Original Research Article

DETERMINATION OF DENGUE VIRUS IGM SEROPREVALENCE, MALARIA POSITIVITY AND SOME HAEMATOLOGICAL PARAMETERS OF HIV INFECTED INDIVIDUALS IN AWKA, NIGERIA.

Abstract:

Dengue fever is regarded as an important neglected Arboviral disease worldwide. This study aimed at investigating the prevalence of dengue IgM seroprevalence, malaria positivity and some haematological parameters of HIV infected individuals attending Chukwuemeka Odumegwu Ojukwu Teaching Hospital Amaku, Awka, Nigeria. A cross sectional study consisting of 188 participants was performed. The demographic data and possible risks factors of the subjects were obtained using well-structured questionnaire. Dengue virus IgM was analysed using ELISA techniques, Malaria parasitaemia was determined using microscopy technique while some haematological parameters were evaluated using heamatology auto analyser (PE-6800 fully auto heamatology analyser procan). Statistical analysis was performed using the statistical package for social (spss) version 25. The result of this study showed that the prevalence of Dengue virus was 38 (20.2%) while that of Malaria was 70(37.2%). The co-infection of Dengue virus IgM antibodies and Malaria parasitemia was 16(8.51%). *Plasmodium falciparium* was seen as the only specie of malaria parasite present in the study. The results of some haematogical parameters of the Dengue virus seropositive participants as compared with the Dengue virus seronegative participants were statistically significant with neutrophils ($p = 0.035$), mean cell hemoglobin concentration (MCHC = $p<0.013$), eosinophils (EOS = $p<0.001$) and mean corpuscular volume (MCV = $p<0.001$). This study suggests that Dengue maybe the emerging cause of fever of unknown origin among populations. This calls for urgent attention, adoption of immediate control measures and public health preventive actions against the disease so as to curb or mitigate the emergence as well as reduce the morbidity and mortality resulting from dengue burden especially in the immunocompromised individuals.

Key words: Dengue virus, Malaria, HIV, Co-infection, Seroprevalence, Immunoglobulin M, Haematological parameters

1. INTRODUCTION:

Dengue virus fever is a mosquito-borne tropical disease caused by the dengue virus [1]. Dengue virus fever is caused by four closely related dengue virus strains 1–4 (DENV 1–4), which are positive-sense, single-stranded RNA viruses that belong to the family Flaviviridae of the genus Flavivirus [2]. Dengue virus has a non-segmented, positive-strand RNA genome of about 10,700 nucleotides with a 5' cap structure and a non-polyadenylated 3' end [3]. The main arthropod vectors for the transmission of the dengue virus (DENVs) are Aedes aegypti and Aedes albopictus which are now known to be extensively spread in both tropics and subtropics [4]. Dengue fever is the most common arthropod-borne and neglected tropical disease which affects over 2.5 billion people at risk of infection worldwide [5]. Infection may be asymptomatic or symptomatic in which case patients may present with dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) [6, 7]. The incubation period is

between 3-15 days and symptomatic patients may present with headache, myalgia, arthralgia, retro-orbital pain, rash, and leucopenia [8]. Early symptoms of Dengue Fever and Dengue Hemorrhagic Fever are indistinguishable, but Dengue Hemorrhagic Fever is associated with hemorrhagic manifestations, plasma leakage, and thrombocytopenia [9]. Dengue Shock Syndrome on the other hand is distinguished from DHF by the presence of cardiovascular compromise, which occurs when plasma leakage into the interstitial spaces results in shock. Dengue Shock Syndrome is a fatal condition with mortality rates as high as 20% in developing countries with limited resources [6]. It is thought that infection with one serotype provides lifelong immunity against that serotype but confers only partial or transient protection against subsequent infection with any of the other three serotypes (Halstead 1988).

More than 2.5 billion people reside in dengue endemic areas worldwide, where approximately 390 million people are infected with a mortality rate surpassing 5–20% in some areas [10, 11]. In those countries, about 50 – 100 million new cases occur annually [12]. The first isolated case of dengue in Nigeria was in the 1960s [13], but dengue is not a reportable disease in Nigeria with most cases often undiagnosed, misdiagnosed as malaria or referred to as fever of unknown cause. Dengue IgM seroprevalence of 30.8% was reported in Nigeria among febrile children [15], while another study in the northern part of Nigeria among healthy children revealed a seroprevalence of 17.2% [15]. The World Health Organization considers dengue as a major global public health challenge in the tropical and subtropical nations [16]. Dengue has seen a 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel and lack of health care facilities [17].

Dengue is endemic in the tropical and subtropical areas of the world, where human immunodeficiency virus (HIV) is pandemic. Dengue and HIV infections might provide an opportunity for exchange of genetic materials and mutations resulting in the emergence of strains with enhanced disease severity. Antibody cross reactivity by viruses of the flaviviridae family may also affect accurate serological diagnosis. The diagnosis of dengue is usually based on its history and its clinical profiles especially haematological investigations. The laboratory hallmarks of dengue include thrombocytopenia, atypical lymphocytosis and hemoconcentration. Despite the overlapping epidemiology, knowledge on the differential clinical manifestations and disease severity between DENV-HIV co-infected and DENV patients is limited. Only a handful of case reports and case series have been published thus far [18]. The dengue virus has been considered an important flavivirus that exhibits a protective role against HIV by transiently inhibiting its replication [19].

Dengue fever is clinically difficult to diagnose, especially in developing countries with no established dengue diagnostic reagents/equipment, and could easily be mistaken for malaria, typhoid fever or pyrexia of unknown origin due to frequent nonspecific illnesses exhibited by HIV-infected individuals [20]. However, there is paucity of research on the clinical presentations and outcomes of dengue infection in HIV-infected persons. This study was conducted to determine the Dengue Virus IgM seroprevalence, malaria positivity and some haematological parameters of HIV infected individuals in Awka, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra state, south east Nigeria. COOUTH is a tertiary health institution serving the high, low and middle income patients. Anambra state was created on 27th August 1991 with population of 4,177,828 and a land mass of 4844 square kilometres. The state capital (Awka) has a land mass of 120 kilometres square and estimated population of about 306,657 according to the 2006 National population census by National Population Commission. The official languages spoken are Igbo and English. The

populace are predominantly civil servants and students though we still have traders, artisans, farmers and also health workers in Awka.

2.2 Study Population

The study population includes 125 HIV positive persons and 63 HIV negative persons seeking medical assistance at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka. The research was conducted in the medical/virology laboratory of the University Teaching Hospital, Awka.

2.3 Sampling Technique and Sample Size Determination

The study samples were randomly collected from the HIV infected individuals and non HIV infected individuals as they come on their appointment dates for their routine check- ups. Sample size was calculated using Daniel's formula [21];

$$N = Z^2PQ / D^2$$

Where,

N= sample size

P= Prevalence rate in percentage 6% [22]

Q= (1-p)

Z= confidence interval of

D= desired level of precision or significance which is equal to 0.05.

$$N = \frac{(1.96)^2 \ X \ 0.06 \ X \ (1-0.06)}{(0.05)^2} = 87$$

Sample size: The study population consists of 188 patients (125 HIV positive and 63 HIV negative patients).

2.4 Research Design

This was a cross sectional study that determined the Dengue Virus IgM seroprevalence, malaria positivity and some haematological parameters among HIV positive and negative individuals attending clinics at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra state, Nigeria.

2.5 Ethical Approval

Ethical approval/clearance for the study was sought from the Health Ethics and Research Committee of the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka. Permission to conduct the study was obtained from the Microbiology Department of Nnamdi Azikiwe University Awka, Anambra State, Nigeria, while oral and written consent was obtained from the respective subjects/clients that presented in the COOUTH's out patients Department and HIV clinic during the course of this study.

2.6 Data Collection

A well-structured and validated questionnaire was given to each of the subjects whose consent were sought before collecting their blood samples. The questionnaire helped to obtain detailed information about the individual's demographic and clinical data.

2.7 Sample Collection

One hundred and eighty eight (188) clinical specimens (venous blood samples) were collected aseptically from 125 HIV positive and 63 HIV negative patients seeking medical attention at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka using a 5mls plastic syringe each and taken to the laboratory for examination within one hour. Two (2) mls of the blood was dispensed carefully in the EDTA container for determination of malaria parasite and Full blood count while the remaining 3mls of the blood was centrifuged at 12,000rpm for 5 minutes and used for the dengue virus IgM ELISA assay.

1.8 Methods of Assay

- Dengue virus IgM ELISA: This was done using the technique by Calbiotech California USA and Mindray ELISA machine was also used for the assay.

- Malaria parasite: This was done using thin and thick film procedures.
- Full blood count: This was done using PE-6800 fully auto haematology analyser (Procan).

2.8.1 Dengue virus IgM ELISA (Calbiotech California USA).

Assay procedure

Enzyme-linked immunosorbent assay (ELISA) technique by calbiotech California USA and Mindray ELISA machine were used for the ELISA assay. 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 was then ready to use. 1:21 dilution of test samples was prepared by adding 10ul of the sample to 200ul of sample diluents and was mixed thoroughly (according to the manufacturer's instructions). Then 100ul of diluted sera, calibrator and controls were dispensed into the appropriate wells and another 100ul of sample diluents were 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 was 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. Then 100ul of Tetramethyl benzidine (TMB) substrate was dispensed and incubated for 10 minutes at room temperature.100ul of stop solution was added. Finally, the patient's optical density (O.D) was read at 450nm using ELISA reader within 15mins. A dual wavelength was recommended with reference filter of 600-650nm.

2.8.2 Assay of 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 2cm 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^{o}, 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 diluted with twice the volume of the buffer solution and then allowed to stain for ten minutes. The slide was washed with tap water, the slide was drained and dried in the air by keeping it in a slanting position [23].

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 observe the film under the microscope using x100 magnification [23].

2.8.3 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 impedance 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 (nonconductive) was diluted in a conductive liquid (diluents), to produce a big difference between them. When the diluents passed through the aperture sensor, electrode was submerged in the liquid on each side of the aperture to create a continuous current. When the cells passed through the aperture, the resistance between the electrodes increased as the volume increased. Passing through the magnification circuit, the voltage signal was magnified and the noise was filtered, then the analytical result was displayed. One count pool and the detection circuit count the white blood cell (WBC), as another count pool and detection circuit count the red blood cell (RBC), platelets (PLT), lymphocytes (LYM) and neutrophils (NEU). The microprocessor of the instrument calculated and analyzed the cells (WBC, RBC, PLT, LYM and NEU) and then gave out the histogram [24].

Principles of haemoglobin measurement

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

2.9 Statistical analysis

Statistical package for social science (SPSS) version 25 was employed in the analysis of the result and the data obtained for different parameters expressed as mean± standard deviation. Parameters were compared between different groups using t-test table. Level of significance was set at $P<0.05$.

3. RESULTS

The Prevalence of dengue virus seropositivity among study participants based on their demographic characteristics is represented in Table 1. Out of the 188 samples studied, 38 (20.2%) were seropositive for dengue virus IgM. The females were 28 (20.1%) while the males were 10 (20.4%). Seroprevalence of dengue virus was observed to be highest 23.0% among the age group of 26-35 years followed by 20.6% among 36-45 years while 56-65 years has the least seroprevalence of 15.4%.

Table 1: Seroprevalence of Dengue Virus based on Demographic characteristics of participants

Total sampled (N=188)		Number Examined	Number Infected (%)
Gender	Male	49	10 (20.4)
	Female	139	28 (20.1)
	Total	188	38 (20.2)
		P–value	0.968
Age	18-25 years	25	4 (16.0)
	26-35 years	61	14 (23.0)
	36-45 years	63	13 (20.6)
	46-55 years	26	5 (19.2)
	56-65 years	13	2 (15.4)
	Total	188	38 (20.2)
		P–value	0.0943

Table 2: The Prevalence of Malaria based on Demographic characteristics of participants

Total sampled (N=188)		Number Examined	Number Infected (%)
Gender	Male	49	10 (20.4)
	Female	139	60 (43.2)
	Total	188	70 (37.2)
		P–value	0.005

Age	18-25 years	25	11 (44.0)	
	26-35 years	61	27 (44.3)	
	36-45 years	63	20 (31.7)	
	46-55 years	26	7 (26.9)	
	56-65 years	13	5 (38.5)	
	Total	188	70 (37.2)	
		P–value	0.436	
Age (± SD) in years	34.5 ± 11.2	38.5 ± 10.5	t= -2.431	0.016

The prevalence of malaria among the study participants based on their demographic characteristics is summarized in Table 2. Out of the 188 participants, 70 were positive for malaria parasitemia giving a prevalence of 37.2%. The difference between the malaria prevalence in males (20.4%) and that of the females (43.2%) was statistically significant at P-value=0.005. The age group of 26-35 years had the highest prevalence of 44.3%, followed by the age group of 18-25 years with 44.0% while age group of 46-55 years had the least malaria prevalence of 26.9%.

The Seroprevalence of dengue virus among the subject group (people living with HIV/AIDS who are accessing drugs from HIV clinic) and control group (people not living with HIV/AIDS who visited the hospital) is shown in Table 3. The result revealed that the Seroprevalence rate of dengue virus among the subject group was 14.4% while that of the control group was 5.9%.

Table 3: Seroprevalence of dengue virus among Subject group (HIV positive patients) and Control group (HIV negative patients)

Total sampled (N=188)	**Number examined**	**Number infected (%)**	**T-Test**	**P-value**
Subject group	125	27 (14.4)	0.664	0.507
Control Group	63	11 (5.9)		
Total	188	38 (20.2)		

The prevalence of malaria among Subject group (HIV positive patients) and Control group (HIV negative patients) is presented in Table 4. Out of 125 participants sampled for malaria among the subject group, 45 persons were infected with malaria whereas 63 participants which represent the control group have 25 persons infected with malaria. The subject and control groups have the prevalence of malaria to be 23.9% and 13.3% respectively.

Table 4: Prevalence of malaria among Subject group (HIV positive patients) and Control group (HIV negative patients)

Total sampled (N=188)	Number examined	Number Infected (%)	T-Test	P-value
Subject group	125	45 (23.9)	–0.491	0.624
Control Group	63	25 (13.3)		
Total	188	70 (37.2)		

The result of the co-infection of dengue virus with malaria among Subject group (HIV positive patients) and Control group (HIV negative patients) shown on Table 5, revealed that out of the 188 participants, 10 persons were found to be co-infected with dengue and malaria among the subject group (125) while 6 persons were co-infected with dengue and malaria in the control group (63). The subject and control groups have 5.32% and 3.19% as their dengue and malaria co-infection prevalence rates respectively.

Table 5: Co-infection of Dengue with Malaria

Total sampled (N=188)	Number examined	Number Co-Infected (%)	T-Test	P-value
Subject group	125	10 (5.32)	0.817	0.500
Control Group	63	6 (3.19)		
Total	188	16 (8.51)		

The haematological profile of the participants (HIV positive patients and HIV negative patients) were presented in Table 6. The table showed that the mean difference between the participants in the MCV (0.001), MCHC (0.013), Monocyte (0.001), Neutrophils (0.035) and eosinophils (0.001) were statistically significant, while the WBC (0.118), RBC (0.988), HGB (0.659), HCT (0.120), PLT (0.380), MCH (0.102), LYM (0.418), BAS (0.914) and MPV (0.468) were not statistically significant. The statistical significance of

MCV and MCHC by implication suggests the fact that the participants have a likelihood to develop anaemia as a result of Vitamin B12 deficiency. The high level of Neutrophils in the haematological profile is an indication that the bodies were not able to fight off infection which may be of bacterial, viral or parasitic origin while higher Eosinophil counts or Eosinophilia is common among individuals with parasitic infections.

Table 6: Haematological parameters of the HIV positive (Subject) and HIV negative (Control) participants

Variable	Control n = 63 (Mean ± SD)	Subject n = 125 (Mean ± SD)	t-test	p-value
WBC	6.62 ± 5.07	5.35 ± 5.35	1.571	0.118
RBC	3.46 ± 0.84	3.45 ± 0.64	0.016	0.988
HGB	12.00 ± 5.58	11.75 ± 2.17	0.442	0.659
HCT	34.53 ± 8.40	36.26 ± 6.42	- 1.561	0.120
PLT	307.48 ± 460.83	255.19 ± 127.03	0.884	0.380
MCV	98.58 ± 11.51	107.13 ± 14.19	- 4.140	**< 0.001**
MCH	32.75 ± 5.04	34.21 ± 6.07	- 1.646	0.102
MCHC	33.08 ± 3.29	32.21 ± 1.46	2.500	**0.013**
LYM	48.67 ± 14.41	50.43 ± 13.95	- 0.812	0.418
MONO	2.15 ± 2.23	7.08 ± 3.27	- 12.137	**< 0.001**
NEUT	50.27 ± 19.26	43.75 ± 20.26	2.119	**<0.035**
EOS	2.37 ± 1.58	4.67 ± 5.62	- 4.251	**< 0.001**
BAS	0.60 ± 0.77	0.61 ± 0.75	- 0.108	0.914
MPV	9.27 ± 1.27	9.14 ± 1.12	0.728	0.468

SD means standard deviation. *The significant values are in bold characters.

Keywords: MCV – Mean Corpuscular Volume = (0.001), MCHC - Mean Corpuscular Haemoglobin Concentration = (0.013), MON - Monocyte = (0.001), NEUT - Neutrophils = (0.035), EOS - Eosinophils =

(0.001), WBC – White Blood Cell = (0.118), RBC – Red Blood cell = (0.988), HGB – Haemoglobin = (0.659), HCT – Hematocrit = (0.120), PLT – Platelets (thrombocyte) count = (0.380), MCH- Mean Corpuscular Haemoglobin = (0.102), LYM – Lymphocytes = (0.418), BAS – Basophils = (0.914), MPV – Mean Platelet Volume = (0.468)

The viral loads of the subjects (HIV positive participants) shown in Table 7 were skewed to the right with mean value of 35633.05 ± 180166.48 and median value of 40.00. This suggests that the subjects who had their viral load values around 40c/ml may have adhered to their drugs or medications strictly while the few who have their viral loads above 40c/ml may be defaulting from taking their medications or are newly enrolled HIV patients who just started their treatment. More so, some previous studies recorded that dengue plays a role in suppressing HIV from progressing to AIDS. Therefore, this might also be the reason why the subjects have their viral loads mostly around 40c/ml.

Table 7: Viral loads among the Subjects (HIV positive participants)

	Viral load						
N	Mean	Median	SD	Minimum value	Maximum value	Skewness	Kurtosis
125	35633.05	40.00	180166.48	19.00	1538127	6.662	48.333

N = no of cases, SD = standard deviation

Table 8 showed the distribution of some risk factors associated with dengue infection and malaria co-infection among the participants. This table reveals that males were positive for dengue IgM with a prevalence of 62.5% while females were positive for dengue IgM with the prevalence of 37.5%. There was a statistically significant difference between males and females with the presence of dengue virus IgM. (X^2=12.0494; *df*=1, *p*=0.005). Dengue virus IgM seropositivity was seen to increase from participants who were within the age range of 26 – 35 years (87.5%: 14/16) compared to participants who were within the age range of 56-65 years ((12.5%: 2/16). 8 out of 48 participants who have their residence close to refuse dump sites were for dengue virus IgM with a prevalence of 50%, while 8 out of the 124 participants who do not have dump sites within their residence were positive for dengue virus IgM with the prevalence of 50%. Data that was generated from participants who had open water storage items were positive for dengue virus IgM with the prevalence of 31.3% while those do not have open water storage items were positive for dengue virus IgM with the prevalence of 68.8%. With regard to constant wearing of trousers and long sleeves, IgM to dengue virus was comparatively higher in participants who usually wore trousers and long sleeves with a prevalence of 56.3% compared to participants who less usually wore same clothes with a prevalence of 43.8%. In terms of constant use of insecticide/mosquito repellents, participants who made constant use of them were observed to be positive for dengue virus IgM with the prevalence of 31.3% while those who don't usually make use of these repellents were positive with a proportionately higher prevalence of 68.8%. Regarding to occupation, dengue virus IgM seropositivity was seen in the highest prevalence of 50.0% amongst the civil servants, while the lowest prevalence of 6.25% was obtained from the self-employed. So therefore, this table clearly portrays that there is no statistically significant association between age, presence of dumpsite, open storage water items and occupation with dengue infection. But there is a statistically significant association between gender,

usually in trousers and long sleeves and constant use of insecticide/mosquito repellents with dengue infection with the p-values of 0.005, 0.010 and 0.043 respectively.

Table 8: Distribution of some risk factors associated with dengue infection and malaria co-infection among the participants

	Uninfected (%) (172)	Infected (%) (16)	χ^2	P–value
Gender				
Male	39	10 (62.5)	12.0494	**0.005**
Female	133	6 (37.5)		
Age				
18-25 years	24	1(6.25)	7.9191	0.095
26-35 years	50	11(68.75)		
36-45 years	61	2(12.5)		
46-55 years	25	1(6.25)		
56-65 years	12	1(6.25)		
Any refuse dump site?				
Yes	48	8 (50)	3.4061	0.060
No	124	8 (50)		
Open water storage items				
Yes	25	5 (31.3)	3.0604	0.075
No	147	11 (68.8)		
Usually in trousers and long sleeves				
Yes	47	9 (56.3)	5.8419	**0.010**
No	125	7 (43.8)		
Constant use of insecticide/				

mosquito repellants.				
Yes			3.9198	**0.043**
No	98	5 (31.3)		
	74	11 (68.8)		
Civil servants			6.8075	0.235
Traders	51	8 (50)		
Students				
Self-employed	50	4 (25)		
Unemployed	16	3 (18.8)		
Artisan	26	1 (6.25)		
	17	…….		
	12	……		

4. DISCUSSION

Dengue virus fever is an important emerging viral disease of the tropical and sub-tropical region with risk of severe infections. It is clear that since the last decades, dengue viral fever has been occurring regularly with periodic surges in a number of cases [25]. Most febrile cases are routinely diagnosed and treated for presumptive typhoid and malaria, or malaria of unknown origin without proper investigation for other health related conditions including viral infections. A definitive diagnosis is confirmed by virus isolation and/or serology [26].

The present study was carried out to determine the Dengue Virus IgM seroprevalence, malaria positivity and haematological parameters of HIV infected individuals at the Chukuwuemeka Odumegwu Ojukwu University Teaching Hospital Awka, Anambra State, Nigeria. In the study, a dengue prevalence of 20.2% was reported among the study participants. This is in contrast with the prevalence of 44.4% reported by Mustapha *et al.* [27] who investigated the survey of malaria and anti-dengue IgG among febrile HIV infected patients attending a tertiary hospital in Abuja. This high prevalence reported by Mustapha *et al.* [27], may be due to the difference in dengue virus serology owing to the fact that they investigated dengue virus IgG, unlike the present study that investigated dengue virus IgM. Immunoglobulin G (IgG) accounts for both previous and present exposure to dengue virus and appears from 5 to 7 days of infection while Immunoglobulin M (IgM) accounts only for current exposure to dengue infections which dissipate or disappear with time. Chukwuma *et al.*[28] who carried out investigation on seroprevalence of dengue virus IgM among children with febrile illness in Nnewi, Nigeria and Abdulaziz *et al*.[29] who investigated the prevalence and factors associated with dengue fever among febrile patients attending

secondary health facilities in Kano Metropolis reported the prevalence of 77.1% and 78.3% respectively. These observed differences in the prevalence reports could be due to the sample sizes used, the type of patients involved (especially when their participants were non HIV patients), age and climatic weather conditions of the environments in which the studies were carried out. Interestingly, the present study closely relates with a study carried out by Adeshina *et al*. [30] who conducted an investigation on the incidence of dengue virus infection on febrile episodes in Ile-Ife, Nigeria and reported a dengue prevalence of 25.7%. The prevalence of dengue virus in this study could be linked to the fact that some of Awka residents live in close proximity to poor drainage system, bad roads with potholes on it, water collecting items like damaged tyres lying around, bushes, uncovered water storage items (mostly people who reside in the villages around), and indiscriminate environmental dumpsites. These things could serve as sites for the breeding of Aedes mosquito (vector for dengue virus infection).
Furthermore, this study reported a prevalence of 8.51% for dengue virus IgM antibodies and malaria parasitemia co-infection among the study participants (subject group has 5.32% while the control group has 3.19%). This value is closely related to the investigation carried out by Idris *et al.* [31] which reported a dengue IgM and malaria prevalence of 10.1% in Maiduguri, Northern Nigeria. Dawurung *et al.* [32] and Idoko *et al*. [33] also reported the prevalence of 2.2% and 1.3% respectively. However, the prevalence of 8.51% for dengue virus IgM antibodies and malaria parasitemia co-infection in this study was lower than the prevalence of 33% and 44.2% reported by Adeleke *et al.,* [34] and Mustapha *et al.* [27] respectively. It is worthy of note that this present study recorded a prevalence of 3.19% for dengue IgM seroprevalence and malaria co-infection amongst non HIV patients (control group). This possibly suggests that dengue does not only affect those living with HIV but also non HIV persons. Thus, anyone who experiences or presents with symptoms similar to that of dengue should also be screened for possible risk of infection by carrying out proper clinical investigations in order to reduce the mortality rate of dengue virus infection among the populace. The prevalence of malaria and dengue co-infection in this study is a possible indication of poor surveillance and vector mitigation measures against dengue vector in the environment. This result is of great importance because Nigeria is one of the few African countries that limit the investigation of febrile illnesses to malaria, typhoid and fever of unknown origin with great negligence to viral infections.

The result of the haematological parameters of the study participants in the present study revealed that the mean corpuscular volume (MCV = <0.001), mean cell hemoglobin concentration (MCHC = 0.013), neutrophils (Neut = 0.035) and eosinophils (EOS = <0.001) were statistically significant while the rest of hematological parameters were not. This study presents that the significant MCV and MCHC indicate B12 deficiency type of anemia which suggests the tendency of the participants being prone to anemia which causes severe headache in people affected. This deficiency could be due to Dengue Virus HIV patients who are on the drug zidovudine which is associated with anaemia [35]. Neutrophils in high volume is regarded as neutropenia, which maybe as a result of bacterial, viral or parasitic infection especially with regards to the HIV infected individuals who are already immunocompromised. Eosinophilia is common in HIV-infected individuals and associated with parasitic infections [36] and drug allergy [37]. Nevertheless, higher eosinophil counts were not significantly correlated with immune activation, altered HIV viral load [38] or thrombocytopenia and granulocytopenia in dengue hemorrhagic fever [39].

Interestingly, this present study reports that the viral loads of the subjects were skewed to the right side of the histogram used to represent it showing that the subjects (HIV patients) had their viral loads mostly around 40c/ml which shows that the suppression of their viral load may be as a result of their strict adherence to medication, being on proper diet or previous studies [40] that suggested that dengue virus (DENV) NS5 protein had a role in HIV-supressing effect during acute infection preventing HIV from progressing to AIDS. Whereas there were some outliers with values above 1,500,000 c/ml with the possibility that they were defaulting by not adhering to their medications or were recently placed on anti-retroviral drugs. However, Hoffman *et al.* [41] reported that there was significant increase in HIV viral load when co-infected with malaria.

There was a statistically significant difference between gender and dengue infection with the p-value of 0.005. The prevalence of dengue virus IgM with regards to gender in this study was higher in males (20.4%) than their female counterparts (20.1%). This observation is in consonance with the works of Ukey

et al. [42] and Adeleke *et al.* [34] who reported higher prevalence of 13% and 46% respectively for males but not in consonance with other studies of Idris *et al.* [31] and Mustapha *et al*., [27] which reported higher prevalence of 18.5% and 49.9% respectively for dengue infection in females than in males. The disparity in the prevalence for gender could be that many men neglect their health related issues until when serious before visiting the hospital [43]. Furthermore, males are generally more exposed to some risk factors such as working in exposed work sites and staying out longer than females especially in drinking bars. Since the dengue vector is day-time feeder; its peak biting periods are early in the morning and in the evening before sunset [12]. Men may be more likely to have a relatively higher prevalence of dengue virus IgM than the women.

There was no statistical significant difference between age and dengue infection. This is in contrast with the study of Peyerl *et al.* [44] who reported that there was a linear association of increasing antibody prevalence with age and the past work of Reiskind *et al*. [45] that showed an age dependent increase of anti-dengue antibodies in exposed population.

Rapid urbanization in Africa has resulted to increase in vector density as a result of human practices that promote mosquito breeding [46]. This present study therefore, necessitates the importance of improved sanitation and proper vector control practices in the homes and work premises.
In general, viral infections suppress the natural immunity of the host, and this often allows opportunistic infections [32]. Therefore, a co-infection of dengue/malaria as observed in this study could be very devastating to HIV-infected persons. This study also recorded 6(3.19%) of malaria/dengue co-infection among the 63 participants in the control group (non-HIV patients) which seems to be the first report of its kind in Awka, Anambra State. So active monitoring and surveillance vector control should be the paramount technical strategy to be adopted for the prevention and control of malaria and dengue infections.

5. CONCLUSION

Considering the fact that malaria is hyperendemic in Nigeria and dengue infections have also been reported in some places, great emphasis should be laid on prevention of mosquito breeding sites and/or bites and other measures adopted at all community levels so as to curb or mitigate the emergence of more severe forms of dengue such as Dengue Haemorrhagic Fever (DHF) and Dengue shock syndrome (DSS) as well as reduce the morbidity and mortality resulting from dengue burden especially in the immunocompromised individuals.

REFERENCES

1. World Health Organisation (2015) Dengue and severe dengue fact sheet N^{o} 117.
2. Coker, R.J. Hunter, B.M. Rudge, J.W. Liverani, M. and Hanvoravongchai, P. (2011). Emerging infectious diseases in Southeast Asia: regional challenges to control. *Lancet Infectious Diseases*, 377:599–609
3. Back, A.T. and Lundikvist, A. (2013). Dengue viruses – an overview. *The Journal of Infection Ecology and Epidemiology,* 310.3402/310.19839
4. McCall, P. J. and Lenhart, A. (2008). Dengue control. *The Lancet infectious diseases* 8: 7-9.
5. Whitehorn, J. and Simmons, C. P. (2011). The pathogenesis of dengue. *Vaccine,* 29:7221–8.
6. Gubler, D. J. (2002). Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in microbiology*. 10: 100-103.
7. Endy, T.P. Anderson, K.B. Nisalak, A. Yoon, I.K. Green, S. and Rothman, A.L. (2011). Determinants of Inapparent and symptomatic Dengue Infection in a prospective Study of Primary School Children in Kamphaeng Phet, Thailand. *Plos Neglected Tropical Diseases*, 5(3): 975.
8. Henchal, E. A. and J. R. Putnak (1990). The dengue viruses. *Clinical Microbiology reviews,* 3: 376-396.
9. Rigau-Perez, J. G. Clark, G. G. Gubler, D. J. Reiter, P. Sanders, E. J. and Vorndam, A.V. (1998). Dengue and dengue haemorrhagic fever. *The Lancet infectious diseases,* 352: 971-977.
10. Bhatt, S. Gething, P. W. Brady, J.O. Messina, J. P, Farlow, J.M. Moyes, C.L. Drake, M.J. Browsnstein, J.S. Hoen, G.A. Sankoh, O. Myers, F.M. George, T.J. William-Wint, G.R. Simmons,

C.P. Scott, T. W Farrer, J.J and Hay, S.I (2013). The global distribution and burden of dengue. *Nature.* 496(7446): p. 504–507.

11. Linares, E. M. Pannuti, C.S. Kubota, L.T. and Thalhammer, S. (2013) Immunospot assay based on fluorescent nanoparticles for dengue fever detection. *The Journal of Biosensors and Bioelectronics*.41:180–185.

12. World Health Organization. (2021). The current global statistics on dengue and severe dengue.
13. Amarasinghe, A. Kuritsk, J.N. Letson, G.W and Margolis, H.S. (2011). Dengue virus infection in Africa. *The Journal of Emerging infectious diseases,* 17: 1349-1354.
14. Faneye, A. Idika, N. Motayo, B.O. Adesanmi, A. and Afocha, E. (2013). Serological evidence of recent dengue virus infection among febrile children in a semi-arid zone. *The American Journal of Infectious Disease,* 9: 7-10.
15. Oladipo, E. K. Amanetu, C. Gbadero, T. A. and Oloke J. K. (2014). Detectable anti-dengue virus IgM antibodies among healthy individuals in Ogbomoso, Oyo state, Nigeria. *The American Journal Infectious Disease*. 10 (2):64-67.
16. World Health Organization (2009). Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva: World Health Organization. 1-147.
17. Guzman, M.G. Halstead, S.B. Artsob, H. Buchy, P. Farrar, J. and Gubler, D. J. (2010) Dengue: A continuing global threat. *The Journal of Nature Review Microbiology*. 8:7–16.
18. Siong, W.C. Ching, T. H. Jong, G.C. Pang, C. S. Vernon, L. J. and Sin L.Y. (2008) Dengue infections in HIV patients Southeast Asian. *The Journal of Tropical Medicine and Public Health.*39:260–265.
19. Watt, G. Kantipong, P. and Jongsakul, K. (2003) Decrease in human immunodeficiency virus type 1 load during acute dengue fever*. Journal of Clinical Infectious* Diseases, 36(8):1067-1069.
20. Baba, M. Saron, M.F. Vorndam, A. Adeniji, J. Diop, O. and Olaleye, D. (2009). Dengue virus infections in patients suspected of malaria/typhoid in Nigeria. *Journal of American Science,* 5(5):129–134.
21. Daniel, W.W. (1999) Biostatistics: A foundation for analysis in the health sciences. 7th edition. New York; John Wiley & Sons.
22. Otu, A. A. Udoh, U. A. Ita, O. I. Hicks, J. P. Egbe, W. O. and Walley, J. (2019). A cross-sectional survey on the seroprevalence of dengue fever in febrile patients attending health facilities in Cross River State, Nigeria. *The Public Library of Science Journal,* 14(14): e0215143
23. Bailey, J.W. Williams, J. Bain, B.J. Parker-Williams, J. and Chiodini, P. (2013). General Haematology Task Force of the British Committee for Standards in Haematology: Guideline: The Laboratory Diagnosis of malaria. *The British Journal of Haematology.* 163(5):573-580.
24. Salvagno, G. L. Sanchis-Gomer, F. Picanza, A. and Lippi, G. (2014). Red blood cell distribution width: A simple parameter with multiple clinical applications. *The journal of Critical Reviews in Laboratory Science*, 52(2);86-105.
25. Ahmed, S. Ali, N. Ashraf, S. Ilyas, M. Tariq, W. and Chotani, R. (2008). Dengue fever outbreak: a clinical management experience. *The Journal of College of Physicians and Surgeons of Pakistan*.
26. Singh, B. (2007) Dengue outbreak in 2006. Failure of public health system? *Indian Journal of Community Medicine*, 32:99-100.
27. Mustapha, J. O, Emeribe, A. U, and Nasir, I.A. (2017). Survey of malaria and anti-dengue virus IgG among febrile HIV-infected patients attending a tertiary hospital in Abuja, Nigeria. *HIV& AIDS*. (Auckland, N.Z) 9,145-151.

28. Chukwuma, G.O, Audu, J.S. Chukwuma, O.M. Manafa, P.O. Ebugosi, R.S. Akulue, J.C. Aneke, J.C. Ahaneku, G.I. Nchinda, G.W. and Esimone, C.O. (2018). Seroprevalence of Dengue virus among children with febrile illness. Nnewi Nigeria. *The Journal of Medical Research,* 4(1):24-30.

29. Abdulaziz, M. M. Ibrahim, A. Ado, M. Ameh, C. Umeokonkwo, C. Sufyan, M.B. Balogun, M.S. and Ahmed, S.A. (2020). Prevalence and factors associated with dengue fever among febrile patients attending secondary health facilities in Kano metropolis, Nigeria. *The Journal of Clinical Experimental Microbiology,* 21(4):340-348.
30. Adeshina, O.A. and Adeniyi, J.A. (2015). Incidence of dengue virus infection in febrile episodes in Ile-Ife, Nigeria. *The African Journal of Infectious Disease,* 10(1):21-24.

31. Idris, A.N. Baba, M.M. Thairu, Y. and Bamidele, O. (2013). Sero-prevalence of dengue type-3 Virus among patients with febrile illnesses attending a tertiary hospital in Maiduguri, Nigeria. *International Journal of Medical Science*, 5(12):560–563.
32. Dawurung, J. S. Baba, M.M. Stephen, G. Jonas, S.C. Bukbuk, D.N. and Dawurung, C.J. (2010). Serological evidence of acute Dengue virus infection among febrile patients attending Plateau State Specialist hospital Jos, Nigeria. *Republic's Opinion*, 2:71–76.
33. Idoko, M.O. Ado, S.A. and Umoh, V.J. (2015) Prevalence of dengue virus and malaria in patients with febrile complaints in Kaduna Metropolis, Nigeria. *British Microbiology Research Journal*, 8(1):343–347.
34. Adeleke, M.A. Muhibi, M.A. Ajayi, E.I. and Idowu, O.A. (2016). Dengue virus specific Immunoglobulin G antibodies among patients with febrile conditions in Oshogbo, South-western Nigeria. *The journal of Tropical Biomedicine,* 33(1):1–7.
35. Phe, T. Thai, S. Veng, C. Sok, S. Lynen, L. and Griensven, V. J. (2013) Risk factors of treatment-limiting anemia after substitution of zidovudine for stavudine in HIV-infected adult patients on antiretroviral treatment. *PLoS ONE*, 8: e60206.
36. Sivaram, M. White, A. and Radcliffe, K. W. (2012) Eosinophilia: clinical significance in HIV-infected individuals. *International Journal of STD & AIDS*, 23:635–638.
37. Colebunders, R. Van Den Eynde, C. Tolo, A. Fleerackers, Y. Vanham, G. Kestens, L. Vervoort, T. and Depraetere, K. (1997). Eosinophilia in patients infected with human immunodeficiency virus. *The Journal of Infectious Diseases,* 175:1283.
38. Chorba, T.L. Nkengasong, J. Roels, T.H. Monga, B. Maurice, C. Maran, and M. Djomand, G. (2002). Assessing eosinophil count as a marker of immune activation among human immunodeficiency virus-infected persons in sub-Saharan Africa. *The Journal of Clinical of Infectious Disease*, 34:1264–1266.
39. Butthep, P. Bunyaratvej, A. and Bhamarapravati, N. (1993). Dengue virus and endothelial cell: a related phenomenon to thrombocytopenia and granulocytopenia in dengue hemorrhagic fever. Southeast *Asian Journal of Tropical Medicine and Public Health,* 24:246–249.
40. Xiang, J. McLinden, J. H. Rydze, R. A. Chang, Q. Kaufman, T. M, Klinzman, D. and Stapleton, J. T. (2009) Viruses within the Flaviviridae decrease CD4 expression and inhibit HIV replication in human CD4+ cells. *The Journal of Immunology*; 183:7860–7869.
41. Hoffman, I.F. Jere, C.S. Taylor, T.E. Munthali, P. Dyer, J.R. Wirima, J.J. Rogerson, S.J. Kumwenda, N. Eron, J.J. Fiscus, S.A. Chakraborty, H. Taha, T.E. Cohen, M.S. and Molyneux, M. E. (1999) The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration. *Acquired Immuno Deficiency Syndrome (Journals)* 13:487–494.
42. Ukey, P. Bondade, S. Paunipagar, P. Powar, R. and Akulwar, S. (2010) Study of seroprevalence of dengue fever in central India. *Indian Journal of Community Medicine*; 35(4):517–519.
43. INTEGRIS Health. (2019) Health and wellness news on Why Don't Men see Doctors? INTEGRIS Health Oklahoma's largest hospital network.www.google.com
44. Peyerl-Hoffmann, G. Schwobel, B. Jordan, S. Vamisaveth, V. Phetsouvanh, R. Christophel, E. M. Phompida, S. Sonneburg, F. V. and Jelinek, T. (2004) Serological investigation of the prevalence of anti-dengue IgM and IgG antibodies in Attapeu Province, South Laos. The Journal of *Clinical Microbiology and Infection*, 10(2):181–184.
45. Reiskind, M. H, Baisley, K. J, Calampa, C. Sharp, T. W. Watts, D. M. and Wilson, M. L. (2001). Epidemiological and ecological characteristics of past dengue virus infection in Santa Clara, Peru. *Tropical Medicine and International Health*, 6(3):212–218.
46. Monath, T. P. (1994) Dengue: the risk to developed and developing countries. *Proceedings of National Academia of Science,* 91:2395-2400.