgG antibodies. Samples with a concentration higher than 0.5 WHO IU/ml were considered positive for anti-Cytomegalovirus IgG antibodies.

2.4 Data Analysis

Data were analysed using Microsoft Excel 2016 version to calculate the results.

3 Results

3.1 Analysis of Study Population.

The total number of women included in this study was 93 (ages 20 to 56). The socio-demographic data for these samples and the number of positive women were stratified and shown in Table 1. The median age in the samples was 25 years (range between 20 to 30). The age group 20 to 30 years constituted the most considerable population making up 55.0%, while 38.0% of the women were aged 31 to 56 years. Married women predominated the study constituting 55.0% of the population compared to 38.0% of the single population. Based on educational background, 33.0% and 60.0% of the women were found to have acquired secondary and tertiary education, respectively. A lower percentage of 24.0% were unemployed, and 69.0% were employed (Table 1).

3.2 Overall Prevalence of Cytomegalovirus IgG Antibodies

Among the 93 women investigated, 89 (95.7%) were seropositive for Cytomegalovirus IgG antibodies.

3.3 Prevalence of CMV IgG Antibodies According to Age

The prevalence of Cytomegalovirus IgG antibodies with age is shown in Table 1. A total prevalence rate of 100.0% (55 out of 55) was recorded in the age group of 20 to 30 years, which had the highest prevalence, while 89.5% (34 out of 38) was found in the age group of 31 to 56 years. The result was statistically significant with age ($p = 0.01$).

3.4 Prevalence of CMV IgG According to Marital Status

Table 1 also shows the seropositivity rate of Cytomegalovirus IgG antibodies according to marital status. Married women comprised a higher number of the population (55), while the single women included in this study were fewer, with a total of 38. Among the 38 single women, all were found to be seropositive (100.0%) for cytomegalovirus, while 51(92.7%) of the 55 married women were seropositive. There was no significant association ($p = 0.09$) between CMV infection and marital status.

3.5 Prevalence of CMV IgG According to Educational Status

The number of women in this study with secondary and tertiary education was 33 and 60, respectively. The women with a secondary level of education had a higher prevalence of 97.0% (32 out of 33) than those with a tertiary level of education which had a prevalence of 95.0% (57 out of 60). This observation is also shown in Table 1. There was no significant association ($p=0.65$) between CMV infection and education.

3.6 Prevalence of CMV IgG Antibodies According to Occupation

Table 1 also shows the prevalence rate of Cytomegalovirus IgG antibodies according to occupation. The unemployed women had a higher prevalence (95.8%; 23 out of 24) than the employed, which had a prevalence of 95.7% (66 out of 69). There was no significant association ($p=0.97$) between CMV infection and occupation.

Table 1 Prevalence of CMV IgG **Antibodies** According to Socio-demographic Parameters of the Women

Parameters	Category	No. Tested	No. Positive for CMV IgG (%)	P value
Age (years)	20-30	55	55 (100.0)	P= 0.01
	31-56	38	34 (89.5)	
Marital Status	Married	55	51 (92.7)	P = 0.09
	Singles	38	38 (100.0)	
Educational Status	Secondary	33	32 (97.0)	P = 0.65
	Tertiary	60	57 (95.0)	
Occupation	Employed	69	66 (95.7)	P = 0.97
	Unemployed	24	23 (95.8)	
Total		93	89(95.7)	

4 Discussion

The prevalence of cytomegalovirus infection varies with the population (Ojide et al., 2012). It is more common in areas of developing countries and areas with low socioeconomic conditions (Ojide et al., 2012). The seroprevalence of CMV among females ranges from 35.0% to 95.0% in different countries (Arun et al., 2012). Previous studies have confirmed that CMV infection is relatively common among women of reproductive age, with seroprevalence ranging from 45.0% to 100.0% (Maingi & Nyamache, 2014). African continents like South America and Asia has one of the highest prevalence of CMV (Cannon et al., 2010; Maingi & Nyamache, 2014). From the previous studies conducted in Africa, CMV prevalence rates in Egypt were found to be 96.0% (Kamel et al., 2013), 85.7% in Tanzania (Mhalu & Haukenes, 1990), 97.2% in Benin (Rodier et al., 1995) and 86.4% South Africa (Bos et al., 1995). Thus, this study sought to determine the seroprevalence of Cytomegalovirus infections among women in Buguma, Rivers State, Nigeria. Among the 93 women investigated, 89 (95.7%) were seropositive for Cytomegalovirus IgG antibodies.

The results obtained from this study show that the seroprevalence rate of CMV infection among women in Buguma, Rivers State is high. The 95.7% prevalence of CMV IgG reported in this study is comparable to the seroprevalence of the 95.7% reported in Madinah, the Kingdom of Saudi Arabia (Mahallawi et al., 2022), the 94.8% obtained in Kaduna State, Nigeria (Yeroh et al., 2015), 93.3% reported in Makurdi, Benue State, Nigeria (Umeh et al., 2015) and 93.2% reported in Karachi, Pakistan (Ibrahim et al., 2016). This result is similar to the seroprevalence of 97.2% reported in Lagos, Nigeria; 98.7% reported in Sokoto State, Nigeria and 98.2% reported in Ilorin, Kwara State, Nigeria (Akinbami et al., 2011; Ahmad et al., 2011; Odebisi-Omokanye et al., 2017). This prevalence agrees with Saraswathy et al.'s (2001) assertion that the African continent has the highest prevalence rate of CMV IgG antibodies.

Studies among pregnant women worldwide have shown different seroprevalence rates for previous infections (Odebisi-Omokanye et al., 2017). In Australia, Belgium, France and Poland, a relatively low seroprevalence rate of 30.0%-60.0% was reported (Basha et al., 2014), while a high CMV seroprevalence of greater than 90.0% was reported in Iran, Turkey, Nigeria and India (Josheghani et al., 2015) and >96.0% among Saudi women in the Asir region (Almaghrabi et al., 2019). Another study performed in Jazan, Saudi Arabia, showed an HCMV IgG antibodies positivity rate of 93.1% (Bakri et al., 2016). In Nigeria, past studies have revealed CMV prevalence of 84.2%, 91.1%, 97.2%, and 98.7% in Bida, Kano, Lagos and Sokoto, respectively, among pregnant women, indicating an increase in prevalence (Odebisi-Omokanye et al., 2017).

This result (95.7%) is similar to the seroprevalence of 97.5% in Sudan (Khairi et al., 2013) and 96.0% in Egypt (El-Nawawy et al., 1996). It is lower than the 98.5% reported by Vueba et al. (2022) in Launda, Angola and 98.7% reported by La et al. (2019) in Seoul, South Korea. The proportion of HCMV IgG-seropositive individuals ranged from 95.7 to 100% in a study by Almaghrabi et al. (2019) in Saudi Arabia. In the Jazan Region of Saudi Arabia, CMV IgG seropositivity was reported to be 93.10% (Bakri et al., 2016). This result is also consistent with reports from other parts of the world, including Nigeria (Redwan&Ahmedi, 2001; Akinbami et al., 2011; Leila et al., 2012; Umeh et al., 2015), in which a high seroprevalence of anti-CMV IgG antibodies (indicating past CMV infection) in women was reported as in this study. On the other hand, results of studies from developed parts of the world reported a low prevalence of anti-CMV IgG antibodies in pregnant women (Tookey et al., 1992; Knowles et al., 2005; Seale et al., 2006). However, the higher seroprevalence of anti-CMV IgG antibodies has been reported in Germany (Lübeck et al., 2010) and northern Italy (De Paschale et al., 2009).

The overall incidence rate of CMV IgG among women reported in Bida, Nigeria (Okwori et al., 2008) was 84.2%, and in Benin City, Nigeria (Ogbaini-Emovon et al., 2013), was 92.0% which were similar with other studies in different African countries; 77.3% in Kenya (Maingi et al., 2014), 92.0% in Nigeria (Ephraim et al., 2013), 87.0% in Gambia (Bello et al., 1991), 60.0% in the United States (Staras et al., 2006) and even in one of the European countries (Satilmis et al., 2007). In Ghana, the prevalence of cytomegalovirus among females was reported to be 70.0% to 93.0% (Adjei et al., 2008). CMV prevalence tended to be highest in South America, Africa and Asia and lowest in Western Europe and the United States (Staras et al., 2006).

The 95.7% reported for CMV IgG antibodies in this study is higher than what was reported elsewhere by some previous studies. Adeiza et al. (2016) reported 86.0% among HIV-positive patients and 72.8% among HIV-negative controls in Zaria, Nigeria. CMV IgG seroprevalence in Buguma, Rivers State, was similar to the 92.0% and 96.0% reported in healthy blood donors from Lagos and Jos, Nigeria, respectively (Alao et al., 2008; Akinbami et al., 2009). It was also higher than the 84.2% reported by Okwori in pregnant women in Bida, the 87.0% reported by Kothari et al. (2002) from India, 62.4% reported by Wujcicka et al. (2014) among Polish women in Lodz and 83.16% reported by Adane and Getawa (2021). Most other studies from Africa and Asia have quoted higher seroprevalence rates (McCormack et al., 1990; Gargouri et al., 2000; Wong et al., 2000) except Adjei et al. (2008) in Accra Ghana, in which CMV IgG seroprevalence was 77.6% in healthy blood donors and 59.2% in HIV/AIDS patients. This lower CMV IgG seroprevalence in these previous studies contrasts with the current study and can be explained by the population used in those studies.

The prevalence of CMV infection observed in this study was similar to that reported in other developing communities but higher than that in the developed ones. The high prevalence rates observed in the above countries suggest that quite several people in the study areas have previously been exposed to CMV, and this is contrary to the pattern seen in Western countries such as Australia, Germany, and the US, where the CMV IgG seroprevalence is around 50.0 to 60.0% (Rodier et al., 1995; Kumar et al., 2008; Lübeck et al., 2010). The possible explanation for this difference may be related to the prevailing socioeconomic, environmental, and climatic factors and the lower HIV prevalence in these countries (Adeiza et al., 2016). This observation may be attributed to the low socioeconomic status and poor hygienic practices, which might significantly increase the rate of CMV infection.

This study's high prevalence of previous infection agrees with several other studies (Adler et al., 1996; Satilmis et al., 2007; Hamdan et al., 2011; Ogbaini-Emovon et al., 2013). However, a higher seroprevalence has been documented by other researchers (Rubina et al., 2004; Tabatabaee & Tayyebi, 2009). These discrepancies may be attributed to differences in socioeconomic setting, and this can be inferred from the work of Stagno and Whitley (1985), which demonstrated that the risk of primary maternal infection was about three times higher among the high-income susceptible women than the lower income group (Ogbaini-Emovon et al., 2013). This observation may be viewed from the point that there are likely more seronegative women among the high social class due to better hygiene than the low social class, making the former more susceptible to primary infection (Ogbaini-Emovon et al., 2013).

Although the seronegativity among these is low (4.3%), they are a critical group with no maternal immunity because the risk of congenital CMV infection is much higher during primary infection in the mother (Griffiths et al., 2001; Fowler et al., 2003). However, recently published data have demonstrated that universal screening for primary maternal infection using IgG avidity testing can help distinguish primary CMV infection from reactivation. It is known that reactivation is usually associated with a meagre rate of vertical transmission (Boppana et al., 1999; Stagno, 2001; Kenneson & Cannon, 2007). Treatment with hyperimmune globulin was efficacious and cost-effective (Cahill et al., 2009; Ogbaini-Emovon et al., 2013).

Analysis of the result by age shows a significant association ($p=0.01$) between CMV and age compared to previous reports that used the ELISA method (Okwori et al., 2008; Stadler et al., 2012; Yeroh et al., 2015). Our findings also pointed to a strong correlation between age and the prevalence of HCMV antibodies, in agreement with the findings of Zhang et al. (2014) in China and Almaghrabi et al. (2019) in Saudi Arabia. A higher prevalence (100.0%) was recorded in the age group of 20 to 30 years than in the 31-56 years age group (89.5%). This result corroborates with Adeiza et al. (2016), who also reported CMV IgG age-specific seroprevalence to be highest in the younger age groups (16-25 years). It also corroborates with Almaghrabi et al. (2019), who reported 100.0% in their age group 15-20 and 25-30 years in the Asir region of Saudi Arabia and Mahallawi et al. (2022), who reported 100.0% in their age group 18-30 years in Madinah region of Saudi Arabia.

This study had no predictable pattern between seroprevalence and age, even though the youngest and oldest ages gave high prevalence. The highest prevalence reported in the younger age group (100.0%) than, the older group (89.5%) in this study is contrary to what was reported by other previous studies. Umeh et al. (2015) in Makurdi, Benue State, Nigeria, reported 100.0% in older women aged 41-50 years and the lowest (85.0%) in younger ones aged 15-20.

Wujcicka et al. (2014), among Polish women in Lodz, reported that the highest IgG prevalence was observed in women above 36 years of age. La et al. (2019) in Seoul, South Korea, reported that age was independently associated with higher HCMV seroprevalence (41-60 years, ≥ 61 years, compared to ≤ 40 years). Our findings agree with previous studies that showed changes in anti-HCMV IgG levels with age (Frasca et al., 2015; Aiello et al., 2017; Almaghrabi et al., 2019; Mahallawi et al., 2022).

Other study results align with ours, showing an inverse correlation between age and anti-HCMV IgG levels among pregnant women in the Kingdom of Saudi Arabia and China (Zhang et al., 2014; Almaghrabi et al., 2019; Mahallawi et al., 2022). The highest seroprevalence among the youngest age group could be explained by the increased sexual activities of this age group, as the virus can be transmitted sexually. In contrast, a more prolonged duration of exposure to the virus might be responsible for the highest prevalence in the older age group (Yeroh et al., 2015).

This observation is similar to the pattern in Ghana, a population similar to ours (Adjei et al., 2008). Seroprevalence rate did not vary in any particular direction or trend with the different age groups, as Ojide et al. (2012) reported in Benin City, Nigeria, and corroborated by Adjei et al. (2008) in Ghana. The clustering of higher CMV IgG and IgM seroprevalence in the younger age groups of HIV-positive patients in this study is in contrast to the pattern seen in HIV-negative controls and other studies carried out in developed countries like the US, where seroprevalence rates increased with age, with a peak around 80 years (Kumar et al., 2008). This observation may be related to the higher force of sexual transmission in the younger age group (Adeiza et al., 2016).

Married women comprised a higher number of the population (55), while the single women included in this study were fewer, with a total of 38. Among the 38 single women, all were found to be seropositive (100.0%) for cytomegalovirus, while 51 (92.7%) of the 55 married women were seropositive. The marital status shows that Cytomegalovirus infection is higher in singles than the married women, which suggests continuous virus circulation within the area. However, no significant statistical association ($p = 0.09$) was observed. This observation is contrary to a study that recorded increased seropositivity among married women compared to single ladies (Douglas et al., 2012; Odebisi-Omokanye et al., 2017). It also corroborates with what was reported in Karachi, Pakistan (Ibrahim et al., 2016), where seroprevalence differed significantly by marital status. Being married (92.7%) also increased susceptibility to the acquisition of CMV infection, perhaps through direct contact with contagious secretions from their children or poor hygiene practised by these women (Kramer et al., 2006; Bate et al., 2010; Maingi & Nyamache, 2014). These risk factors were similar to those found in previous studies (Hamdan et al., 2011; Maingi & Nyamache, 2014).

The women with secondary education had a higher prevalence of 97.0% than those with tertiary education, which had a prevalence of 95.0%. The seroprevalence rate increased insignificantly ($p=0.65$) with an increase in education, with a higher degree of seropositivity observed in the group with tertiary education. The increase in seroprevalence disagrees with previous reports (Hamdan et al., 2011; Yeroh et al., 2015; Odebisi-Omokanye et al., 2017) showed that illiterate women are at higher risk of CMV infection due to contact with contagious secretions from their children and poor hygienic practice. Wujcicka et al. (2014), among Polish women in Lodz, reported that primary and professional education was significantly associated with HCMV IgG prevalence rates.

The unemployed women had a higher prevalence (95.8%) than the employed, which had a prevalence of 95.7%. Seroprevalence of CMV infection by employment status, one of the socioeconomic status predictors, was higher among the unemployed. This observation is similar to what Yeroh et al. (2015) reported in their study: a strong association between employment status and CMV infection. This finding in this study deviates from that of Tebuka et al. (2019), who reported a higher prevalence among workers than students in their study in Mwanza. Wujcicka et al. (2014), among Polish women in Lodz, reported that financial status and occupational risk related to contact with children were unrelated to the prevalence of IgG antibodies. Socioeconomic status is a risk factor for CMV infection (Brooks et al., 2007; Yeroh et al., 2015). The reason for this is probably because high socioeconomic status implies the ability to acquire education and afford better and healthy living conditions, which decreases exposure to the virus (Yeroh et al., 2015)

5 Conclusion

The high prevalence of cytomegalovirus among women cannot be overemphasised. As shown in this study, women of childbearing age are highly susceptible to this infection. Therefore, they are advised to undergo pre-natal and antenatal check-ups since the infection could be asymptomatic. This study has shown that the prevalence of CMV IgG was age dependent, as it could be found among different age groups. In general, this study showed a high seroprevalence rate of CMV infection among women in Buguma, Rivers State, Nigeria; and is likely to reflect the overall high prevalence among adult Nigerians. The CMV status should be investigated, especially among women of reproductive age before pregnancy, and seronegative females should be vaccinated.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no competing interests exist.

Statement of ethical approval

All authors declare that all experiments have been examined and approved by the University of Port Harcourt and Rivers State University Teaching Hospital Research Ethics committees. Therefore, the study is performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

Statement of informed consent

All authors declare that informed consent was obtained from all individual participants included in the study.

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