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Prevalence of Dengue Virus Infection Among Febrile Outpatients Attending University of Maiduguri Teaching Hospital in Borno State, Nigeria

[1]T.M. Hamisu,[2]A.D. El-Yuguda,[3]M.B Abubakar,[4]Y.M. Shettima,[5]M.M. Maina, [6]M.Y. Zanna,[7]S.S. Baba,[8]A. Andrew,[9]i.C. Terhemen
Virus Research Laboratory, Department Of Veterinary Microbiology, Faculty Of Veterinary Medicine, University Of Maiduguri, P.M.B 1069 Maiduguri, Nigeria.

Abstract: *Dengue fever is a zoonotic arthropod-borne viral disease caused by Dengue fever virus (DENV) of the Genus Flavivirus and the FamilyFlaviviridaethat is endemic in Africa and beyond. The illness could be fatal especially among children and depleted patients.This study was designed to diagnose recentDengue virus infectionsamong febrile patients attending University of Maiduguri Teaching Hospital using ELISA kits for the detection of DENV IgM antibodies and NS1 antigens. Ninety one (91) venous blood samples were randomly collected from patients attending Maiduguri Teaching Hospital for malaria test between January and May, 2016. The samples were analysed using Enzyme Linked Immunosorbent Assays for dengue virus IgM antibodies (manufactured by Inverness Innovations Australia Pty Ltd) and for Dengue virus NS1antigens (manufactured by Bio-Rad, France). The results showed a prevalence rate of 37.4% for DENV IgM antibodies and 9.9% for DENV NS1 antigens, with 3.3% of the subjects testingpositive for both IgM and NS1 antigen. Females were observed to have higher IgM prevalence rate of 41% and males showed higher NS1 antigen prevalence rate of 11.1%. The highest prevalence rate of 76.9% was recorded for IgM in the age bracket of 1-14 years. Samples from urban areas have the highest IgM antibodyprevalence rate of 41.4%, however, rural dwellers have the highest NS1 antigen prevalence rate of 11.3%. The presenting complaint with the highest IgM prevalence rate of 50% was headache + fever + nausea, while headache only had the highest NS1 antigen of 22.2%. The highest prevalence rate of 47.4% for IgM and 50% for NS1 antigen were recorded in March and May, 2016 respectively. This study therefore showed a high prevalence of IgM to Dengue fever virus indicating its circulation among febrile patients attending University of Maiduguri Teaching Hospital. Due to the probable misdiagnosis and subsequent treatment of dengue fever with other diseases such as malaria, dengue fever is shown to be prevalent in the study area. It is therefore recommended that febrile patients attending Maiduguri Teaching Hospital should be tested for dengue fever virus.*

Keywords: *Dengue Fever Virus,Enzyme linked Immunosorbent assay,IgM antibodies, NS1 antigen, Febrile patients,University of Maiduguri Teaching Hospital, Nigeria*

I. Introduction

Dengue fever is a mosquito-borne viral infectioncaused by Dengue virusbelongingto genus *flavivirus*, in thefamily *Flaviviridae*. Dengue virus (DENV) containsnon-segmented, positive sense single stranded RNA genome [1]. The dengue virus (DENV) has four distinct serotypes. While infection with one DENV serotype typically provides livelong immunity against the same serotype, it does not provide lasting immunity against the other serotypes [2]. Dengue fever virus is primarily transmitted by *Aedes eagypti* [3]. The virus is maintained in the forests of Southeast Asia and Africa by transmission from female *Aedes* mosquitoes of species other than *Aedes aegypti*to her offspring and to lower primates [4].Clinical manifestations associated with dengue fever ranges from subclinical infection to the potentially fatal dengue shock syndrome [5]. Dengue infection is also associated with anomalies in certain haematological and biochemical parameters. Specifically, leucopenia, thrombocytopaenia, haemo-concentration and elevated serum transaminases can be present in acute dengue virus infection [6]. The dengue viruses are now arguably the most important arthropod-borne viruses from medical and public health perspective [7]. Currently, about 96 million of the estimated 390 million infections that occurred annually in the world, have some clinical manifestations [8]. Around 2.5 billion people in the world are at risk of infection as more than 100 countries are affected by dengue outbreaks [9]. The laboratory diagnosis of dengue is achieved by detecting either viral components or host antibodies mounted against the virus. Thus, for patients presenting early (day 1-7 of illness), the detection of viral non-structural protein 1 (NS1) in sera is recommended [10]. Dengue-specific antibodies of diagnostic importance are immunoglobulins M and G (IgM and IgG). In primary infections, dengue-specific IgM can be detectable as early as day 4 of illness and dengue-specific IgG by the fourteenth day [10; 11]. In secondary infections both IgG and IgM appear as early as day 2 of illness [11].

Dengue is usually not among the differential diagnoses of acute febrile illness in most African countries because, among other reasons, malaria is the most prominent endemic febrile illness in Africa and does not require complex clinical and laboratory diagnostic facilities [12; 13]. Since the first isolation of DENV in Nigeria [14], there has been massive under-reporting of this infection possibly due to unavailability of sufficient diagnostic tools in the health institutions [15] and low awareness by health care providers despite reports of the virusbeen actively circulating in various parts of the country[16; 15].Many patients with fever are designated as having fever of unknown origin or malaria or typhoid and remain without a diagnosis even if they fail to respond to antimalarial or antityphoid drugs. Under these prevailing practices, there is a real potential of misdiagnosing dengue fever [17; 18]. This studywastherefore undertaken to determine the prevalence of dengue fever in the study area.

II. Materials And Methods

Sample Collection

Ninety one venous blood samples were collected into sterile plain vacutainer tubes from febrile outpatients attending University of Maiduguri Teaching Hospital from the month of January to May, 2015. The samples were kept at room temperature and sera were harvested by centrifugation at 2,000 revolutions per minute for 5 minutes. The harvested sera were stored in cryotubes at -20 ^{0}C until tested.

Laboratory Analyses

Enzyme linked immunosorbent assay (ELISA) kits were used for the detection of dengue virusIgM antibodies and NS1 antigen according to the manufacturer's protocol.Briefly, a concentrated pool of dengue 1-4 antigens was diluted with antigen diluents. This is followed by an addition of equal volume of the horseradish peroxidase (HRP) conjugated monoclonal (MAb) antibody. The antigen-MAb complex was added to the assay plate and incubated. A substrate system, TMB chromagen, was added to the microwells after washing the plate. The reaction was stopped after adding stop solution (1N H_2SO4). A colour development after stopping the reaction was an indication of positive test for anti-dengue IgM antibodies in the samples. For dengue NS1 antigen, both samples and controls were incubated simultaneously with the conjugate for 90 minutes at 37 ^{o}C in the microplate wells sensitized with MAb. Formation of MAb-NS 1-MAb/peroxidase complex is an indication of presence of NS1 antigen in the samples and is demonstrated by distribution in each well of a chromogenic solution which initiated a colour development reaction. The enzymatic reaction was stopped by adding stop solution (1N H_2SO4) after 30 minutes of incubation. The optical density reading obtained was proportional tothe amount of NS1 antigen in the sample. The presence of NS1 antigen in an individual sample is determined by comparing the optical density reading of the sample to the optical density of the calibrator.

III. Data Analyses

Results were presented in tables and charts.All the samples with index value of <0.9 were considered negative, while samples with index values of 0.9-1.1 and >1.1 were considered equivocal and positive respectively for dengue IgM ELISA test. For dengue NS1 antigen, samples with ratio value of <0.5 were considered negative (or nonreactive); samples with ratio value of $0.50 \leq$ ratio < 1.00 were considered equivocal to dengue NS1 and samples with ratio value of ≥ 1.00 were considered positive (reactive to dengue NS 1 antigen).

IV. Result

Out of the 91 samples collected,34 (37.4%) samples were positive for DENV IgM and 9 (9.9%) were reactive toDENV NS1 antigen.The gender distribution of the positive samples showed the females had DENV prevalence rates of 41.1% for IgM and 9.6% for NS1 antigen, while the males had22.2% IgM and 11.1% for NS1antigen (Table 1). Table 2 shows the distribution of DENV IgM and NS1 antigen prevalence rates based on age. The age group 1-<15 years recorded the highest prevalence rate (76.9%) for IgM, while the age group 15-<30 had the highest DENV NS1 antigen prevalence. The IgM prevalence rate for DENV among the subjects was observed to be higher among the extreme age groups of 1-<15 (76.9%) and ≥ 60 (59%), followed by the 15-<30 (40%) and 45-<60 (31.8%), and the least was in the 30-<45 (16.7%). However, the DENV NS1 antigen prevalence was highest among the age group 15-<30 (20%), followed by the 30-<45 (8.3%) and 1-<15 (7.7%). The distribution of DENV prevalence rates among different localities showed the rural areas has 35.5% and 11.3% for IgM antibodies and NS1 antigens respectively, while urban areas recorded 41.4% and 6.8% for IgM antibodies and NS1 antigens respectively (Table 3). No statistical difference ($p<0.05$) noted in the prevalence of DENV among the two localities. Table 4 shows patients with different categories of complaint among which those presenting with headache + fever + nausea recorded the highest IgM prevalence rate of 50%, while the highest NS1 antigen prevalence rate of 22.2% was recorded in patients with chief complaint of headache only. The optical density readings for the ELISA IgM antibodies and NS1 antigens showed the positive samples with

OD values 05 -< 1.0 have 58.2% and 86% frequencies for IgM and NS1 antigen respectively, by the OD values 1.0 - < 1.5 (Figure 1). Whereas the NS1 antigen positive samples gave OD values of up to ≤ 1.5, the IgM antibodies positive samples showed OD values of up to 2.5. The prevalence rates of DENV depicted by IgM antibodies only, NS1 antigen only or combined IgM antibodies and NS1 antigens is presented in table 5. The samples showing IgM antibodies only scored 37.4% , NS1 antigen only 9.9% and combined IgM antibodies and NS1 antigen 3.3%. The monthly distribution of the DENV prevalence rates showed the month of March recorded the highest prevalence rate for IgM antibodies of 47.6%. For NS1 antigen, the highest prevalence of 50% was recorded in May (Table 6).

Table 1: Prevalence of dengue IgM and NS1 antigen base on sex distribution among febrile patients attending University of Maiduguri Teaching Hospital (UMTH)

Sex	No. Tested	No. (%) positive for IgM	No. (%) positive for NS 1
Male	18	4 (22.2)	2 (11.1)
Female	73	30 (41.1)	7 (9.6)
Total	91	34 (37.4)	9 (9.9)

Table 2: Prevalence of dengue IgM and NS1 antigen based on age bracket among febrile patients attending University of Maiduguri Teaching Hospital (UMTH)

Age (in years)	No. Tested	No. (%) positive for IgM	No. (%) positive for NS1
1- <15	13	10 (76.9)	1 (7.7)
15-<30	30	12 (40)	6 (20)
30-<45	24	4 (16.7)	2 (8.3)
45-<60	22	7 (31.8)	0 (0)
≥ 60	2	1 (50)	0 (0)
Total	91	34 (37.4)	9 (9.9)

Table 3: Prevalence of dengue virus IgM and NS1 antigen based on location among febrile patients attending UMTH

Location	No. Tested	No. (%) positive for IgM	No. (%) positive for NS1
Rural	62	22 (35.5)	7 (11.3)
Urban	29	12 (41.4)	2 (6.9)
Total	91	34 (37.4)	9 (9.9)

Table 4: Prevalence of dengue virus IgM and NS1 antigen based on clinical complains presented among febrile patients attending UMTH

Complains	No. Tested	No. (%) +ve for IgM	No. (%) +ve for NS1
Headache	9	4 (44.4)	2 (22.2)
Headache + fever	38	16 (42.1)	1 (2.6)
Headache + fatigue	38	11 (29.0)	5 (13.2)
Headache + fever + nausea	6	3 (50.0)	1 (16.7)
Total	91	34 (37.4)	9 (9.9)

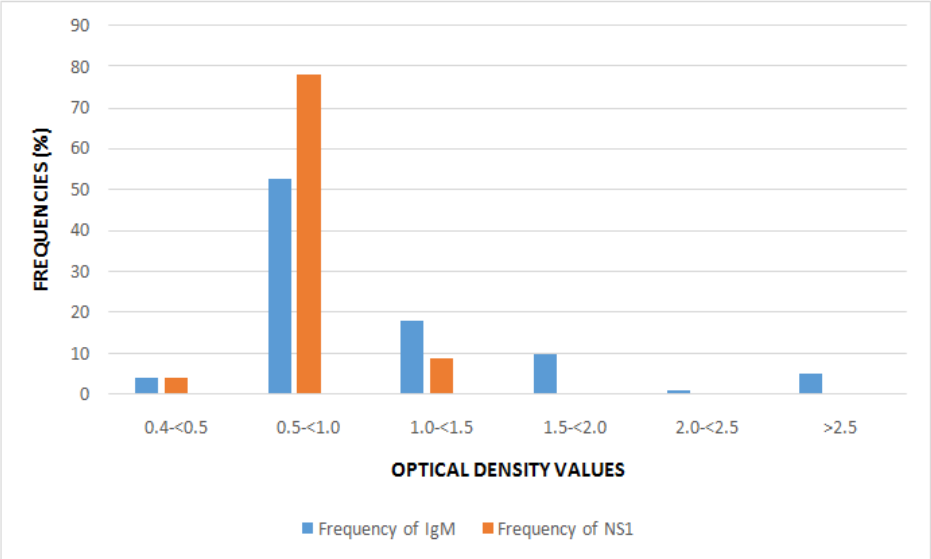

Figure 1: Histogram showing prevalence of dengue virus IgM and NS1 antigen based on recorded optical density among febrile patients attending

Table 5: Prevalence of dengue virus monotypic and polytypic infection with IgM, NS1 and IgM + NS1 among febrile patients attending UMTH

IgM antibodies only	NS1antigen only	IgM + NS1
34 (37.4%)	9 (9.9%)	3 (3.3%)

Table 6: Monthly distribution of dengue virus infection among febrile patients attending UMTH

Months	No. Tested	No. (%) positive for IgM	No. (%) positive for NS1
January	20	5 (25)	5 (25)
February	22	10 (45.5)	0 (0)
March	21	10 (47.6)	1 (4.8)
April	22	7 (31.8)	0 (0)
May	6	2 (33.3)	3 (50)
Total	91	34 (37.4)	9 (9.9)

V. Discussion

The high seroprevalence of DENV infection among febrile patients observed in this study goes to show the endemicity of DENV infection in the study area. This finding has further supported the previous reports of misdiagnosis of this disease among patients in the study area. The DENV IgM antibody prevalence rate of 37.4% recorded in this study is higher than the17.2% reported by Oladipo *et. al*.[18] in a study conducted in Ogbomosho, Nigeria, 30.8% reported by Faneye *et al*.[19] and the 0.6% reported by Baba *et al.*[20] all in Nigeria. However, this result is lower than the 51.9% observed by Bello *et al*.[21]. In addition, this study recorded a prevalence rate of 9.9% for DENV NS1 antigen that is higher than the 2.2% reported in Jos, Nigeria [16] and 5% in Mali [22] and also lower than the 35% in Ibadan Nigeria[23].These differences could be due to differences in the geographical locations of the study sites and the time of the year this study was carried out. Although this study was carried out during the dry season between January and May, 2016, however lack of rainfall doesn't preclude presence of stagnant water bodies occasioned by poor drainage and waste disposal systems conditions that favour the Aedes spp. mosquito to breeds in this area.

The socio-demographic characteristics of the subjects under studyrevealed that females showed higher prevalence rate of 41% for DENV IgM as compared to the males who recorded higher prevalence rate (11.1%) for DENV NS1 antigen.The higher IgM prevalence recorded in females is in agreement with Bello *et al.*[21] who reported a prevalence rate of 51.09% in females against 48.9% in males in Kaduna state, Nigeria. However, this observation contrasts that of Oladipo *et al.* [18] who reported a higher prevalence in males than females. The differences in prevalence rates between the genders could be attributed to differences in sample size in the different studies. The higher prevalence of dengue NS1 in males than females reported in this study is in disagreement with Dawurung *et al.*[16] who reported higher prevalence of dengue NS1 in females than males in Jos, Nigeria.The age bracket 1-14 years recorded the highest prevalence rate (76.9%) for DENV IgM antibodies. This result, although higher than the 28.6% reported by Oladipo *et al*.[18] and 35% by Garg *et al.*[24], tallies with their observation on the age bracket that recorded the highest prevalence rates. The findings of this study also agrees with the reports of Shah *et al*.[25] and Anderson *et al.*[26]who also observed a higher prevalence of dengue virus among age group 1-15 years. The higher prevalence in this age bracket may not be unconnected with the outdoor activities of this age group. The age bracket 15-29 has the highest NS1 antigen prevalence rate (20%) among the various age brackets sampled.

In this study, subjects from urban areas recorded the higher DENV IgM antibody prevalence rate of 41.4%. This is in agreement with Oladipo *et al.*[18]in Nigeria and Collenberg *et al.* [27] in Burkina Faso, who documented higher DENV prevalence rates among urban dwellers. This could be related to poor urbanization planning and poor sanitary conditions associated with cities in most African countries[14]. However, the highest prevalence rate of NS1 antigen (11.3%) observed in this study was from rural areas. This may not be unconnected with their exposure due to various farming activities in which rural dwellers are known for.

Result from this study showed that patients with multiple complaint of headache + fever + nausea recorded the highest IgM prevalence rate of 50% and those with complaint of headache alone had the highest prevalence rate (22.2%) of DENV NS1 antigen. Although differences between these results may likely be influenced by the differences in the number of patients tested per complaint, but it shows that many cases of dengue fever virus may be misdiagnosed for other ailments. The result of this study indicates that up to 3.3% of the subjects were suffering from DENV infection as at the time of collecting the samples. This is evidenced by the detection of DENV IgM antibodies and NS1 antigens in same subject. It was also observed in this study that the cases of DENV infection is increasing in the study area, going by previous studies that showed the infection to occur only during the rainy season [15]. The authors suggest that Public enlightenment and periodic nationwide surveillance and screening of febrile patients for dengue fever be carried out.

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