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THE FIRST SEROLOGICAL EVIDENCE OF RECENT DENGUE VIRUS INFECTION AMONG HIV-INFECTED PATIENTS ATTENDING THE UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL, RIVERS STATE, NIGERIA

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ABSTRACT

Dengue fever has grown to be a significant public health issue. By testing HIV-infected patients for IgM antibodies to the dengue virus, the study sought to determine its serological evidence in Port Harcourt Nigeria. Infected patients with HIV who were seen at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria, were the subject of this cross-sectional study. A total of 94 HIV-positive patients were enlisted, and after gaining consent, approximately 5 ml of whole blood was taken. Enzyme Linked Immunosorbent Assay was used to detect the presence of IgM antibodies to the dengue fever virus (ELISA). IgM antibodies prevalence to the dengue fever virus was found to be 35.1%. Females (43.6%) had a higher prevalence. Age-wise, the group of those under 25 showed a higher prevalence (46.7%). Single people (37.9%), people with tertiary degrees (52.9%), those without jobs (42.9%), and people who live in cities (37.8%) all had higher incidence rates. Several sociodemographic characteristics and dengue fever did not differ significantly ($p > 0.05$), although sex ($p = 0.04$), employment ($p = 0.02$), and place of residence ($p = 0.001$) were statistically linked to the prevalence of dengue IgM. This study found significant serological evidence of IgM antibodies to the dengue virus, indicating that HIV-infected patients in Port Harcourt, Nigeria, were carriers of this virus. Further research on this virus is required to build on the relationship between age, marital status, education, and most importantly, the seasonal variation of the virus in terms of infection rates.

Keywords: Dengue IgM, HIV, Patients, Public health concern

INTRODUCTION

Dengue fever, a viral infection has become a significant public health issue (Bello et al., 2016). Moreover, the majority (>70%) of febrile infections, including dengue, are likely to be misdiagnosed and treated as malaria due to the large endemicity of malaria in Nigeria (Ayukekbong, 2014). For the purpose of controlling infectious diseases, public health programs must understand risk factors. It has been challenging to assess, for example, the difference in infection rates between men and women (Goh et al., 1987; Agarwal et al., 1999; Wali et al., 1999). Because normal laboratory diagnosis is not performed, dengue surveillance is compromised in Nigeria. PCR, serological tests, and culture may all be used in the laboratory to diagnose dengue (Back & Lundkvist, 2013).

Dengue is currently one of the most prevalent viral infections spread by mosquitoes, with significant human and financial implications (Guzman et al., 2010; WHO, 2012; Onyedibe et al., 2018). About half of the world's population is now at risk of dengue with an estimated 100–400 million infections

occurring each year (WHO, 2023). In recent decades, dengue has become much more common, with cases reported to the WHO rising from 505 430 cases in 2000 to 5.2 million cases in 2019. Dengue cases are underreported since a large percentage of them are asymptomatic, moderate, and self-managed (WHO, 2023). In many instances, other febrile infections are incorrectly diagnosed (Bhatt et al., 2013; WHO, 2023). According to one model, there are 390 million dengue virus infections each year, of which 96 million results in clinical symptoms (Brady et al., 2012; WHO, 2023). According to a different study on the incidence of dengue, 3.9 billion people worldwide could contract dengue viruses (WHO, 2023).

In the WHO Regions, the illness is currently endemic in more than 100 countries. The regions most severely impacted include the Americas, South-East Asia, and Western Pacific, with Asia accounting for over 70% of the worldwide disease burden. Explosive outbreaks of dengue are occurring, and the disease is moving to other regions, including Europe (WHO, 2023).

The first case of dengue fever occurred for the first time in Nigeria in the 1960s (Carey et al., 1971; Onyedibe et al., 2018). Within the months of August 1964 and December 1968, febrile individuals were found to have 32 different dengue virus strains in the same Ibadan (Carey et al., 1971; Onyedibe et al., 2018). Since then, numerous further investigations have discovered dengue virus infections in various national geographic locations (Fagbami et al., 1977a, b; Faneye et al., 2013; Idris et al., 2013; Oyero & Ayukekbong, 2014; Adesina & Adeniji, 2016; Onyedibe et al., 2018).

In a study conducted in Nigeria, samples primarily taken during the early 1970s were used to determine the prevalence of flavivirus infections in children and adults from urban and rural locations (Amarasinghe et al., 2011). Faneye et al. (2013) reported a prevalence of 30.8%, Srinivas et al. (2013) reported 17.7%, Oladipo et al. (2014) reported 17.2% in Ogbomoso, and Baba et al. (2013) reported 0.6%. (2009). The findings of these investigations highlight the significance of monitoring dengue fever virus (Bello et al., 2016).

Dengue is still not a reportable disease in Nigeria and in most African nations, with most cases going untreated or being misdiagnosed as malaria, despite reports of infections and severe dengue (Amexo et al., 2004; Amarasinghe et al., 2011; Onyedibe et al., 2018). Malaria is known to be the primary cause of acute febrile disease in many healthcare facilities in Africa, with enteric fever a distant second (Baba et al., 2009; Dawurung et al., 2010; Baba et al., 2013; Idoko et al., 2014; Onyedibe et al., 2018). Most fever screening procedures at these facilities only check for enteric fever and occasionally malaria, ignoring numerous additional bacterial and viral causes of fever in addition to dengue fever virus and other acute febrile disorders (Baba et al., 2009; Dawurung et al., 2010; Baba et al., 2013; Idoko et al., 2014; Onyedibe et al., 2018).

Many febrile diseases are treated as presumed cases of malaria in Nigeria, frequently without a complete physical examination or test confirmation. As a result, even when given antimalarial medications, many patients with fever are labelled as having malaria or fever of unknown cause and do not receive a test diagnosis. The scarcity of reasonably priced diagnostic tools is largely to blame for this condition. This situation shows that many DENV infections go undetected or are even

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misdiagnosed. Furthermore, Omatola et al. (2021) reported finding the dengue vector Aedes aegypti in Nigeria's Savannah region. Although particular demographic characteristics, albeit age and gender have not been mentioned among febrile patients in Nigeria, individual differences in exposure to dengue-infective bites may be associated to prevalence. The purpose of this study was to identify HIV-infected individuals at the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria, who had IgM antibodies specific to the dengue fever virus.

MATERIALS AND METHODS

Study Area

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH) located in Alakahia, Rivers State, Nigeria. UPTH is a tertiary healthcare institution that serves patients from high, low, and middle-income backgrounds, both within and outside of Rivers State, Nigeria.

Study Design

This study was performed to provide serological evidence of dengue virus among HIV-infected patients attending the University of Port Harcourt Teaching Hospital, Nigeria. The study constituted a total number of 94 patients within the ages of 20-70 years and the relevant socio-demographic characteristics (age, sex, marital status, educational level, occupations and location of residence) were obtained from a structured questionnaire.

Eligibility Criterion

Male and female HIV-infected Nigerians who consented to participate in the study were recruited irrespective of age group. Non-HIV-infected patients were excluded from this study.

Specimen Collection and Preparation

Five millilitres (5 mls) of whole blood sample were aseptically drawn out by venipuncture from the patient using a new sterile syringe and placed into a sterile EDTA tube and the samples were taken into the Virus & Genomics Research Unit. The blood samples were centrifuged to separate the serum from the red blood cell. The serum was aspirated using a disposable hand pipette into sterile Eppendorf tubes which were labeled with codes. The sera were stored at -20^{O}C for a few days after collection.

Serological Analysis

In accordance with the manufacturer's instructions, ELISA kits from DIA.PRO Diagnostic Bioprobes (Milano) - Italy were used to test for IgM antibodies to dengue fever virus in the serum.

Analysis of Data

The data generated from the questionnaire and the result from the serological analysis were analyzed using SPSS version 22.0. Frequencies and percentages were generated, and categorized variables were compared using chi-square test. A p-value less or equal to 0.05 was considered as statistically significant at 95% confidence interval.

RESULTS

General Characteristics of Participants

Ninety-four (94) participants were tested for dengue virus IgM antibodies. Of these 94 participants, 39 (41.5%) were males and 55 (58.5%) females. Majority (43.6%) were in age group 36 to 55 years, married (37.2%), not in school (73.4%), unemployed (67.0%) and resided in rural communities (60.6%) as shown in Table 1.

ELISA Results

Of the 94 participants whose sera were tested for dengue fever virus IgM antibodies using ELISA, 35.1% (33/94) were positive and 64.9% (61/94) were negative, thus giving an overall prevalence of 35.1% (Table 1).

Prevalence of Dengue fever Virus IgM Antibodies as it relates to Sociodemographic factors

Based on sociodemographic factors, the ELISA results were analysed for sex, age, marital status, educational level, employment (occupation) and location of residence. Only sex (p = 0.04), occupation (p = 0.02) and location of residence (p = 0.001) were statistically associated with prevalence of Dengue IgM antibodies (Table 1).

Prevalence of Dengue fever Virus IgM Antibodies in Relation to Sex

Based on gender, a total of 24 of the female participants were positive for dengue fever virus IgM antibodies giving a prevalence of 43.6% while 9 of the male participants were positive giving a prevalence of 23.1%. The difference observed in the prevalence between male and females was statistically significant (p=0.04) in Table 1.

Prevalence of Dengue Virus IgM Antibodies in Relation to Age

The result was analyzed according to age and the highest prevalence (46.7%; 7/15) was found among participants who were in age group <25 years, and the lowest (11.1%; 1/9) was found among participants in age group 56 years and above. There was no statistically significant difference between age and the presence of IgM antibodies to dengue fever virus (p=0.33) as shown in Table 1.

Prevalence of Dengue Virus IgM Antibodies in Relation to Marital Status

In terms of marital status, IgM antibodies to dengue fever virus was detected highest with a prevalence of 37.9% (11/29) among the singles, followed by the married participants (37.1%; 13/35) while the lowest prevalence of 30.0% (9/30) was recorded among the widowed/divorced. There was no statistically significant difference between marital status and the presence of IgM antibodies to dengue fever virus (p=0.77) as shown in Table 1.

Prevalence of Dengue Virus IgM Antibodies in Relation to Educational Level

In terms of educational level, IgM antibodies to dengue fever virus was detected with the highest prevalence of 52.9% (11/29) among the tertiary education, followed by those with secondary education (40.0%; 2/5) and those not in school (31.9%; 22/69), while the lowest prevalence of 0.0% was recorded among those with primary education. There was no statistically significant difference between educational level and the presence of IgM antibodies to dengue fever virus (p=0.22) as observed in Table 1.

Prevalence of Dengue Virus IgM Antibodies in Relation to Employment Status

In terms of occupation, IgM antibodies to dengue fever virus was detected with the highest prevalence of 42.9% (27/63) among the unemployed while the lowest prevalence of 19.4% (6/31) was recorded among the employed. There was statistically significant difference between occupation and the presence of IgM antibodies to dengue fever virus (p=0.02) in Table 1.

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Prevalence of Dengue Virus IgM Antibodies in Relation to Residence

According to location of residence, the highest prevalence (37.8%; 27/37) was found among participants who were in urban areas and the lowest (33.3%; 6/57) was found among participants in rural areas. There was statistically significant difference between location of residence and the presence of IgM antibodies to dengue fever virus (p=0.001) as seen in Table 1.

Table 1: Serological Evidence of Dengue Fever Virus in Relation to Sociodemographic Characteristics of the Participants

Variables	No. Tested (%)	No. Positive (%)	Chi-square Analysis
Sex			
Males	39(41.5)	9 (23.1)	P = 0.04
Females	55(58.5)	24 (43.6)	
Age (years)			
<25	15(16.0)	7(46.7)	P = 0.33
26 - 35	26(27.7)	9(34.6)	
36 – 55	41(43.6)	16(39.0)	
56 & above	9(9.6)	1(11.1)	
Marital Status			
Singles	29(30.9)	11(37.9)	P = 0.77
Married	35(37.2)	13(37.1)	
Widowed/divorced	30(31.9)	9(30.0)	
Education			
Primary	3(3.2)	0 (0.0)	P = 0.22
Secondary	5(5.3)	2(40.0)	
Tertiary	17(18.1)	9(52.9)	
Not in School	69(73.4)	22 (31.9)	
Occupation			
Employed	31(33.0)	6 (19.4)	P = 0.02
Unemployed	63(67.0)	27(42.9)	
Location (residence area)			
Rural	57(60.6)	6(33.3)	P = 0.00
Urban	37(39.4)	27(37.8)	
Total	**94(100.0)**	**33(35.1)**	

DISCUSSION

A significant emerging illness in both tropical and subtropical areas is dengue fever (Chukwuma et al., 2018). More than 70% of febrile cases in Nigeria, where malaria is widespread, are treated presumptively as malaria, and this is frequently done without a laboratory evaluation for other potential causes of fever (Onyedibe et al., 2018).

According to the results of this study, 35.1% of participants who were enrolled at the chosen hospital in Rivers State, Nigeria had been exposed to the dengue fever virus, as shown by the presence of IgM antibodies in their serum. The presence of IgM in the blood of these patients suggests recent virus infection and a high frequency of vector mosquito bites (Bello et al., 2016). The prevalence of 35.1% found in this study is higher than what was reported

in other studies conducted in Nigeria. Faneye et al. (2013) reported a prevalence of 30.8%, Srinivas et al. (2013) reported 17.7%, Oladipo et al. (2014) reported 17.2% in Ogbomoso, Baba et al. (2009) reported 0.6%, Adeshina and Adeniyi (2016) reported 25.7% in Ile-Ife, Nigeria, and 20.2% in Awka, Nigeria (Okoye et al., 2021). The prevalence is also greater than that found in a Singaporean study (Yew et al., 2009), which found a frequency of 2.65%. Yet, it is lower than the 78.3% in Kano Metropolis, Nigeria (Abdulaziz et al., 2020), the 77.1% in Nnewi, Nigeria (Chukwuma et al., 2018), and the 51.9% in Kaduna state, Nigeria (Bello et al., 2016).

Age, marital status, and educational attainment were found to have no s with the dengue fever virus in this study ($p > 0.05$). However, this study demonstrated that among the study participants, gender, employment (occupation) status, and place of residence were sufficient predictors of dengue fever virus immunoglobulin at $p < 0.05$. Hence, there might be additional elements that haven't been investigated. The lack of statistically significant variations ($p > 0.05$) in the occurrence of dengue fever virus among some of the parameters (age, marital status and education) evaluated may indicate that everyone in the study area, regardless of status, age, marital status, or level of education, is at risk for infections (Ahmadu et al., 2020).

According to gender, female participants had a greater prevalence of dengue virus IgM antibodies (43.6%) than male participants (23.1%). This study revealed that sex is a possible predisposing factor, with more females than males contracting the infection. This finding is consistent with Bello et al. (2016) who observed that sex was a predisposing factor, with more females contracting the disease than males. It also agrees with earlier research by Idris et al. (2013) and Mustapha et al. (2017) which indicated a greater prevalence for dengue infection in females than in males.

Oladipo et al. (2014) found a higher prevalence in males than females, which conflicts with our finding. Similarly, Brown et al. (2009 observed a link between sex and dengue fever in which all the males tested positive but none of the ladies did, differing from the finding of this study. Also, in certain earlier investigations, males were shown to have a higher prevalence of dengue infection than females (Gupta et al., 2005; Kumar et al., 2010; Ukey et al., 2010; Oladipo et al., 2014; Adeleke et al.,2016; Okoye et al., 2021). , The difference in the prevalence between men and women may be due to the fact that more men wait until major health problems arise before going to the hospital (INTEGRIS Health, 2019). Also, these disparities in prevalence between the sexes could possibly be explained by the different sample sizes used in the studies, where more samples from women were taken, the participants' occupations, and exposure to the virus' vector (Bello et al., 2016).

According to this study, age did not appear to be related to the dengue fever virus ($p > 0.05$). Although though there was no correlation between age and dengue fever virus, it was shown that participants under the age of 25 years had a larger prevalence while patients over the age of 56 years had the lowest frequency. This finding is comparable to that made by Baba et al. (2009), who found no correlation between age and dengue. It also supports the findings of Oladipo et al. (2014) who found that in Ogbomoso, Oyo State, Nigeria, people aged 0 to 15 years had the highest prevalence of anti-dengue virus IgM antibodies, as well as those of Garg et al.

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(2017) who found that dengue seroprevalence rose with age, from 40.7% in children aged 5 to 73.4% in 10-year-olds.

However, it contradicts with research by Okoye et al. (2021) and other international studies (Anderson et al., 2011; Garg et al., 2011), which found that the prevalence was higher among people aged 26 to 35 in Awka, Nigeria. Our findings also differ from those of Peyerl-Hoffmann et al. (2004), who found a linear relationship between age and rising antibody prevalence, and from earlier research by Reiskind et al. (2001), which revealed an age-dependent rise in anti-dengue antibodies in populations exposed to the disease.

In terms of marital status, there was a higher frequency of IgM antibodies to Dengue fever virus among singles than among married people and widowed or divorced people. This result supports the findings of Adesina and Adeniji (2016), who found that seropositive Dengue fever virus IgM antibodies were more common in single persons. Additionally, it refutes the finding of Kolawole et al. (2017) that married people had higher seropositivity rates for Dengue fever virus IgM antibodies.

Regarding educational attainment, university education holders (52.9%) had a greater prevalence of IgM antibodies to dengue fever virus than other groups with 0% incidence in primary education. However, a study by Chukwuma et al. (2018) found that primary school students (41.9%) had the highest seropositivity of the dengue virus. Nonetheless, this study is in line with some earlier research on dengue, such as that by Teixeira et al. (2012), who reported that there was no dengue infection among preschoolers in Brazil. It goes against the findings of Adesina and Adeniji (2016), who found that people with no formal education were more likely to contract dengue fever. Kolawole et al. (2017), who had the same viewpoint as Adesina and Adeniji (2016), disagreed with it as well.

Regarding occupation, the highest frequency of IgM antibodies to dengue fever virus was found to be 42.9% in the unemployed, while the lowest prevalence of 19.4% was found in the employed. In contrast, Oladipo et al. (2014) observed that Ogbomoso, Oyo State, Nigeria had the greatest prevalence of anti-dengue IgM antibodies among civil personnel. According to their study, there is no correlation between work and the dengue fever virus ($p > 0.05$). The highest prevalence of IgM antibodies to dengue fever virus was seen among the unemployed participants in the present study links occupation to dengue fever virus infection. This finding contrasts with that made by Bello et al. (2016), who found no connection between dengue and employment.

Compared to employed people who were less exposed to the virus, the unemployed were more likely to get dengue fever. This contradicts Bello et al. (2016), who claimed that farmers had a greater prevalence of the dengue virus. This finding suggests that the virus predisposes the unemployed to the virus due to their involvement in other activities.

A higher prevalence was found among urban rather than rural residents after the data was broken down by place of residence. It supports the findings of Oladipo et al. (2014), who also noted the highest frequency of anti-dengue IgM antibodies among urban residents in Ogbomoso, Oyo State, Nigeria. This can be due to bad city planning, which gives mosquitoes additional places to reproduce. Industrialization might potentially have a significant impact on vector control, accelerating the spread of the virus in metropolitan areas (Oladipo et al., 2014). Due to human activities that encourage mosquito

breeding, rapid urbanization in Africa has increased vector density (Monath, 1994).

When compared to other research, the prevalence found in this study was higher, which could be explained by the different geographic locations and seasons at which the studies were conducted (Bello et al., 2016).

CONCLUSION

According to this study, a significant percentage of HIV-infected patients (35.1%) at the University of Port Harcourt Teaching Hospital (UPTH) had the dengue virus. This finding implies that the dengue fever virus is spreading among HIV-infected patients in Port Harcourt, Nigeria. Females had a higher rate of dengue fever virus. Age, marital status and educational background were not linked to the presence of the dengue fever virus. This study's high prevalence finding raises serious public health issues. This expands on the information already known on the prevalence of dengue fever virus in Nigeria. Africa, especially Nigeria, where dengue virus infections are prevalent, should implement the dengue virus elimination program. In order to manage the infection effectively, it must be correctly and quickly detected. HIV-infected patients should also undergo extensive screening for dengue fever virus in addition to malaria and typhoid, to sustain effective therapy. Further research on this virus is required to build on the relationship between age, marital status, education, and most importantly, the seasonal variation of the virus in terms of infection rates.

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All authors hereby declare that all experiments have been examined and approved by the University of Port Harcourt and University of Port Harcourt Teaching Hospital Research Ethics committees and have, therefore, been 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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