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SEROPREVALENCE OF DENGUE VIRUS INFECTION AMONG FEBRILE PATIENTS ATTENDING SOME HOSPITALS WITHIN ADAMAWA STATE IN NORTH-EASTERN NIGERIA

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ABSTRACT

Dengue is a major public health concern throughout tropical and sub-tropical regions of the planet. The actual numb of dengue cases is underreported and many are wrongly classified. The study therefore aimed at establishing the seroprevalence of Dengue virus (DENV) infection in Adamawa state. The study was a cross-sectional study where socio-demographic data and blood samples were collected from three selected government public health facilities. The blood samples were allowed to clot, the sera were separated and screened for dengue virus using ELISA. Data were analyzed using Statistical Package for Social Sciences version 20 (SPSS 20). Of the 600 participants, 65% (390/600) had IgM to dengue virus indicating infection with Dengue virus. The IgM was detected with the highest prevalence in Mubi (81.5%: 163/200) while Numan (52%: 104/200) had the lowest. The distribution of DENV infection according to gender shows more exposure in males (66.79%: 181/271) than females (63.61%: 201/316) though the difference was not significant ($P<0.05$). Individuals between the age group 11-20 years had the highest prevalence (67.39%: 62/92) of DENV infection while the lowest was recorded among those who were aged 51 and above (59.34%: 54/91). The high seroprevalence of IgM to Dengue virus found among febrile patients attending public health facilities in Adamawa state indicates the circulation of the virus in the state and its potential endemicity in this North-eastern state of Nigeria.

Keywords: *Seroprevalence, Dengue virus, Febrile Patients, Adamawa, Nigeria*

INTRODUCTION

Dengue virus (DENV) is one of the important mosquito-borne viral diseases in humans (1). It is transmitted by infected *Aedes aegypti* which also serves as a vector for the transmission of other flaviviruses such as the yellow fever virus (YFV), Zika, and Chikungunya viruses. These mosquito species are abundant in Northeastern Nigeria (2). Dengue virus is a positive sense, single-stranded RNA virus of the family Flaviviridae which is comprised of more than 70 viruses. The viruses have four antigenically distinct serotypes, DENV 1- 4 (3). The DENV has a broad geographic range, circulating on every continent except Antarctica (4). Infection may be asymptomatic and symptomatic patients may present with fever, severe headache, backache, joint pains, nausea and vomiting, eye pain, rash, liver enlargement, and haemorrhagic manifestations (5). The incubation period is between 3-15 days and the virus appears to have no detrimental effect on the *Aedes* mosquito, which remains infected for life (6).

Infection with DENV elicits antibody production with immunoglobulin M (IgM) which develops acutely and is short-lived, while long-lasting immunoglobulin G (IgG) develops shortly thereafter (7). Dengue is a major public health concern throughout tropical and sub-tropical regions of the world. Population growth, a sudden increase in migration, globalization, poverty, climate change, and urban slums are factors that contribute to vector breeding and the rise in dengue outbreaks (8). The actual number of dengue cases is underreported and many cases are misclassified. One estimate indicates that 390 million dengue infections occur per year of which 96 million clinically manifest globally (9). Another study on the prevalence of dengue estimates that 3.9 billion people in 129 countries are at risk of the infection (9).

Several reports also, suggested that climate change could swell up dengue transmission by 2080s to additional 2 billion people worldwide, in the absence of changes in all other determining factors (10). Also, it has been estimated that about 500,000 people with severe dengue such as Dengue Haemorrhagic Fever and Dengue Shock Syndrome (DHF and DSS) require hospitalization each year and an estimated 2.5% of those affected would die (11).

The distribution of the dengue fever infection in places like Borno State has been reported: however, few works have documented the infection due to the dengue virus in Adamawa state, North-Eastern Nigeria (11,17). The study aimed at establishing the prevalence of DENV in Adamawa State and determining the demographic factors associated with the

infection. This is hoped to provide stakeholders with the data needed to proffer solutions and initiate appropriate control measures.

MATERIALS AND METHODS

Study Area and study Population

The study was carried out in three purposively selected Local Government Areas (LGAs) in Adamawa State (Figure 1). Adamawa state is located between latitude 9°14′N and longitude 12°28′E. The state is traversed by large Rivers; Benue, Gongola, and Yadzarem thus, prone to flood water and swampy terrain, especially during the rainy season (12). The study areas are also characterized by high population movement due to upheavals in the Northeast with many people storing water around their homes and dumping solid waste in the open gutter and close to water bodies, which provide a suitable environment for the breeding of *Aedes* species and interaction with humans (13). The study populations included both male and female febrile patients of all ages, assessing health services at the three selected public health facilities.

Ethical Clearance and Data Collection

Ethical approval for the study was obtained from the Ethical Committee of the Adamawa State Ministry of Health and the University Ethical Committee on the Use of Human and Animal Subject for Research, ABU, Zaria (Ref. N0. S/MoH/1131/1). The questionnaire was used to obtain data on socio-demographics, possible risk factors, and clinical information. The questionnaire was piloted around the actual communities of study and administered by trained interviewers.

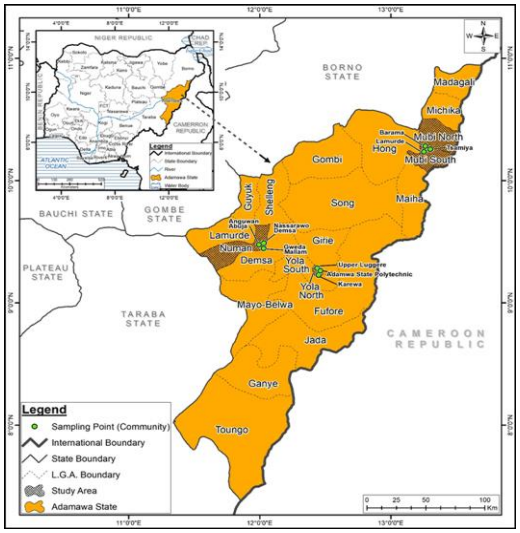

Figure 1 Map of Adamawa State showing Study Area
Source: Modified from the Administrative Map of Adamawa State

Inclusion and Exclusion Criteria

All consented patients of all ages, presenting with symptoms of fever (>38°C), severe headache, neck and back pain, and abdominal pain attending the selected hospitals were included in the study. On the other hand, patients who did not give their consent to participate and those who did not present with fever or febrile condition were excluded.

Sample Size Determination

The sample size used for the study was determined using the equation $n = Z^2(1-p)/2d^2$ of Cochrane as modified by Kogi (14) and DENV prevalence of 74.4%

obtained from previous studies in Borno (15). The blood sample size calculated for dengue was 196; however, a total of 600 samples were used for the study.

Blood Samples Collection and Processing

Five millilitres (5ml) of blood were collected with the help of trained research assistants (phlebotomists) from consenting patients attending the selected public health facilities by venepuncture, the samples were collected in clean labelled sample bottles and allowed to clot at room temperature (30^{o}C). To obtain serum, each blood sample was separated by centrifugation at 1000rpm for 10 minutes and stored at -20°C (16).

Detection of IgM Antibodies by ELISA

All serum samples were screened for dengue virus according to the manufacturer's protocols using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Diagnostics Automation/Cortez Diagnostics, Inc. Woodland Hills, USA). Samples and reagents were brought to room temperature (15-25°C) before use. A 25ml wash buffer (20x) was diluted with 250ml of reagent-grade water. A 96-well microplate was used. The wells were labelled as follows: a reagent blanks well, 2 controls, and samples wells. Hundred micro litters (100µl) of controls, samples, and 40µl Rheumatoid Factor (RF) absorbent were dispensed into the microplate wells and thoroughly mixed. The plate was incubated for 10 minutes at room temperature. The contents were shaken and washed 3 times with the diluted wash buffer and 2 drops of enzyme conjugate were added to each well. Exactly 100µl of Enzyme Conjugate was added to each well and incubated at room temperature for 10 minutes. The plate was vigorously washed 3 times with the wash buffer and blot dried on an absorbent paper. Two (2) drops of substrate Tetramethyl-benzidine (Chromogen) were then added to each well, and incubated for 10 minutes at room temperature. Two (2) drops of stop solution were added to each well and mixed appropriately by gently tapping the strip holder. The Optical Density (O.D) of each well was read by setting the microplate reader wavelength at 450nm. (AccuDiag™ Dengue IgM ELISA Kit-Catalog Number: 8117-35).

An Optical Density ratio of 0.3-1.0 was interpreted as Negative, but when greater than 1.0 was interpreted as a positive result. The negative result indicates that there is no detectable antibody in the specimen while the positive result revealed the formation of a specific antibody against DENV.

Data Analysis

The data obtained from the questionnaire and the result of the laboratory tests were analyzed using the Statistical Package for

Social Sciences version 20 (SPSS 20). Odds ratios (OR) for the association of variables with Dengue virus infection were analyzed using Epi-Info^{TM}. The results were presented using charts and tables. A P value of ≤ 0.05 was considered significant at a 95% confidence interval.

RESULTS

Serological results were available for 600 individuals that participated in the study, 200 each from Mubi, Yola, and Numan. Of the 600 participants, 65% (390/600) were IgM positive for dengue virus infection (Figure 2). The highest prevalence was observed in Mubi (81.5%: 163/200) and the lowest (52%:104/200) in Numan (Figure 3). There was a higher prevalence among male patients (66.79%: 181/271) than females (63.61%:201/316) but the difference was not statistically significant ($\chi^2 = 0.518$, p=0.472; OR = 1.151; 95%CI= 0.818-1.619) (Table 1).

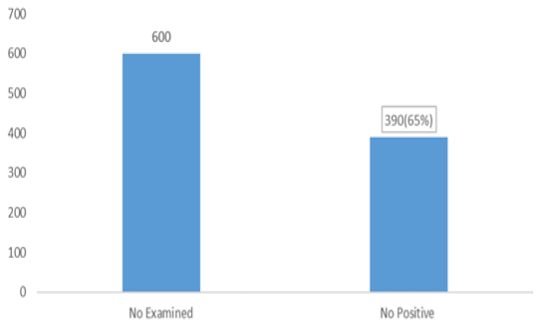

Figure 2: Prevalence of Dengue virus infections in Adamawa, North-East Nigeria

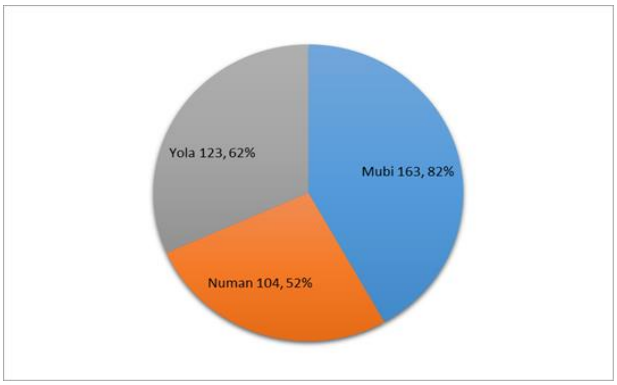

Figure 3: Distribution of Dengue virus infection in relation to the study area

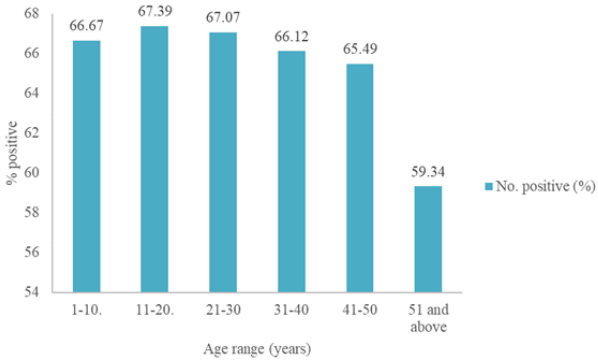

Figure 4: Distribution of Dengue virus infection with respect to the age group of the study population

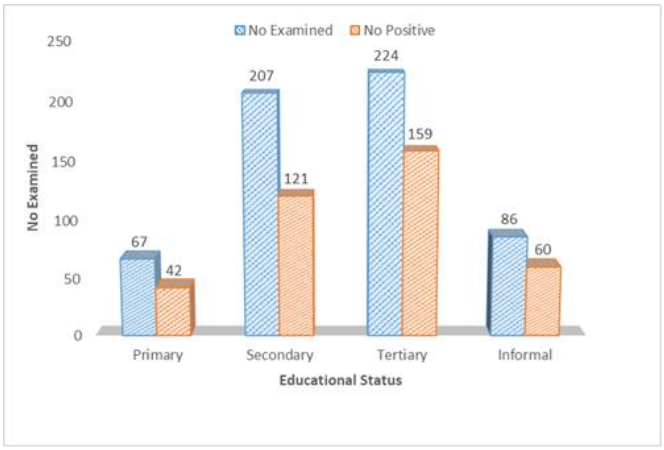

Figure 5: Distribution of Dengue Virus infection in Respect to the Educational Status of the Study Population

Table 1: Distribution of Dengue virus infection by demographic factors among febrile patients in Adamawa state

Dem. Factor	Total	No. Positive (%)	χ^2	P-value	Odd Ratio	95%CI
Gender						
Male	271	181 (66.79)	0.518	0.472	1.151	0.818-1.619
Female	316	201 (63.61)	0.518	0.472	0.869	0.618-1.223
Total	**587**	**382 (65.08)**				
Occupation						
C/Servant	165	114 (69.09)	0.976	0.323	1.239	0.841-1.829
Unemployed	141	84 (59.57)	2.799	0.094	0.701	0.473-1.039
Business	119	76 (63.87)	0.138	0.711	0.902	0.591-1.376
Farmers	82	52 (63.41)	0.122	0.726	0.888	0.546-1.447
Others	56	44 (78.57)	3.948	0.047	2.036	1.048-3.953
Total	**563**	**370 (65.72)**				
Marital status						
Married	367	247 (67.30)	1.273	0.259	1.244	0.875-1.769
Singles	215	134 (62.3)	1.273	0.259	0.804	0.565-1.142
Total	**582**	**381 (65.46)**				

Key: Dem. = Demographic

There was a marked difference in the distribution of DENV infection by age group as shown in Figure 4. Individuals within the age group 11-20 years had the highest prevalence (67.39%: 62/92) while the lowest IgM antibody was detected in those who were 51 and above (59.34%:54/91). In terms of the level of education, IgM to Dengue fever was detected with the highest prevalence (70.98%; 159/224) among those with tertiary education (Figure 5).

Based on occupation, IgM to Dengue fever was detected with the highest prevalence of 78.57% (44/56) among patients engaged in other occupations, and the lowest prevalence of 59.57% (84/141) was observed among the unemployed. There was no significant difference (χ^2 = 1.273, p=0.259) between the seroprevalence of DENV infection and marital status (Table 1).

DISCUSSION

In this study conducted to screen IgM antibodies against dengue virus infection among febrile patients in Adamawa state, a seroprevalence of 65% was obtained indicating exposure to DENV infection. This seroprevalence obtained is high and could be due to the following reasons. Firstly, the samples were collected from febrile patients with indicative symptoms during the period of highest mosquito activities, July to October. Secondly, the insurgency in Nigeria might have forced infected persons from neighbouring states and some of the affected areas to settle in the study cities for safety leading to overpopulation and an increase in the risk of transmission. Thirdly, socio-economic activities could have encouraged trans-border transmission as Nigeria shares borders with Cameroon where DENV IgM prevalence of 61.4%, 24.2%, and 9.8% have been reported in the following towns, Douala, Garoua, and Yaounde in Cameroon (22).

The 65% seroprevalence obtained in this study is high compared to 0.5% earlier reported in Borno (17), 51.9% reported in Kaduna State (18), 35% reported in Ibadan (19), 30.8% in Kwara State (20) and 17.2% reported in Ogbomoso, Oyo State all in Nigeria (6). The prevalence obtained in

this study is lower than the 78.3% prevalence reported among febrile patients attending secondary health facilities in Kano (21), 77.1% reported among children with febrile illness in Nnewi, Anambra State, and 74.4% among Internally Display Persons (IDPs) in Northeast all in Nigeria (15). These variations in the prevalence may be related to the fact that infections tend to vary from one locality to another depending on the level of the associated risk factors and the seasons.

The highest seroprevalence was obtained in Mubi (82%). This corroborates the work of Isa *et al.* (23) who reported high DENV infection in *Aedes* mosquitoes in Mubi. Therefore, it can be said that DENV infection is highly endemic in the study areas and if not checked, could lead to a silent epidemic and consequently, Dengue Shock Syndrome (DSS) and Dengue Haemorrhagic Fever (DHF) complications in some patients due to secondary infection.

The difference in DENV IgM prevalence in males and females was found not to be significant, although slightly higher in males than females. This agrees with the findings of Faneye *et al.* (24), Oladipo *et al.* (6), and Chukwuma *et al.* (25) who reported a higher prevalence of DENV in males than females. The finding contrasts that of Bello *et al.* (17) who found a higher prevalence in females than males. The reason for the discrepancies obtained is unclear, but this could be due to the level of exposure of males to infected *Aedes* mosquito vectors during work or leisure. In addition, it might not be unconnected to male-female differences in disease severity in different areas (26).

Dengue affects humans of all ages worldwide and causes a significant public health problem. During the present study, a comparison was made between the different age groups. The highest seroprevalence of DENV IgM was observed in the age group 11-20, although not statistically significant. This is probably because the study included more patients within this age group. The result agrees with the findings of Sharma *et al.* (27) who found a higher prevalence in individuals between the age group 11-20 years in Delhi, India. There are reports of the high burden of the dengue virus in young children and late adolescents (23). Studies suggest that multiple serotypes exposure over time usually results in the development of immunity (multitypic immunity) in adults while the younger generation becomes completely susceptible individuals. Thus, mono-typically immune individuals are more likely to be from the younger age group (28).

In terms of the level of education, IgM to dengue fever was significantly highest among patients with tertiary education, a

finding that contrast with the report of Abdulaziz *et al.* (21) who found an association between DF with a low literacy level in Kano. However, participants with a lower level of education in this study appeared to have been less infected. The diurnal nature of the vector is likely to be responsible for this observation as most educated persons work in offices that harbour these invading mosquitoes. Thus, lower education plays a protective factor rather than a risk factor and was not related to dengue infection in this study.

The DENV IgM was significantly associated with patients who were either artisans, students, labourers, or apprentices. These individuals are more likely to encounter the mosquitos' vectors due to the nature of their occupation and daily activities. Most of these are not well educated on the danger of the diurnal biting mosquitoes; making them more susceptible to these bites and possible acquisition of the viral agents. Additionally, these categories of patients often patronize secondary health facilities. Abdulaziz *et al*. (21) reported dengue fever among the lower socio-economic class, who usually receive treatment in secondary public health facilities in Kano.

CONCLUSION

This study found the seroprevalence of DENV IgM among febrile patients attending public health facilities in Adamawa state to be 65% with the highest prevalence in Mubi (81.5%). This is an indication of the potential endemicity of DENV infection in Northeastern Nigeria. Generally, the difference in the infection in males as compared to females did not differ significantly. Higher seroprevalence of DENV IgM antibodies was observed among patients within the age group 11-20 years. Age, level of education, and marital status were not significantly associated with the presence of DENV IgM but the occupation of the participants was.

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CONFLICT OF INTEREST

The authors declare no conflicting interest regarding the publication of this paper.

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