

Full Length Research Paper

Sero-prevalence of dengue type-3 Virus among patients with febrile illnesses attending a tertiary hospital in Maiduguri, Nigeria

Idris A. N.^{1*}, Baba M. M.², Thairu Y.¹, Bamidele O.²

¹Medical Laboratory Department, University of Abuja Teaching Hospital, Gwagwalada, F.C.T Abuja.

²Department of Medical Laboratory Science, College of Medical Sciences, University of Maiduguri, Nigeria.

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Biomedical researches available to date indicate that Dengue viruses (DENV) could be among the etiologies of acute fevers in Nigeria. Dengue viruses are mosquito-borne and exist in four serotypes (DENV1 to DENV4) which immunologically do not cross protect but cross react. Dengue fever is clinically difficult to diagnose especially in the developing countries with no established diagnostic facility and could easily be mistaken for malaria and/or typhoid etc. The objective of this study is to determine whether Dengue Type-3 virus is circulating in this community; to determine its significance in febrile illnesses and also to encourage periodic surveillance work on the viral infection. The method adopted was: 256 serum samples from suspected cases of malaria and/or typhoid were collected from patients seeking for medical assistance at the University of Maiduguri Teaching Hospital, Nigeria. Cell culture-based Microneutralization assay was used to test all the sera for DENV-3 neutralizing antibodies. Out of 256 samples 26 (10.1%) had neutralizing antibodies to DENV-3 virus. Among the seropositive patients, prevalence of DENV-3 antibodies was significantly higher in female patients (18.5%) compared to males (6.3%). The highest antibody titre recorded in this study was 1:320 while the lowest was 1:10. Majority of the seropositive patients were ≥ 20 years (14.5%). Co-circulation of dengue virus with malaria and/typhoid in North-eastern Nigeria has been suspected. The findings of this study suggest the need to conduct further research on dengue virus in order to confirm its involvement or exclude it from being responsible for febrile illnesses.

Key words: Dengue type-3 virus, microneutralization assay, dengue haemorrhagic fever, dengue shock syndrome, Maiduguri.

INTRODUCTION

Biomedical researches available to date indicate that Dengue viruses (DENV) could be among the aetiologies of acute fevers in Nigeria. Dengue virus (DENV) is a small, non-segmented, single stranded, positive-sense, enveloped RNA virus measuring 50nm in diameter. Comprise of four closely related but antigenically distinct serotypes (DEN-1 to -4) which immunologically do not cross protect but cross react.

They belong to the genus Flavivirus, family Flaviviridae

(Messer, 2003). They are Arthropod borne or more appropriately mosquito-borne (day-biting *Aedes aegypti* and *Aedes albopictus*). It's a pathogen that causes Dengue Fever, Dengue Haemorrhagic Fever and Dengue Shock Syndrome by virtue of secondary (or heterotypic) infection and subsequent antibody enhancement process (Domingo et al., 2006). First reported and confirmed epidemics were in 1780 by American scientist Benjamin Rush (Halstead 2009). Rush coined the term "break-

*Corresponding author E-mail: eedris888@yahoo.com. Tel: +2348030522324

bone" fever to describe the intense symptoms reported by one of his patient (Halstead 2009).

Current estimate shows that 50 to 100 million infections occur worldwide each year with 100,000 patients being hospitalized. The dengue viruses are now arguably the most important arthropod-borne viruses from medical and public health perspective (Rothman, 2000).

In Africa, Dengue virus was first isolated in Ibadan, Nigeria around 1960 (Amarasinghe et al., 2011). Since then there have been several reports of isolated outbreaks of Dengue infection till date although previous reports have indicated massive under-reporting of this infection possible due to unavailability of sufficient diagnostic tools in our health institutions (Baba and Talle, 2011).

The inadequate drainage system and indiscriminate solid waste disposal close to households in Maiduguri metropolis has led to the presence of stagnant water bodies and water collected in tin (and other metals) container and vehicle tires etc. which serve as breeding sites for this mosquito vectors and indeed some being the agents of dengue virus transmission.

The increase in the number of susceptible individuals in this part of the country has increased the risk of human to mosquito transmission; hence mosquito to man transmission is already on the border scale. With these, there may be possible association between dengue type-3 virus infections with the present rise in malaria/typhoid fever cases reported to most healthcare centers (CDC, 2012).

MATERIALS AND METHODS

Study Area

This is a descriptive cross-sectional study that was carried out in the W.H.O national polio reference laboratory, University of Maiduguri Teaching Hospital, Borno State, Nigeria. The study was approved by the Ethical Research Committee of the University of Maiduguri Teaching Hospital, Nigeria. Maiduguri, the capital city of Borno state Nigeria, is situated at the North-East of Nigeria sharing borders with neighboring countries such as Niger Republic, Chad and Cameroon. It also shares borders with neighboring states such as Adamawa, Yobe and Gombe states. This city is in the Sahel savannah with high temperature climatic condition for almost seven months of the year and very little rainfall.

Study Population

Serum samples of patients who sort for laboratory investigations of malaria parasite and salmonellae widal reaction tests were recruited into this study. A total sample size of 256 sera was collected from these patients within the period of March, 2011 to November, 2011.

Sample size

The sample size was determined using the following equation as described by (Naing et al, 2006):

$$n = \frac{z^2 P (1 - P)}{d^2}$$

Where n = sample size for sera.

z = statistics for a level of 95% confidence interval = 1.96

P = Prevalence rate = 0.67% (Baba et al., 2009)

d = precision (allowable error) = 5%=0.05

$$\text{Thus } n = \frac{(1.96)^2 \times 0.67 (1 - 0.67)}{(0.05)^2} = \frac{3.8416 \times 0.67 \times 0.33}{0.0025} = 339$$

The calculated sample size was 339. But due to tight security situation in the study area, a total of 256 samples could be collected from the test subjects in Maiduguri, Nigeria.

METHODOLOGY

Specimen collection and storage

5ml of Peripheral blood was collected from the patients by venipuncture into a new sterile plain container. The blood sample was appropriately spun to separate the serum from the cells. Each of the separated serum measuring up to 1 ml was appropriately labeled and stored in an ultra-cool freezer at -76°C pending their laboratory analyses.

Determination of tcid50 of dengue type-3 virus

The procedure is performed to determine the infectious titer of dengue type-3 virus which can cause cytopathic effects (CPE) in tissue culture (BHK-21) over a reasonable period of 3 to 15 days while cells in culture remain viable. The TCID50 for dengue type-3 virus obtained was 0.1 (10TCID50). The wild dengue type-3 virus was then diluted with serum-free medium at a dilution factor of 1:10, that is, 3 ml of the virus in 27 ml of the serum-free medium.

Microneutralization test

A microneutralization assay was used to detect dengue type-3 virus antibody activities in vitro using a 96-well, flat-bottomed microtiter plates. Aliquot samples were heat inactivated at 56°C for 30 min, treated with PenStrep antibiotics and stored in a freezer for 24 h before their usage. Individual treated serum sample was dispensed into wells of each row of the microtiter plate leaving the last column as the control, that is, 88 wells used per microtiter plate.

Diluted virus was then carefully dispensed into every well containing the test serum. That is with the exclusion of the control column. Individual virus-serum mixture were subsequently added on the already propagated BHK-21 monolayer cells then sealed with non-toxic sealer and incubated at 37°C for 7 days whilst observing for cytopathic effect starting from day 3.

The wells that came down with CPE in day 3 and 4 were recorded as NEGATIVE while those with no CPE up to day 10 were recorded as POSITIVE. Consequently, those recorded as negative were sorted-out and taken for microtitration.

In microtitration, individual positive samples were serially diluted using serum-virus mixture and serum-free medium on a fresh BHK-21 cell line (starting from 1:10 to 1:320 dilutions). The microtitre plate was sealed, incubated for 15 days and observed for CPE or no CPE using inverted microscopes. Subsequently, antibody titer values are calculated. All the aforementioned procedure was done on BSCL-2 and on ice packs.

Data analysis

All generated data was analyzed using Statistical package for social sciences SPSS version 16.0 for windows, tables were used to illustrate relationship of variables and comparisons made using the

Table 1. Summary of neutralizing antibodies to dengue type-3 virus in the test subjects.

Sera	Total number of samples tested	Positive sera	Titer level of sera					
			1/10	1/20	1/40	1/80	1/160	1/320
Acute phase	256	26	2	6	8	6	2	2

Table 2. Age distribution of neutralizing antibodies to dengue type-3 in acute phase sera.

Age range	Total no. of patient tested	Positive patients	Positive sera (%)
0- 9	23	2	7.7
10- 19	42	3	11.5
20- 29	69	6	23.1
30- 39	36	14	53.8
40- 49	40	1	3.80
50- 59	27	-	0.00
≥ 60	19	-	0.00
Total	256	26	100.0

Table 3. Gender distribution of neutralizing antibodies to dengue type-3 virus in acute phase sera.

Sex	Total number tested	Positive sera tested	Positive sera tested (%)
Male	175	11	42.3
Female	81	15	57.7

Chi-square test, a p-value of <0.05 was reported as statistically significant.

RESULTS

A total of two hundred and fifty six samples were used for this research. Out of this number, 175 (68.3%) were males and 81 (31.7%) were females, none of these patients came back for convalescent serum collection. 26 (10.2%) of these acute phase sera tested positive to dengue type-3 virus, out of which 11 (6.3%) were males and 15 (18.5%) were females in a ratio of 1:3.

The highest antibody titer recorded from the work was 1:320 (in two patients) whereas the lowest antibody titer gotten was 1:10 (in two patients) as shown in Table 1.

Table 1 is a presentation of all the titer values obtained from the positive acute phase sera with the lowest titer being (1:10) and the highest titer being (1:320). The prevalence of the DENV-3 neutralizing antibodies was 10.1% with a P value <0.05. This implies that there is statistical relationship between DENV-3 and its corresponding neutralizing antibodies.

Table 2 shows age distribution with respect to the number of subjects positive to neutralizing antibodies to dengue-type 3 virus. From Table 2, individuals in the age group 30-39 had the highest incidence of infection, 14

(53.8%). While none of the individuals in the age range 50 ≥ had the neutralizing antibodies.

Table 3 shows the Gender distribution of Dengue type-3 virus neutralizing antibodies among patients with febrile illnesses in Maiduguri. From the table female subjects recorded a higher prevalence rate with 15 seropositivity (18.5%) while male recorded 11 seropositivity (6.3%) in a ratio of 3:1.

DISCUSSIONS

The results of this work with a seroprevalence of 10.1% are indicative of potential endemicity of Dengue type-3 virus infection in Maiduguri. This is a significant public health finding as this supports previous hypothesis of low clinical detection rate of Dengue in potentially endemic regions in Nigeria due to clinical oversight and lack of appropriate diagnostic facilities (Baba and Talle, 2011) with a prevalence of 0.5% and more recently by Faneye et al., (2013) where they demonstrated DENV seroprevalence of 30.8%.

The prevalence of Faneye et al., (2013) was overwhelmingly high as compared to ours because their result was spread across all the serotypes while ours was only on one serotype (DENV-3). It is thus worthy of note that Dengue viruses has been shown to be actively

circulating in various parts of Nigeria (Dawurung et al., 2010; Baba and Talle, 2011) but, there is paucity of published work on DENV-3 in Nigeria and Africa at large.

With regards to gender distribution, the prevalence of DENV-3 antibodies in females (18.5%) was significantly higher than in males (6.3%), (P value > 0.05). Considering chi-square statistical analysis, there is no statistical relationship between DEV-3 antibodies and gender distribution (P value > 0.05). This data is in agreement with previous research (Shu-yuan, 2007).

Similarly, higher prevalence of DENV-3 neutralizing antibodies was observed in age group ≥ 20 years as compared to younger age groups, this value is in agreement with some past studies done on dengue such as that of Teixeira et al. (2008) who reported on recent shift in age pattern of dengue hemorrhagic fever in Brazil likewise by Fagbemi et al., (1977). This is expected because the mosquito vector for Dengue which is responsible for the transmission of the infection *Aedes aegypti*, is a predominantly day biting, outdoor vector, as a result adults are at higher risk of infection because they spend majority of their time outdoors.

CONCLUSION AND RECOMMENDATION

Co-circulation of dengue type-3 virus with malaria and/typhoid in North-eastern Nigeria has been suspected. The findings of this study suggest the need to conduct further research on dengue virus in order to confirm its involvement or exclude it from being responsible for febrile illnesses. Our inability to get paired sera for the analysis is regretted as this would have given insight into the ability to demonstrate a four-fold increase in rising titer between acute and convalescent sera as suggested by WHO, (2007).

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