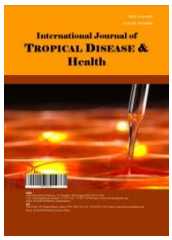

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Detection of Dengue Virus IgM Seropositivity and Malaria Co-infection among Individuals Resident on the Banks of River Niger in Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NIM, UKC, CGO and COM designed the study of this research, performed the research for the manuscript. Authors UKC, COM, MPO, NIM and CGO performed the analyzed data, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Dengue virus (DENV) is the cause of Dengue fever and it is a mosquito-borne, RNA virus. This study was aimed at determining Dengue virus Immunoglobulin M seropositivity and malaria co-infection among residents of the river Niger Banks.

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Materials and Methods: A cross sectional study consisting of 96 subjects was performed. The subjects were recruited using the convenience sampling technique. Ethical approval was obtained and informed consent was sought from study participants. Questionnaire was administered to determine basic demographic information. Dengue Virus IgM was analysed using ELISA techniques. Malaria parasitaemia was detected using microscopy while packed cell volume (PCV) and haemoglobin (Hb) analysis were evaluated using manual methods. Statistical analysis was performed using the statistical package for social sciences (SPSS) version 21.

Results: The results showed that the prevalence of Dengue virus and malaria seropositivity among the study population was 17.7% and 36.5% respectively. The incidence of malaria was higher in those who were dengue virus negative (33.3%) than in those who tested positive to Dengue virus (3.1%). Dengue virus seropositive patients had significantly lower PCV (37.5±3.7) and Hb (12.9±1.14) compared to those who were negative to dengue virus, 38.8±3.5 and 13.3±1.17 respectively.

Conclusion: Our report has revealed that dengue virus is an emerging cause of febrile illness among the study population. This could be due to the nature of their environment which supports the breeding of different species of mosquito. This calls for urgent intervention and large scale research to confirm the circulating strains of the Dengue virus.

Keywords: Dengue virus; Aedes mosquito; malaria; Immunoglobulin M; River Bank.

1. INTRODUCTION

"Dengue virus (DENV) is the cause of dengue fever. It is a mosquito-borne, single positive-stranded RNA virus of the family *Flaviviridae*; genus *Flavivirus"* [1]. "Dengue fever is caused by four Dengue virus serotypes, namely dengue virus 1, 2, 3, and 4 (DENV 1–4)" [1]. A new serotype, DENV-5 was announced in October, 2013 [2]. "The *Aedes aegypti* and *Aedes albopictus* are the principal dengue mosquito vectors. Despite serologic evidence of DENV infections in several countries, the burden of dengue remains poorly documented across Africa" [3]. "In Nigeria recent reports suggest that DENV could be a major cause of acute fevers, although many people presenting with fever to health facilities get treated with an antimalarial without confirmatory tests due to the overlap in symptoms between malaria and dengue. The virus is transmitted to humans through the bite of infected female mosquitoes, though humans are not capable of transmitting the disease and are not contagious" [4]. "Only one vaccine for dengue is currently approved in 11 countries including Mexico, the Philippines, Indonesia, Brazil, El Salvador, Costa Rica etc. Developing a vaccine against the disease is challenging" [5]. "There are four different serotypes of the virus that can cause the disease and the vaccine must immunize against all four types to be effective [6]. Malaria is the most important tropical parasitic infection in humans all over the world, and very common in developing countries especially in the tropical zones. Malaria is caused by the Plasmodium parasite" [7]. The parasite can be transmitted to humans by infected mosquitoes during blood meal. Malaria has been found to be much prevalent among residents of River Niger banks due to flooding and poor hygienic state of the environment and a consequent increased rate of mosquitoes in the community [8]. So far, there has not been any report of confirmed outbreak of Dengue Fever among residents of River Niger banks, but due to the increasing reports of recurrent fever and high rate of mosquitoes in the area, the need arises to assess the rate of exposure and prevalence of Dengue Fever and malaria parasitaemia among residents of River Niger banks. Also, there is paucity of information regarding the seroprevalence of Dengue virus immunoglobulin M among residents of river Niger banks in Anambra state. This research was therefore an attempt to estimate the prevalence of current DENV infections and malaria parasitaemia among residents of River Niger banks in Anambra state and to understand the risk factors associated with DENV infection.

2. MATERIALS AND METHOD

2.1 Study Design

A cross sectional study was conducted to determine the presence of Dengue virus IgM antibody in exposed individuals along the River Niger banks, Anambra State, as well as possible co-infection with malaria. The subjects were chosen by a convenience sampling technique.

2.2 Study Area

"The Okpoko community in Ogbaru Local Government Area (LGA) of Anambra state was chosen as the study area for this research. Ogbaru is one of the Local Government Areas in Anambra State and consist of several towns as well as sub-towns and villages that made it up. Towns that make up the local government are Atani, Akili-Ogidi, Akili-Ozizor, Amiyi, Mputu, Obeagwe, Ohita, Odekpe, Ogbakugba, Ochuche Umuodu, Ossomala/Ossomari, Ogwu-aniocha, Umunankwo, Umuzu, Okpoko, Ogwu Ikpele. Ogbaru has its local government headquarters in Atani. The Okpoko town was selected for this study. Ogbaru People are traditionally fishermen, farmers as well as known warriors from its history. The Ogbaru people also share clan lineage and boundaries with its people in Asaba, Delta state and Ndoni Rivers. The Ogbaru people consider River Niger waters that run through its lands as their territorial lands. Ogbaru land is neighbored by Onitsha, a major commercial city in South-Eastern Nigeria. The Ogbaru LGA is a known flood-prone area as a result to its nearness to River Niger as well as its low and flat topography with slope angles of 1^{o}-3^{o}" [9].

2.3 Study Population

A total of 96 subjects were recruited for this study which comprised of apparently healthy individuals both males and females who are aged 15 years and above, residing in Okpoko community, Ogbaru LGA, Anambra State.

2.4 Duration of Study

The study was carried out over a period of three months, from August to October.

2.5 Specimen Collection and Storage

About 5 millilitres of venous blood was collected aseptically by a vene-puncture from each consenting subject and dispensed into sterile ethylene di-amine tetra-acetic acid (EDTA) anti-coagulant containers. Each specimen was labeled with the subject's initials and laboratory identification number. Blood specimens were immediately transported to the laboratory. The packed cell volume (PCV), haemoglobin estimation as well as thick film for malaria microscopy were carried out and the remaining blood specimens were separated by low-speed centrifugation at 500 x g for 5 minutes. The plasma for Dengue virus estimation was aseptically transferred into labeled sterile cryovials and stored at -20^{0}C until ready for analysis. Specimens were processed within a week of collection and were analyzed at the Molecular Research Laboratory, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

2.6 Method of Specimen Processing

The Enzyme Linked Immuno-Sorbent Assay (ELISA) technique was used to screen for Dengue virus-specific IgM antibody in the samples. The ELISA kit was procured from the Melsin Medical Company Limited, Kuancheng District, Changchun 130000, Jilin Province, China. The test was carried out according to manufacturer's instructions. The optical density (OD) or absorbance was read at 450nm using a microtitre plate reader.

"Malaria parasites were tested for by preparing thick and thin blood films which were stained with 3% Giemsa for 30 minutes and allowed to air-dry and viewed under the microscope using the ×100 objective magnification. The haemoglobin level was estimated using the haemoglobincyanide (HiCN) technique. About 20µl (0.02 ml) of the blood samples were dispensed into 4 ml of Drabkin's neutral diluting fluid, mixed and allowed at room temperature for 4-5 minutes, protected from sunlight, to allow for lysis of red blood cells and release of haemoglobin. The absorbance of the lysates was read colorimetrically at 540nm wavelength. The reference range for men is 13.0 – 18.0g/dl and that of women is 12.0 – 15.0g/dl [10]. The packed cell volume (PCV) of the subjects was also determined. Here, anticoagulated blood in a glass capillary of specified length, bore size, and wall-thickness was centrifuged in a microhaematocrit centrifuge at RCF 12000 xg for 5 minutes to obtain a constant packing of the red cells. The PCV value was read using the microhaematocrit reader. The reference range for men is 0.40 – 0.54 l/l (40.0 – 54.0%) and 0.36 – 0.46 l/l (36.0 – 46.0%)" [10].

2.7 Data Analysis

The various results obtained were presented in tables. Data were presented as percentages and mean ± standard deviation. The statistical package for social science (SPSS) version 21, was used for data analysis. Simple prevalence and chi-square analysis were used where

necessary. Pearson correlation was used to compare some results. The level of significance was set at 95% and 0.05 confidence interval.

3. RESULTS

Table 1 summarizes the demographic characteristics of the respondents. Majority, (69.8%), of the respondents were females, while 30.2% were males. About 42.7% of the respondents were between the ages of 15-25 years, 27.1% were between 26-35 years, 16.7% were between 36-46 years, while 13.5% were 47 years and above. The respondents who had secondary education, (58.3%), were the dominant group followed by those with primary education (19.8%) and those with tertiary education were (13.5%), while those with no formal education form the least group (8.3%). Majority, (50.0%), of the participants are traders, followed by students, (28.1%). About 9.4% of the participants were Artisans, while 6.3% were farmers and 6.3% were civil servants. The table also shows that 47.9% of the respondents were single, 45.8% were married, 4.2% were widowed, while 2.1% were divorced.

Fig. 1 shows that the prevalence of Dengue virus seropositivity and malaria parasitemia among the study population. DENV seropositivity (detectable IgM antibody to Dengue virus by ELISA) is 17.7%. About 82.3% were seronegative for the virus (no detectable IgM antibody to dengue virus by ELISA). About 36.5% of the respondents were malaria positve, while 63.5% were negative.

Table 2 shows the Chi-square analysis indicating association between Dengue virus - malaria co-infection. The incidence of malaria was higher in those who are Dengue virus seronegative (33.3%) than in those who are Dengue virus seropositive (3.1%). Chi-square test indicated non-significant association ($p = 0.062$) between Dengue virus and malaria co-infection.

Table 1. Demographic characteristics of the respondents (n = 96)

Characteristics	Frequency	Percentage (%)
Age		
15-25 years	41	42.7
26-35 years	26	27.1
36-46 years	16	16.7
47 years & above	13	13.5
Gender		
Male	29	30.2
Female	67	69.8
Level of Educational		
No formal education	8	8.3
Primary	19	19.8
Secondary	56	58.3
Tertiary	13	13.5
Marital Status		
Single	46	47.9
Married	44	45.8
Widowed	4	4.2
Divorced	2	2.1
Occupation		
Artisan	9	9.4
Trader	48	50.0
Civil servant	6	6.3
Farmer	6	6.3
Student	27	28.1
Total	96	100

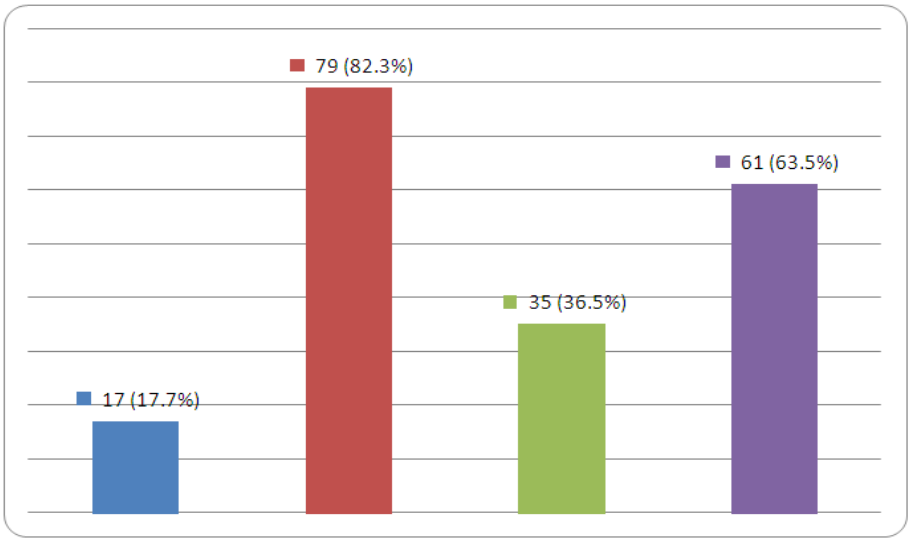

Fig. 1. Seroprevalence of Dengue virus and malaria parasitaemia among residents of the River Niger banks

Table 2. Association of dengue virus and malaria co-infection

Subjects' Dengue virus status	Malaria		Total (%)	Chi square test		
	Positive (%)	Absent (%)		X^2	df	P-value
Seropositive	3(3.1)	14(14.6)	17(17.7)	3.47	1	0.062
Seronegative	32(33.3)	47(49.0)	79(82.3)			
Total	**35(36.5)**	**61(63.5)**	**96 (100)**			

Table 3 compares the blood parameters of the Dengue virus positive and Dengue virus negative subjects. Independent t-test indicated that subjects who had dengue virus had significantly lower PCV (p = 0.000) and Hb (p = 0.000). Result is presented as mean ± standard deviation.

4. DISCUSSION

In this study, the Dengue virus-specific IgM was detected in 17 out of the 96 sampled individuals who may have been exposed to *Aedes* mosquito bite by reason of the characteristic predisposing unhygienic environment of communities close to river banks. The Okpoko community of Ogbaru LGA of Anambra State was selected as the study area. The prevalence percent was 17.7% as shown in the bar plot. This finding is in contrast to the study carried out by Chukwuma et al. [11] who investigated "the Dengue virus seroprevalence among children with febrile illness in Nnewi, Nigeria and reported a prevalence rate of 77.1%. This present study was carried out among predominantly adult population whom are apparently healthy. A similar study carried out in Cross River state, Nigeria indicated that 46 (25.7%) of the 179 had detectable IgM antibodies to Dengue virus" [3]. "Also a study of Dengue virus and malaria co-infection in febrile subjects in Ilorin, Nigeria shows that 46.0% of the total subjects enrolled in the study were positive for Dengue virus IgM antibodies" [12]. "However, lower serological prevalence has been reported also in Nigeria and beyond". A study by Idoko et al. [13] on "the serological survey of Dengue virus Immunoglobulin M among febrile patients in Kaduna metropolis, Nigeria, showed 1.8% prevalence. A similar study carried out in clinically suspected patients in Nepal showed 8.5%" [14]. "This indicates that the serological prevalence of Dengue detected in this study is significant enough to be seen as a public health concern. Recent reports suggest that Dengue is a growing public health problem in Nigeria, the magnitude of which needs to be more clearly defined" [15].

The prevalence of malaria in this study is 36.5%. However, of the 17 subjects who were seropositive to DENV, only 3(3.1%) of them tested positive to malaria. The incidence of malaria was higher in DENV seronegative subjects. Chi-square test shows non-significant

Table 3. Comparison of the blood parameters of the Dengue virus seropositive and Dengue virus seronegative patients

Variables	Dengue Virus Negative	Dengue Virus Positive	t-test	P-Value
PCV	38.8±3.56	37.5±3.73	41.2	0.000
Hb	13.3±1.17	12.9±1.14	46.6	0.000

association between Dengue virus and malaria concurrent infection in this study, as the p-value was higher than the alpha level (Σ^2, 3.47; p = 0.062) as seen in the second table. In contrast, the study by Otu et al. [3] reveals a 20.7% DENV and malaria concurrent infection among their sampled population in Cross River, Nigeria. Also, the study by Chukwuma et al. [11] shows that "about 86.8% of their sampled population had malaria and Dengue virus co-infection". "However, in a study by Osarumwense et al. [16] on the prevalence of Dengue virus and malaria co-infection among HIV-infected patients within South-Eastern Nigeria, 13.02% of the subjects were seropositive for DENV, 55.8% were positive for plasmodium spp. and only about 2.7% were positive for both Dengue virus and plasmodium spp. which is in tandem with the findings in this study". Kolawole et al. [12] also reported about 2.84% prevalence for concurrent Dengue and malaria infections. In another study carried out in an epidemic district in India, about 1,980 blood samples were collected to check for DENV seroprevalence and malaria parasitaemia and only 22(3.0%) cases were identified as Dengue – malaria co-infection cases [17]. These as well support our finding in this study.

Also, the blood parameters (PCV and Hb) were significantly reduced (p = 0.000) in subjects who were seropositive to DENV than the seronegative ones as shown in the last table. This finding is validated by that of Chukwuma et al. [11]. In their study, the red blood cell (RBC) and Hb values were significantly reduced in the infected subjects. This, therefore, suggests the tendency of infected subjects being prone to anaemia. In a study by Ferede et al. [18], low Hb level was observed, but there was increased haematocrit (PCV). Achalkar [19] and Mohd and Shamin [20] also reported an increased haematocrit among Dengue virus positive patients in their independent studies. This might, according to Ferede et al. [18], be related to increased severity and is elucidated by the haemoconcentration due to increased intravascular plasma permeability which is the basic pathophysiological change in Dengue. However, the low PCV (haematocrit) found in this study may indicate a high prevalence of anaemia in the study area, especially with a significant malaria parasitaemia detected in the study, given the environmental condition of the area which supports mosquito breeding, as validated by the study carried out by Ogalue et al. [8] in the study area.

5. CONCLUSION

In conclusion, this study revealed a significant DENV-IgM seroprevalence among the study population which could be one of the causes of acute undifferentiated fever among residents of River Niger banks Anambra state. Dengue fever is a rare occurrence, as well as Dengue – malaria co-infection. So detecting this prevalence level, especially in this vulnerable population calls for a serious public health concern. The implication of this is a potential risk of Dengue Shock Syndrome (DSS) as multiple serotypes could be circulating among the human population in the study region putting them at risk of immune mediated DSS when previously infected persons become reinfected with a heterologous serotype [21]. This study recommends that Nigerian government should initiate Dengue virus surveillance and commence an integrated vector control programme especially in slums and communities at river banks where the swampy nature of their environment encourages mosquito breeding, and also provide resources at hospital laboratories in Nigeria to facilitate early diagnosis and management of dengue patients. Also, physicians should consider the possibility of dengue cases when examining febrile patients.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

CONSENT AND ETHICAL APPROVAL

Informed consent was sought and obtained from the subjects prior to data and sample collection. Well-structured questionnaires were used for data collection. Ethical approval for this study was obtained from the ethics committee of the College of Health Sciences, Nnamdi

Azikiwe University in accordance with the Helsinki declaration by the World Medical Association on the ethical principles involving human subjects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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