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Seroprevalence of dengue virus among children with febrile illness in Nnewi, Nigeria

Chukwuma GO[1], Audu JS[1], Chukwuma OM[1], Manafa PO[1], Ebugosi RS[1], Akulue JC[1], Aneke JC[2], Ahaneku GI[3], Nchinda GW[4], Esimone CO[5]

1 *Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus*

2 *Department of Haematology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

3 *Department of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

4 *Chantal Biya International Reference Center for Management and Prevention of HIV/AIDS Yaounde Cameroun*

5 *Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State Nigeria*

Abstract

Background: Dengue fever is regarded as the most important arboviral disease worldwide. **Aims and Objectives**: This study was designed to investigate the prevalence of denque virus seropositivity among children with febrile illness at the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. **Study Design**: A cross sectional study consisting of 96 subjects was performed. The subjects were recruited using the systematic sampling technique. Ethical approval was obtained from the Ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi and informed consent was sought from study participants. **Setting**: This study was conducted at the Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State Nigeria. **Materials and Methods**: The demographic data and dietary pattern of the subjects was obtained using well structured questionnaire. Dengue Virus IgM was analysed using ELISA techniques. Malaria parasitaemia was determined using microscopy techniques while Haematological parameters were evaluated using haematology auto analyser (PE-6800 fully auto haematology analyser Procan). **Statistics**: Statistical analysis was performed using the Statistical package for social sciences (SPSS) version 24. **Results**: The results showed a prevalence of 77.1% Dengue Virus seropositivity among children with febrile illness in the study population with a greater prevalence found among the males (54.1%) than female (45.9%) subjects. The dengue virus seropositive participants had significantly greater IgM levels compared with the seronegative participants (2.02 ± 0.76 vs 0.84 ± 0.28; $p < 0.001$). Children who had dengue virus had significantly greater WBC ($p = 0.017$), esinophil ($p<0.001$), but lower RBC (<0.001), Hb ($p = 0.001$) and basophil ($p = 0.001$) compared with dengue virus negative children. The result showed a strong positive association between anemia and dengue viral status (χ^2, 6.31; $p = 0.012$) with dengue virus seropositive partcipants at greater risk (OR, 3.52; $p = 0.015$) of developing anemia compared to those who had no dengue virus. More so, the incidence of anemia was higher in those who had malaria and dengue virus co-infection (86.8%) compared with those who had no malaria and were Dengue virus negative (7.5%) and those who presented with malaria but had no Dengue virus (5.7%). Furthermore, the result indicates a significant association ($p = 0.005$) between dengue virus seropositivity and malaria co-infection with anemia. **Conclusion**: Our report has revealed that dengue virus is an emerging cause of fever among the study population. This calls for urgent attention and large scale research to confirm the circulating strains of the dengue virus

Keywords: Seropositivity, Dengue Virus, Malaria, Anaemia, Co-infection.

***Corresponding author:**
Dr. George O. Chukwuma
Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus
Email: georgechuma[at]yahoo.com

INTRODUCTION

Dengue virus fever is an infectious disease caused by any of the four dengue virus serotypes: DENVs 1–4. It is a mosquito-borne disease and is primarily transmitted to humans by the female *Aedes* mosquito. The disease is mainly concentrated in tropical and subtropical regions, putting nearly a third of the human population, worldwide, at risk of infection [1]. Infection with Dengue virus (DENV) results in varying degrees of pathological conditions, ranging from mild asymptomatic dengue fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which may turn fatal [2]. Dengue virus fever is the most rapidly spreading mosquito-borne viral disease with an estimated incidence of 390 million cases per years [3, 4]. It is regarded as the most important arboviral disease worldwide [5] and it is estimated that every year between 2.5-3.6 billion people in over 125 endemic countries are at risk including 120 million travelers to these regions [6, 7]). About 2 million cases evolve to dengue hemorrhagic fever and about 20,000 may culminate to death [6, 8]. The first isolated case of dengue virus in Nigeria was in the 1960s [9, 10], but dengue virus fever is not a reportable disease in this country with most cases often undiagnosed,

misdiagnosed as malaria or referred to as fever of unknown origin. Dengue virus IgM seroprevalence of 30.8% was reported in Nigeria among febrile children [11], while another study in the north among healthy children revealed a seroprevalence of 17.2% [12]. Dengue virus is often mistaken for Malaria, because they have similar symptoms. In a setting where diagnostic testing is conducted, such as the GeoSentinel Surveillance Network, malaria was found to be the predominant cause of systemic febrile illness among travelers returning from sub-Saharan Africa (622/1,000 patients) compared with dengue virus (7/1,000) [13]. Many patients in Africa with fever are designated as having fever of unknown origin or malaria and remain without a diagnosis even if they fail to respond to antimalarial drugs. Under these prevailing practices, there is a real potential of misdiagnosing dengue fever as malaria. Dengue fever is usually not among the differential diagnoses of acute febrile illness in Africa. Reasons for this lack of inclusion are as follows: 1) malaria is the most prominent endemic febrile illness in Africa and does not require complex clinical and laboratory diagnostic facilities; 2) a low awareness of dengue virus fever may contribute to health care workers not considering the disease; 3) dengue virus fever is not a reportable disease in most countries in Africa; 4) dengue virus fever surveillance and diagnostics are not widely and consistently available throughout Africa; and 5) funding for surveillance and other research activities pertaining to dengue virus in Africa are limited [14]. For these reasons, improved surveillance and laboratory diagnosis of fevers in Africa is a priority and first step in assessing the incidence of dengue virus seropositivity in Africa. This study determined the seroprevalence of Dengue virus in Nnewi Nigeria. The results of this work has shown that dengue virus is a major cause of fever of unknown origin in the study environment. It may be important to conduct a large scale research to determine the circulating strains of the virus.

MATERIALS AND METHODS

Study Area

This study was carried out at the paediatric clinic of Nnamdi Azikiwe University teaching hospital (NAUTH) Nnewi, Nnewi North Local Government Area Anambra State Nigeria. NAUTH is a tertiary health institution serving the high, low and middle income patients. Nnewi is a commercial town that is largely populated with about 200,000 people. The official language spoken are Igbo and English. The town is divided into four major villages namely: Umudim, Uruagu, Nnewichi and Otolo. The populace are predominantly traders, house wives, students and health workers.

Research Design

This is a cross sectional study performed to determine the dengue virus seropositivity level among children with febrile illness attending paediatric clinic at the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria. The study included 96 children aged 0-5 years randomly selected from paediatric clinic of the Nnamdi Azikiwe University Teaching Hospital. Systematic random sampling was employed by selecting after every third child.

Ethical Consideration

The ethical approval for this research was obtained from the Nnamdi Azikiwe University Teaching Hospital ethical committee in accordance with the Helsinki declaration by the World Medical Association(WMA) on the ethical principles for medical research involving human subjects [15]; after which the subject were served questionnaires.

Informed Consent

Consent of the parents/guardians of the subjects were sought and obtained using an informed consent form.

Inclusion Criteria

Children with febrile illness within the ages of 0 – 5years only.

Exclusion Criteria

Children who may have febrile illnesses, but are above the age of 5years

Methods of Assay

- Dengue virus IgM ELISA: Enzyme-linked immunosorbent assay (ELISA) technique by calbiotech California USA and Mindray ELISA machine were used for the ELISA assay.
- Malaria parasite: using thin and thick film procedure.
- Full blood count: Automated

Sample Collection

About 5mls of venous blood was collected by venepuncture from the subjects, 2mls was dispensed in EDTA container for the determination of Malaria Parasite and Full blood count then 3mls of whole blood was centrifuged at 12,000rpm for 5 minutes for the dengue virus IgM ELISA assay. The sample was stored using plain container.

Dengue Virus IgM ELISA (Calbiotech California USA)

All specimens and kit reagents were kept at room temperature (18-26^{o} C) and gently mixed. The desired number of coated strips were placed into the holder. Negative control, positive control and calibrator were then ready to use. 1;21 dilution of test samples were prepared by adding 10ul of the sample to 200ul of sample diluents and mixed thoroughly. Then 100ul of diluted sera, calibrator and controls were dispensed into the appropriate wells. Inaddition, 100ul of sample diluents was dispensed for the reagent blank in 1A well position. The holder was tapped to remove air bubbles from the liquid and thoroughly mixed. It was then incubated for 20 minutes at room temperature. Fluid was removed from all wells and the wells were washed three times with 300ul of 1x wash buffer, and then blotted on absorbance paper or paper towel. Then 100ul of enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature. Enzyme conjugates were removed from all wells. The wells were washed three times with 300ul of 1x wash buffer and then blotted on absorbance paper or paper towel. Then100ul of TMB substrate was dispensed and incubated for 10 minutes at room temperature. Finally, 100ul of stop solution was added. The patients O.D was read at 450nm using ELISA reader within 15mins. A dual wavelength is recommended with reference filter of 600-650nm.

Calculation of result: Calibrator Factor (CF) value was checked on the calibrator bottle. Cut-off value= Calibrator OD X Calibrator factor (CF). Ab (antibody) index of each determination was calculated by dividing the O.D. value of each sample by cut-off value.

Interpretation: The following is intended as a guide to interpretation of dengue virus IgM test results;

<0.9	No detectable antibody to dengue virus IgM by ELISA
0.9-1.1	Borderline positive. Follow up testing is recommended if clinically indicated.
>1.1	Detected antibody to Dengue virus IgM by ELISA.

Malaria parasite using thin and thick film

Thick film: Two drops of anticoagulated blood was placed on a clean glass slide. With a corner of another slide, the drops were mixed in a circular motion over an area about two cm in diameter to prevent formation of fibrin strands that may obscure the parasites after

staining. The film was allowed to dry in air at room temperature.

Thin film: A drop of anticoagulated blood was placed at the edge of a clean slide. Using another glass slide as the spreader, the spreader was held at an angle of 45^0, the blood making contact with the edge of the spreader and pushed forward immediately making a thin film, with a good tail.

Staining of thin blood film: The slide was covered with Leishman stain for two minutes. The stain was then diluted with twice the volume of the buffer solution and then allowed to stain for ten minutes. The slide was then washed with tap water, the slide was then drained and dry in the air by keeping it in a slanting position.

Staining of thick blood film:

Here, the film was covered with 3% giemsa stain for 30minutes. The slide was subsequently washed with clean water, allowed to dry and observed and the film scored under the microscope using x100 magnification.

Estimation of Full Blood Count (FBC)

This was performed using PE-6800 fully auto haematology analyser (Procan), which Is a three part differential blood cell counter. It operates on the principle of electrical impedence as blood cells are counted and sized, while haemoglobin is determined by Colorimetric method.

Principle of cell count

The principle of the instrument is based on the measurement of changes in electrical resistance (impedence) produced by a particle passing through an aperture sensor. The sample blood (non conductive) is diluted in a conductive liquid (diluents), to produce a big difference between them. When the diluents passed through the aperture sensor, electrode are submerged in the liquid on each side of the aperture to create a continuous current. When the cells pass through the aperture, the resistance between the electrodes increases as the volume increase. Passing through the magnification circuit, the voltage signal will be magnified and the noise will be filtered, then the analytical result will be displayed. One count pool and the detection circuit count the WBC, as another count pool and detection circuit count the RBC and PLT. The microprocessor of the instrument calculates and analyses the cells (WBC, RBC and PLT) and then gives out the histogram.

Principles of haemoglobin measurement

Adding lyse in the blood, the red blood cells will rapidly be broken down and release haemoglobin. Haemoglobin and the lyse form a new mixture which can absorb the wavelength of 540nm. The concentration of sample haemoglobin is calculated by comparing the absorbency between pure diluents and the sample (haemoglobin-lyse mixture).

Sample size calculation

Sample size will be calculated using Daniel's formula [16]:

$$N = \frac{Z^2PQ}{D^2}$$

Where,
N= Sample size
P=Prevalence rate in percentage 30.8% [11]
Q = (1-p)
Z= confidence interval of 95% which is equivalent to confidence coefficient of 1.96
D=desired level of precision or significance which is equal to 0.05.

$$N = \frac{(1.96)^2 \times 0.308 \times (1-0.308)}{(0.05)^2} = 328$$

Sample Size

The study population consists of 96 children with febrile illnesses.

Statistical Analysis

Statistical package for social science (SPSS) version 24 will be employed in the analysis of the result and the data obtained for different parameters expressed as mean± standard deviation. Parameters will be compared between different groups using paired t-test table. Level of significance will be set at $P<0.05$.

RESULTS

Table 1: Demographic characteristics of the children with febrile illness (n = 96)

Characteristics	Frequency	Percent
Age		
0-6 months	20	20.8
7-12 months	10	10.4
1-3 years	31	32.3
4-5 years	35	36.5
Sex		
Males	49	51.0
Females	47	49.0
Weight*		
0 -1.9 kg	20	20.8
2.0 -3.1 kg	10	10.4
3.1 – 4.0 kg	31	32.3
>4.0 kg	35	36.5
Level of Education		
Creche	28	29.2
Pre-Nursery	6	6.3
Nursery	28	29.2
Primary	34	35.4

*Mean weight of children = 2.8 ± 1.13 kg

This table summarizes the demographic characteristics of the children with febrile illness. Majority (36.5%) of the children were between the ages of 4 – 5 years, followed by those aged 1 – 3 years (32.3%). Those age 7-12 months had the least percentage (10.4%). There were more males (51%) compared with females (49%). A greater percentage of the children weighed >4kg, followed by those weighing 3.1 – 4.0 kg (32.3%), while the least weight category was 2.0 – 3.1 kg (10.4%). Majority (35.4%) of the children were in primary school, while those in pre-nursery had the least percentage (6.3%).

Table 2: Demographic characteristics of the respondents*

Characteristics	Frequency	Percentage
Marital Status		
Monogamy	72	75.0
Single Parent	18	18.8
Divorcee	6	6.3
Level of Education		
Primary	16	16.7
Secondary	52	54.2
Tertiary	28	29.2
Socio-economic Status		
Low Income Earner	19	19.8
Average Income Earner	69	71.9
High Income Earner	8	8.3
Occupation		
Trader	60	62.5
Civil Servant	21	21.9
Others	15	15.6
Type of Accommodation		
2-4 Bedroom Apartment	25	26.0
Self-Contained Apartment	49	51.0
Single Room Apartment etc	22	22.9

*care giver

The demographic characteristics of the respondents (care-giver) are summarized in Table 2. Majority (75%) of the respondents were monogamous, while the divorcees had the least percentage (6.3%). The respondents who had secondary education (54.2%) were the dominant group followed by those with tertiary education (29.2%) and primary education (16.7%). A greater percentage (71.9%) of the respondents were average income earners, while high income earners had the least percentage (8.3%). Majority (62.5%) of the participants were traders followed by civil servants (21.9%). The major type of accommodation lived in by participants was self contained apartment (51%), while those living in single room apartment had the least percentage (22.9%).

Table 3: Selected pre-clinical findings and status of the children

	Frequency	Percentage
Feeding habit		
Breast feeding	19	19.8
Weaning	10	10.4
Solid	64	66.7
Uncertain	3	3.1
Is the child on any drug?		
Yes	65	67.7
No	31	32.3
Does the child experience high body temperature?		
Yes	96	100.0
No	0	0
The type of treatment administered for high temperature		
Paracetamol	36	37.5
Damping the body with tepid water	25	26.0
Brought him/her immediately to the hospital	32	33.3
Nothing	3	3.1
Who takes care of the child?		
Mother	56	58.3
Housemaid	31	32.3
Auxillary nurse	9	9.4

This table shows selected pre-clinical findings and status of the children. Majority of the children were fed on solid food (66.7%); were on drugs (67.7%); experienced high body temperature (100%); were administered with paracetamol due to the high temperature (37.5%); were taken care of by their mothers (58.3%).

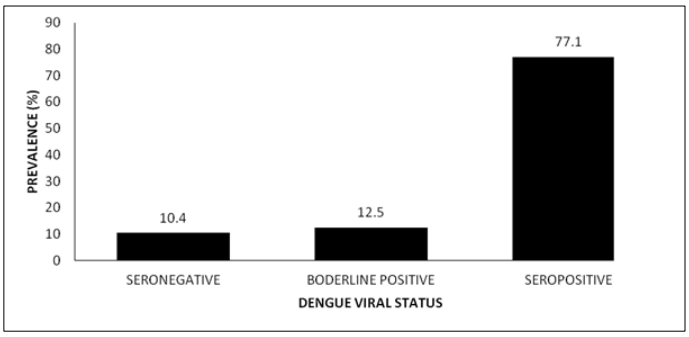

Figure 1: Seroprevalence of Dengue virus among children with febrile illness

The prevalence of Dengue Virus seropositivity among children with febrile illness is shown in Figure 1. Seventy-seven (77.1) percent of the children were dengue virus positive (detectable antibody (IgM) to dengue virus by ELISA), 12.5% fell within the borderline positive, while 10.4% were seronegative for the virus (no detectable antibody (IgM) to dengue virus by ELISA).

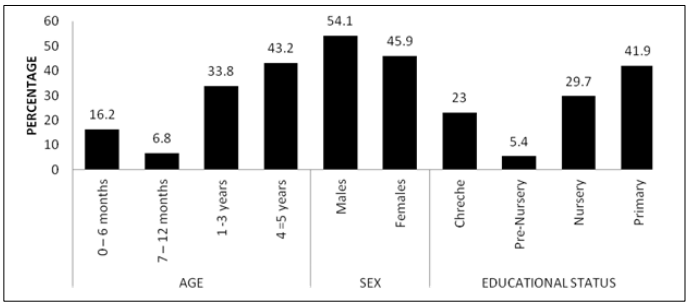

Figure 2: The incidence of dengue virus seropositivity according to age sex and educational status of the children with fibrille illness.

Figure 2 shows the incidence of dengue virus seropositivity according to age, sex and educational status of the children with fibrille illness. Seropositivity of dengue virus was most prevalent in children aged 4-5 years (43.2%); greater in males (54.1%) compared with females (45.9%); and most prevalent among primary school children (41.9%).

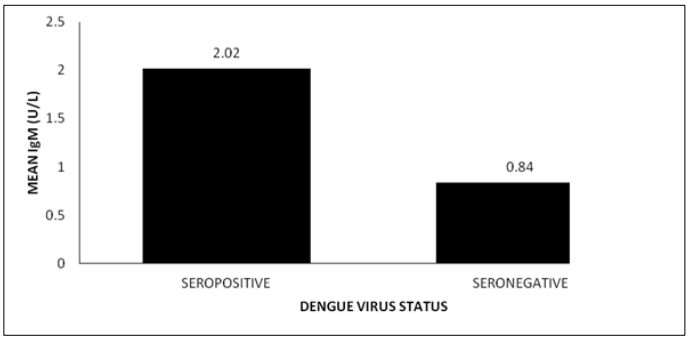

Figure 3: The mean IgM levels of the seropositive and seronegative patients

This figure shows the mean IgM levels of the seropositive and seronegative patients. Independent sample t-test indicated that the dengue virus positive patients had significantly ($p < 0.001$) greater IgM level (2.02 ± 0.76) compared with the seronegative patients (0.84 ± 0.28).

Table 4: Comparison of the blood parameters of the dengue virus seropositive and dengue virus seronegative patients

Variables	Dengue Virus Negative	Dengue Virus Positive	t-Statistics	P-Value
White Blood Cells ($x10^3$ cells/mm^3)	12.67 ± 4.66	15.42 ± 4.94	-2.43	0.017
Red Blood Cells ($x10^6$ cells/mm^3)	4.22 ± 0.81	3.51 ± 0.69	4.15	<0.001
Hemoglobin (g/dl)	12.66 ± 2.29	11.04 ± 1.98	3.37	0.001
Lymphocytes (%)	23.52 ± 2.16	23.90 ± 2.52	-0.67	0.503
Monocytes (%)	5.72 ± 0.42	5.71 ± 0.45	0.05	0.955
Neutrophils (%)	48.80 ± 7.41	47.24 ± 6.66	0.97	0.331
Esinophils (%)	1.35 ± 0.62	2.11 ± 0.48	-6.23	<0.001
Basophils (%)	0.85 ± 0.38	0.59 ± 0.31	3.29	0.001

Table 4 compares the blood parameters of the dengue virus positive and dengue virus negative patients. Independent t-test indicated that children who had dengue virus had significantly greater WBC (p = 0.017), esinophil (p<0.001), but lower RBC (<0.001), Hb (p = 0.001) and basophil (p = 0.001) compared with dengue virus negative children. In contrast, Lymphocyte, monocyte and neutrophil levels did not differ statistically between the two groups.

Table 5: Univariate analysis and multivariate logistic regression test indicating association and risk of Dengue virus seropositivity with anemia

Patients' Dengue Viral Status	Anemia			Chi Square Test			Logistic Regression Test	
	Present N (%)	Absent N (%)	Total N (%)	χ^2	df	P-value	OR (95% CI)	P
Sero-positive	46 (86.8)	28 (58.1)	74 (77.1)	6.31	1	0.012	3.52 (1.2-10.9)	0.015
Sero-negative	7 (13.2)	15 (41.9)	22 (22.9)				1	
Total	53 (100)	43 (100)	96(100)					

OR = Odds Ratio; χ^2 = Chi-square

This table shows the association of dengue virus positivity in children with febrile and the odds of developing anemia using chi square and logistic regression analyses respectively. Chi-square test shows that anemia was associated with dengue viral status (χ^2, 6.31; p = 0.012). Logistic regression shows that dengue virus seropositive patients were at greater risk (OR, 3.52; p = 0.015) of developing anemia compared to those who had no dengue virus.

Table 6: Association of dengue virus and malaria co-infection with anemia

	ANEMIA		Total N (%)	Chi-Square Test		
	Absent N (%)	Present N (%)		X^2	Df	p-value
Malaria and Dengue Virus Negative	13 (30.2)	4 (7.5)	17 (17.7)			
Malaria and Dengue Virus Positive	25 (58.1)	46 (86.8)	71 (74.0)	10.55	2	0.005
Malaria Positive and Dengue Virus Negative	5 (11.6)	3 (5.7)	8 (8.3)			
Total	43 (100.0)	53 (100.0)	96 (100.0)			

Table 6 shows Chi-square analysis indicating association between dengue virus - malaria co-infection with anemia. The incidence of anemia was higher in those who had malaria and dengue virus co-infection (86.8%) compared with those who tested malaria and Dengue virus negative (7.5%) and those who presented with malaria but had no Dengue virus (5.7%).Furthermore, Chi-square test indicated significant association (p = 0.005) between dengue virus and malaria co-infection with anemia. Logistic regression further indicated that those who had Malaria and Dengue Virus co-infection were at greater risk of anemia compared with tested malaria and Dengue virus negative (OR [95% CI], 5.98 [1.57 - 24.6]; p = 0.003), but not significantly at greater risk compared to those who presented with malaria but had no Dengue virus (OR [95% CI], 3.06 [0.57 – 17.96]; p = 0.248).

Table 7: The effect of dengue virus – malaria co-infection on hematological parameters

	Malaria and Dengue Virus Positive	Malaria and Dengue Virus Negative	Malaria Positive and Dengue Virus Negative	F – Statistics	P – Value
WBC	15.42 ± 4.94	11.38 ± 2.33	15.39 ± 7.03	4.93	0.009
RBC	3.51 ± 0.69	4.10 ± 0.54	4.47 ± 1.20	9.40	<0.001
Hb	11.03 ± 1.98	12.38 ± 1.34	13.23 ± 3.64	6.13	0.003

Analysis of variance (ANOVA) indicated significant differences in WBC, RBC and Hb among the three groups. Post-hoc multiple comparison test further indicated that Dengue Virus – malaria co-infected patients had significantly greater WBC compared with Dengue Virus – malaria negative patients (p = 0.007), but not with Malaria Positive-Dengue Virus Negative patients (p = 1.0). In addition, Dengue Virus – malaria co-infected patients had significantly lower RBC compared with Dengue Virus – malaria negative patients (p = 0.011), and Malaria Positive-Dengue Virus Negative patients (p = 0,002). Furthermore, Dengue Virus – malaria co-infected patients had significantly lower Hb compared with Malaria Positive-Dengue Virus Negative patients (p = 0.016) but not with Dengue Virus – malaria negative patients (p = 0.053).

DISCUSSION

Dengue virus fever is an important emerging disease of the tropical and sub-tropical regions today. It is clear that since last decade, dengue viral fever has been occurring regularly with periodic surges in a number of cases [17]. The differential diagnosis associated with dengue fever include a wide variety of viral which includes Chikungunya, bacterial, Rickettsial and parasitic infections that produce a similar syndrome. A definitive diagnosis is confirmed by virus isolation and/or serology [18]. In the present study, the prevalence rate of Dengue Virus seropositivity among children with febrile illness was found to be seventy-seven and one percent (77.1%). This finding is in contrast to the study carried out by [11] who investigated the serological evidence of recent dengue virus infection among febrile children in a semi-arid zone and reported a prevalence rate of 30.8%. Again, Ahmed *et al*., in Chittagong found 63% anti-dengue IgM positive and 68% anti-dengue IgG positive cases in 2017 which is different from our studies [19]. However, Nagi *et al*., in Pakistan showed 73% anti-dengue IgM positive patients in 2011 which is very similar to our findings [20]. Interestingly, from previous reports, Dengue virus has been shown to be actively circulating in various parts of Nigeria [21, 22]. There is also evidence of high vector density in densely populated Nigerian cities [23]. This combined, justifies the high prevalence rate of 77.1% recorded in our current study. Our report of 77.1% is indicative of potential endemicity of Dengue virus seropositivity in Nnewi. This is a significant public health finding as this supports previous hypothesis of low detection rate of Dengue virus in potentially endemic regions in Nigeria due to clinical oversight and lack of appropriate diagnostic facilities [22]. The implication of this is a potential risk for 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 [24]. However, the Seropositivity of dengue virus was most prevalent in children aged 4-5 years (43.2%); greater in males (54.1%) compared with females (45.9%); and most prevalent among primary school children (41.9%). This is in line with the study of [11] who reported a higher prevalence rate of Dengue virus infection in male than female children. This is however not in agreement with some past studies done on dengue such as that of [25] who reported on sero-incidence of Dengue infection in pre-school children in Brazil. With regards to Age group, a higher prevalence was observed in age group 4-5 years, in respect to other age groups. This is anticipated because the mosquito vector responsible for the transmission of the virus, *Aedes egypti*, is a predominantly day biting, outdoor vector, as a result younger children are at a lower risk of infection because they spend majority of their time indoors unlike the older children who are old enough to play and spend more time outdoors. In addition, rapid urbanisation in Africa has resulted in increase in vector density as a result of human practices that promote mosquito breeding [26]. The present study therefore, highlights the importance of proper vector control and improved sanitation practices around households and school premises. Furthermore, Independent sample t-test indicates that the dengue virus positive patients had significantly ($p < 0.001$) greater IgM level (2.02 ± 0.76) compared with the seronegative patients (0.84 ± 0.28). Again, Independent t-test indicated that children who had dengue virus had significantly greater WBC (p = 0.017), esinophil (p<0.001), but lower RBC (<0.001), Hb (p = 0.001) and basophil (p = 0.001) compared with dengue virus negative children. In contrast, Lymphocyte, monocyte and neutrophil levels did not differ significantly between the two groups. These findings may be explained by the fact that the immune system of the infected subjects triggers the production of more anti-bodies in order to combat the invading foreign bodies in the subjects. More so, the RBC and Hb values were significantly reduced in the infected subjects which suggests the tendency of infected subjects being prone to anaemia. Interestingly, the result shows that anemia was associated with dengue virus seropositivity (χ^2, 6.31; p = 0.012) and that dengue virus seropositive patients were at a greater risk (OR, 3.52; p = 0.015) of developing anemia compared to those who had no dengue virus. Again, the incidence of anemia was higher in those who had malaria and dengue virus co-infection (86.8%) compared with those who tested malaria and Dengue virus negative (7.5%) and those who presented with malaria but had no Dengue virus (5.7%).There is also a significant association (p = 0.005) between dengue virus and malaria co-infection with anemia. Logistic regression further indicates that those who had Malaria and Dengue Virus co-infection were at greater risk of anemia compared with tested malaria and Dengue virus negative (OR [95% CI], 5.98 [1.57 - 24.6]; p = 0.003), but not significantly at greater risk compared to those who presented with malaria but had no Dengue virus (OR [95% CI], 3.06 [0.57 – 17.96]; p = 0.248) Finally, analysis of variance (ANOVA) indicates significant differences in WBC, RBC and Hb among the three groups. Post-hoc multiple comparison test further indicates that Dengue Virus – malaria co-infected patients had significantly greater WBC compared with Dengue Virus – malaria negative patients (p = 0.007), but not with Malaria Positive-Dengue Virus Negative patients (p = 1.0). In addition, Dengue Virus – malaria co-infected patients had significantly lower RBC compared with Dengue Virus – malaria negative patients (p = 0.011), and Malaria Positive-Dengue Virus Negative patients (p = 0,002). Furthermore, Dengue Virus – malaria co-infected patients had significantly lower Hb compared with Malaria Positive-Dengue Virus Negative patients (p = 0.016) but not with Dengue Virus – malaria negative patients (p = 0.053).

CONCLUSION

We have reported sero-prevalence of acute Dengue virus infections in children, from Nnewi North, Anambra State, Nigeria for the first time. Our report has revealed that Dengue virus seropositivity may have reached high levels among this population of children, which is indicative of potential endemicity of the infection in the general population of Eastern Nigeria. This finding calls for urgent government attention into the prevailing factors responsible for this observed

trend, as well as immediate control measures and public health preventive action against this disease in other to prevent emergence of the more severe forms of Dengue virus disease, such as DHF and DSS among the population of southeast Nigeria. Inaddition, large scale research needs to be done to determine the circulating serotypes and strains of the virus in the study environment. This will help to determine if routine screening for dengue virus should be included in diagnosis of fever of unknown origin.

Conflict of interest

The authors declare that there is no conflict of interest.

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Research constraints

The unavailability of funding was a major drawback in this research. The sample size had to be reduced due to unavailability of research grant.

Author's contribution

C.G.O., A.J.S., C.O.M., designed research, A.J.S., M.P.O., E.R.S., A.J.C., A.J.C., performed research, A.G.I., C.G.O., analysed data, C.G.O., N.G.W., E.C.O., wrote paper.

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