Co-Infection of Dengue Virus and *Plasmodium Falciparum* in Malaria Patients Attending Mission Hospitals in Enugu State, Nigeria

EZE, PAUL EJIKE[1], OGUDU, EMMANUEL O[2], OZOCHIKELU, CORNELIUS C[3]
[1]*Department of Microbiology, Alex Ekwueme Federal University Ndufu Alike, Ikwo, Ebonyi State.*
[2]*Federal Ministry of Health, National Arbovirus and Vectors Research Centre, Enugu, Enugu State.*
[3]*Department of Science Laboratory Technology, Federal Polytechnic Ohudo, Enugu State.*

Abstract- ***Arboviruses are currently recognized as a global threat to human and public health, causing widespread morbidity worldwide, particularly in Africa. Among the emerging infectious diseases, arboviral diseases, especially dengue viral disease, are of significant importance. The study is aimed to determine the co-infection of dengue virus and P. falciparum among residents of Enugu metropolis, Enugu State, Nigeria. The study adopted a cross sectional survey and was carried out at the major Mission owned hospitals within Enugu Metropolis. The hospitals include Ntasi Obi Ndi Nò N'afufu hospital, Trans Ekulu, Mother of Christ Specialist hospital, Ogbete and Annunciation Specialist hospital Emene. A total of 300 blood samples were collected from the volunteered participants, eighty (80) samples from Ntasi Obi hospital, one hundred samples (100) from Annunciation hospital and one hundred and twenty samples (120) from Mother of Christ hospital. Out of 300 samples analysed, 62% were positive for P.falciparum using RDT while 66.7% were positive by microscopy. Comparatively, across the hospitals, Mother of Christ had the highest prevalence rate of P.falciparum representing 29% followed by Annunciation hospital 19.7% and then Ntasi Obi hospital 13.3%. Molecularly, the Plasmodium spp was confirmed using conventional PCR technique as Plasmodium falciparum at 207bp. On the other hand, the study also revealed that the prevalence rate of dengue across the hospitals were, Mother of Christ 8.1%, Annunciation 4.3% and Ntasi Obi 3.8%. The study revealed that female participants were more seropositive to both Dengue IgG 72% and Dengue IgM 64% than their male counterparts IgG 28% and IgM 36% (P = 0.038). Co-infection rate of dengue IgM and P. falciparum was 12.2% and the distribution across the hospitals was Ntasi Obi 2.1%, Annunciation 4.6% and Mother of Christ 5.5%. The study generally suggested that co-infection of dengue in malaria patients is possible and complications can be severe if they co-exist.***

Indexed Terms- ***P.falciparum, Arboviruses, Dengue.***

I. INTRODUCTION

Arboviruses are currently recognized as a global threat to human and public health, causing widespread morbidity worldwide, particularly in Africa (WHO, 2018). Among the emerging infectious diseases, arboviral diseases, especially dengue viral disease, are of significant importance (WHO, 2015). The complex cycle involving vectors, viruses, and hosts remains unchanged and leads to unpredictable epidemiological patterns (Gan and Leo, 2014). Various diseases transmitted by mosquitoes, such as Malaria, Yellow Fever (YF), Dengue Fever (DEN), Chikungunya (CHIK), and West Nile viruses, pose a risk to the human population (CDC, .2010). These diseases are endemic in regions where over two billion people reside globally, excluding southeast Nigeria where dengue fever has recently been reported (WHO, 2010). Malaria, transmitted by infected female *Anopheles* mosquitoes, is a leading cause of acute febrile illness (AFI) in Africa (WHO, 2022). The vectors responsible for transmitting malaria parasites are mainly nocturnal and indoor biters, with peak biting hours between 6 pm and 10 pm, as well as early morning hours between 4 am and 6 am. In Nigeria, *Plasmodium* is responsible for over 95% of malaria infections, while other species (*P. ovale, P. malariae, P. knowlesi and P. malariae*) play a minor role or are absent among Nigerian residents (Mohaptra, *et al.,* 2012). On the other hand, the transmission of dengue virus occurs through

infected female *Aedes* mosquitoes, which are primarily diurnal and outdoor biters. Co-infection with multiple diseases is possible in regions where the respective vectors coexist (Mohaptra, *et al.*, 2012). Given the presence of the mosquito species responsible for transmitting these infections in Nigeria, co-infection is highly likely (Ayorinde, *et al.*, 2009). The spread of these diseases has been facilitated by urbanization, migration, and climate change, increasing their impact on human populations (WHO, 2010). Arboviral infections are often misdiagnosed and treated as malaria due to their similar clinical presentations. Cases of co-infection with arboviruses and malaria parasites have been reported in Nigeria (Senn *et al*., 2011), Senegal, and among European travelers in Senegal, Guinea, and Sierra Leone (Charrel *et al*., 2005). Concurrent infection with malaria and dengue has been documented since 2005 (Arya *et al*., 2016; Deresinski *et al*., 2006; Carme *et al*., 2009), and it tends to be more severe than single infections, characterized by hematologic abnormalities like thrombocytopenia and anemia, which are known risk factors for severe dengue fever and/or malaria (Epelboin *et al*., 2012). Dengue fever, also known as break bone fever, is an infectious tropical disease caused by the dengue virus. There are four strains of the virus, namely DENV-1, DENV-2, DENV-3, and DENV-4. It is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti* and recently *A. albopictus* (Chen and Wilson, 2010).

These febrile diseases, malaria and dengue, have become significant public health concerns worldwide, especially in Africa, due to their endemic nature and similarities in signs and symptoms, such as fever, severe joint and muscle pains, headache, sore throat, malaise, nausea, and an irritating rash (Baba *et al*., 2013). Because of these similar symptomatic presentations, individuals infected with either of these pathogens could be misdiagnosed if clinical considerations alone are relied upon without laboratory investigations. The reverse transcription-polymerase chain reaction (RT-PCR) is the preferred method for early detection and confirmation of viruses in clinical samples, particularly when symptoms overlap. Accurate diagnosis of infectious diseases is crucial for their control (WHO, 2011). Managing malaria can be complicated due to the coexistence of other disease-causing agents that can also cause febrile symptoms. Treatment may be prolonged, and drug resistance may occur due to different levels of drug interactions. The management of dengue virus disease primarily involves supportive therapy (Domingues, 2009).

II. AIM OF THE STUDY

To determine the co-infection of dengue virus and *P. falciparum* among residents of Enugu metropolis, Enugu State, Nigeria.

Specific Objectives of the Study

a) To determine the distribution of *P. falciparum* among patients visiting mission-owned hospitals in Enugu metropolis.
b) To detect the presence of dengue virus among residents of Enugu State using ELISA technique.
c) To determine the rate of dengue virus co-infection with *Plasmodium falciparum*.

III. MATERIALS AND METHODS

Study Area:

The study was carried out at the major Mission owned hospitals within Enugu Metropolis. The phlebotomy unit of each of the hospitals was used as the sample collection point. Enugu State is located in South-Eastern Nigeria and the city has a population of 722,664 according to the 2006 Nigerian census. The total area of the State is 7,161 km^2 (2,765 sq. mi) and the State shares borders with Abia State and Imo State to the south, Ebonyi State to the east, Benue State to the northeast, Kogi State to the northwest and Anambra State to the west.

Study Population and design

The study adopted a cross-sectional survey and demographic and other relevant information of the patients was extracted using a structured questionnaire. Patients visiting the major mission owned hospitals within Enugu metropolis were recruited for the study. The hospitals purposively selected for the study include, Ntasi Obi Ndi Nò N'afufu hospital, Trans Ekulu, Mother of Christ Specialist hospital, Ogbete and Annunciation Specialist hospital Emene. Both those clinically diagnosed of malaria and the apparently healthy

patients were recruited for the study. Since the vectors for dengue virus transmission are majorly outdoor biters, children below the age of 2 years were not recruited for the study as they are meant to spend most of their time indoors.

Sample Size

A total of 300 blood samples were collected from the volunteered participants, eighty (80) samples from Ntasi Obi hospital, one hundred samples (100) from Annunciation hospital and one hundred and twenty samples (120) from Mother of Christ hospital. The sample size was calculated using the formula below.

$$\text{Sample size (n)} = \frac{Z^2P(1-P)}{d^2}$$

Z = is standard normal variate (at 5% type 1 error ($P<0.005$) it is 1.96 and at 1% type 1 error ($P<0.001$) it is 2.58). As in majority of studies P values are considered significant below 0.005 hence 1.96 is used in formula.

P = Expected proportion in population based on previous studies or pilot studies.

d = Absolute error or precision.

Study Design:

The study adopted a cross sectional design and the demographic and other information of the patients was extracted using a structured questionnaire.

Ethical Consideration:

Ethical approval was duly collected from the Catholic community that owns the hospitals that was selected for the study. An informed consent was obtained from all the participants. The aim of the study was clearly explained to the participants in the language he or she understands before specimen collection. In cases where the participant cannot speak, hear or communicate effectively due to health impediment, the closest relative was permitted to provide us with the information needed. Samples were collected from only clients who gave their consent either directly or through relatives.

Specimen Collection:

Three milliliters (3ml) of whole blood was collected aseptically through venopuncture using sterile 5 ml syringe and transferred into a sterile EDTA container already properly labeled. The samples were transported to National Arbovirus and Vectors Research Centre (NAVRC) Enugu for sample processing and analysis.

Specimen Processing/Analyses:

Microscopy

All laboratory processing and analysis of the samples were done at NAVRC Laboratory. Thick smear was made on a clean grease-free slide and stained properly using Giemsa stain. After air-drying the stained smear was viewed under the light microscope to determine the malaria parasite load.

Serology Assay (Rapid Diagnostic Test and ELISA)

A rapid diagnostic testing (RDT) technique was used to detect *Plasmodium falciparum* antibody using a commercially prepared serology test kit. The plasma was harvested from each sample that were positive for Malaria microscopically and stored frozen at -20^{o}C. The malaria positive samples were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) to determine the ones that are also seropositive to Dengue Virus both Imunnoglobulin G (IgG) and immunoglobulin M (IgM). The kit for the ELISA analysis was purchased commercially from Inqaba Biotec West Africa. The manufacturers' protocol for the analysis was strictly followed and the optical density read out from the ELISA reader (Biobase 10, ELISA reader). Internal controls included in the commercial kits were Positive controls (PC), Negative controls (NC) and Cut-off value (COV). The NC, the OD value of each specimen and the COV was used to calculate the real values of the specimen to determine seropositivity and seronegativity of the specimen to dengue virus.

Dry Blood Spot (DBS):

Seventy microliter (70µl) of whole blood in EDTA container of the malaria-positive samples (for both Microscopy and RDT) was placed on DBS card and properly labeled. It was allowed to air-dry at room temperature inside a hood for 24hrs, then packaged inside a clean zip-lock bag and stored at -20^{o}C.

Molecular Assay:

The samples on DBS card was used for molecular confirmation of *Plasmodium falciparum* from the positive RDT samples. *P. falciparum* was detected using conventional PCR technique. The primers

sequences used for the plasmodium detection are; forward primer *(Pf1) 5'-agc gtg atg aga ttg aag tca g-3'* and the reverse primer (*Pf2*) *5'-ccc taa acc ctc taa tca ttg tc-3'*. The primers were designed from NCBI sequence data base and synthesized at Inqaba Biotec West Africa. The DNA was extracted fro using ZymoResearch kit purchased from BioLabs England. The manufacturers' protocol was strictly followed for the DNA extraction. The following amplification conditions were adopted during amplification process; initial denaturation @95ºC for 5min, denaturation @95ºC for 30 sec, annealing @55ºC for 30 sec, elongation @72ºC for 1min, final elongation @72ºC for 5 min and final hold @4ºC for 7min. The protocol was adopted and modified from Mohanty *et al.,* 2009. After amplification, the amplicons were stained with ethidium bromide and run on 2% agarose gel for 1hr30mins at 120V. The stained DNA bands were visualized under an ultraviolet transilluminator.

IV. RESULTS

Out of the 300 samples analyzed it was found that 62% were positive for *Plasmodium falciparum* using PF rapid diagnostic kit but 66.7% were positive for malaria on microscopy. Comparatively, across the hospitals, Mother of Christ had the highest prevalence rate of *P.falciparum* representing 29% followed by Annunciation hospital 19.7% and then Ntasi Obi hospital 13.3% using rapid diagnostic testing kit. Table.1. Majorly, the participants in this study were traders 58(31.2%), followed by civil servants 52(28%), students 41(22%), Farmers 28 (15.1%), Drivers 4(2.2%) and those with unspecified occupation (others) 3(1.6%).

Molecularly, the *Plasmodium spp* was confirmed molecularly using conventional PCR technique as *Plasmodium falciparum* at 207bp Fig 1. Statistical prevalence of *Plasmodium falciparum* using molecular technique was not determined in this present study.

The study revealed that female participants were more seropositive to both Dengue IgG 72% and Dengue IgM 64% than their male counterparts IgG 28% and IgM 36% ($P > 0.038$). The study also revealed that patients within the ages of 31-40 years of age had the highest seroprevalence rate of 8.6% followed by those within the age of 11-20 years representing 6.5% ($P = 0.001$). Majority of the IgG seropositive patients were traders 15.6% and civil servants 10.2% ($P < 0.001$) Table 2. Similarly, IgM seropositive patients were more prevalent within the age bracket of 31 – 40 years and 21 – 30 years representing 5.4% and 4.3% respectively ($P > 0.05$). Occupationally, traders were more seropositive 8.6% followed by students 3.8% ($P > 0.001$) Table 3.

Table 1: Demographic characteristics of study population

Variable	Number Sampled (N)	Percentage (%) Infected with malaria
Gender		
Males	105	11.7
Females	195	21.7
Total	300	
Age group		
5-10	35	11.7
11-20	46	15.3
21-30	59	19.7

31-40	60	20.0
41-50	56	18.7
50&aboe	44	14.7
Total	300	
Occupation		
Farmer	47	15.7
Civil Servant	76	25.3
Student	61	20.3
Trader	78	26.0
Driver	15	5.0
Others	23	7.7
Total	300	

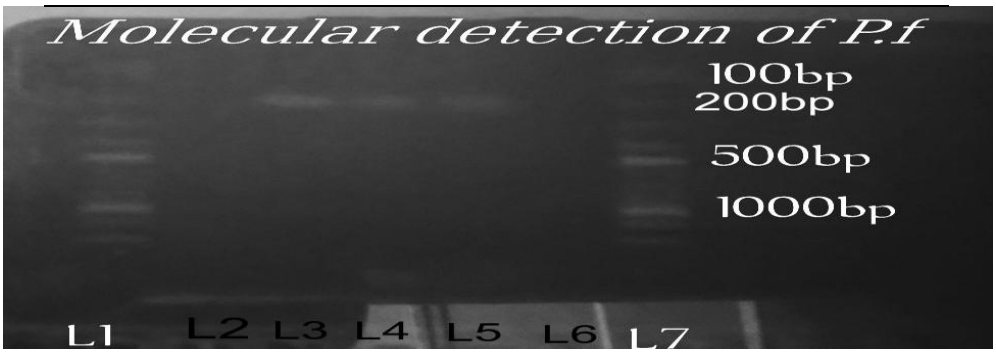

Figure 1: Molecular detection of *P. falciparum*

Molecular confirmation of *Plasmodium falciparum* from the three hospitals within Enugu Metropolis. The *Pf* is confirmed at 205bp as shown above. L1 and L7 are the 100bp DNA ladder.

Table 2: The Seroprevalence of Dengue Immunoglobulin G (IgG) according to gender, age group and occupation.

Variable	Number Sampled (%)	Negative (%)	Positive (%)	χ^2	P-value
Gender					
Males	76	51(67)	25 (33)		
Females	110	68 (62)	42 (38)	4.313	0.038
Age Group					
5-10	15	12 (80)	3 (20)		
11-20	26	11 (42)	15 (58)		
21-30	39	27 (69)	12 (31)		
31-40	46	30 (65)	16 (35)	18.59	0.001
41-50	36	24 (67)	12 (33)		
50+	24	15 (44)	9 (56)		
Occupation					
Farmer	28	21 (75)	7 (25)		

Civil Servant	52	33 (64)	19 (36)		
Student	41	31 (76)	10 (24)	34.11	0.000
Trader	58	29 (50)	29 (50)		
Driver	4	3 (75)	1 (25)		
Others	3	2 (67)	1 (33)		

Difference between variable statistically significant at P-value ≤ 0.05

Table 3: The Seroprevalence of Dengue Immunoglobulin M (IgM) according to gender, age group and occupation.

Variable	Number Sampled (%)	Negative (%)	Positive (%)	χ^2	P-value
Gender					
Males	76	63(83)	13 (17)		
Females	110	89(81)	21 (19)	18.82	0.170
Age Group					
5-10	15	12 (80)	3 (20)		
11-20	26	19 (73)	7 (27)		
21-30	39	31 (79)	8 (21)		
31-40	46	36 (78)	10 (22)	80.91	0.151
41-50	36	32 (89)	4 (11)		
50+	24	22 (65)	2 (17)		
Occupation					
Farmer	28	24 (86)	4 (14)		
Civil Servant	52	46 (88)	6 (12)	18.64	0.001
Student	41	34 (83)	7 (17)		
Trader	58	42 (72)	16 (28)		
Driver	4	4 (100)	0 (0)		
Others	3	2 (67)	1 (33)		

Difference between variable statistically significant at P-value ≤ 0.05

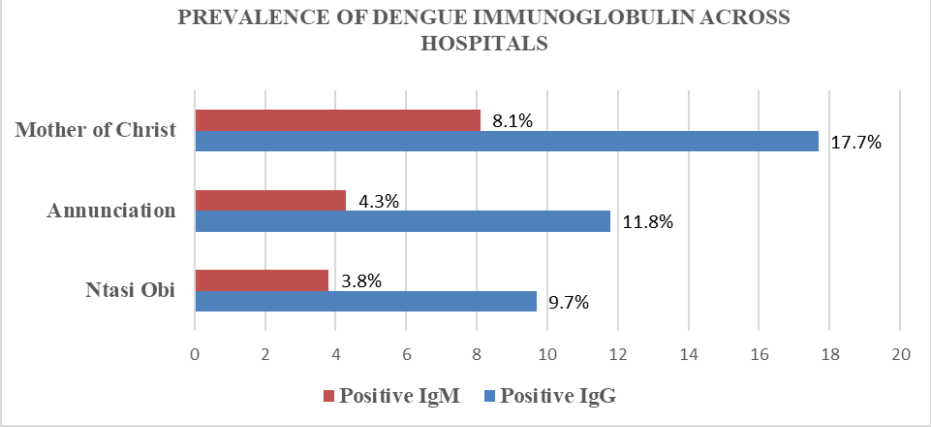

Figure 2: Prevalence of dengue immunoglobulin across hospitals

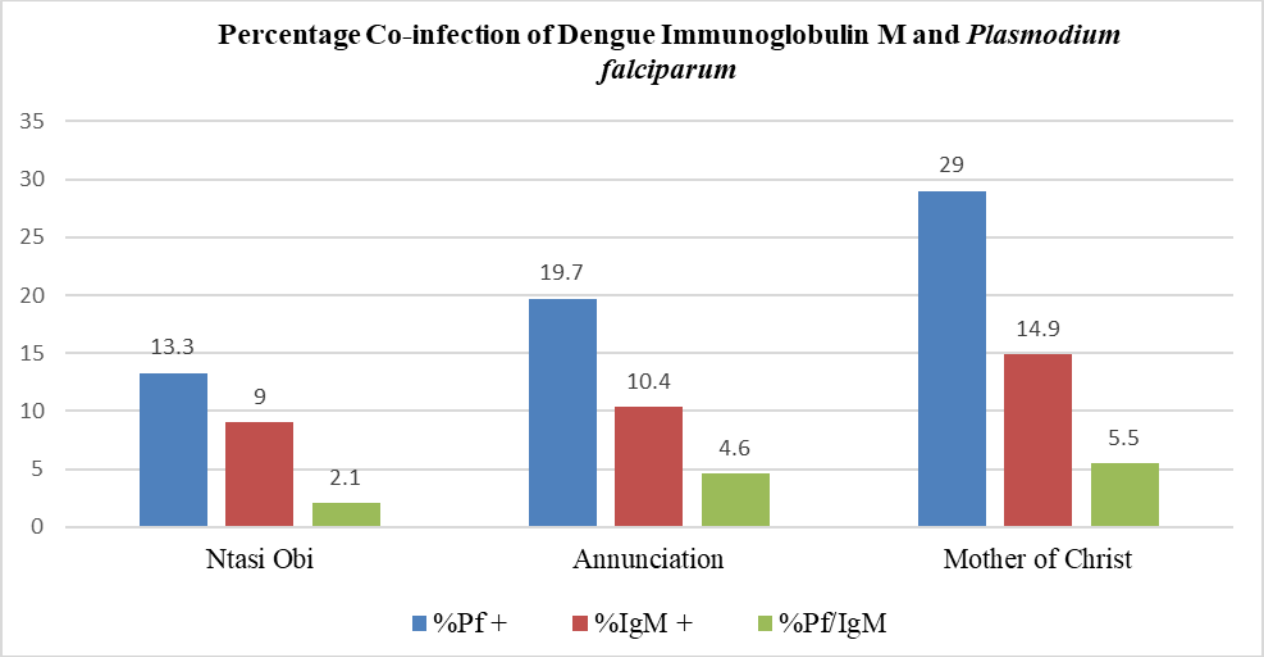

Figure 3: Prevalence of dengue IgM and Pf Co-infection

V. DISCUSSION

Plasmodium infection can be complicated when it co-exists with other viral pathogens like arboviruses. This study was targeted at determining the prevalence of dengue virus infection in patients that have *Plasmodium* infection. In this present study it was found that 66.7% was positive for malaria and 62% of it were confirmed to be *Plasmodium falcipurium.* The high prevalent rate contradicts an earlier report of 21.1% by Tadesse *et al.* (2022). This discordant may be due to seasonal variation, age difference and geographical location.

The study also showed that Mother of Christ hospital had the highest prevalent rate of plasmodium followed by Annuciation hospital and then Ntasi Obi hospital. This outcome could be as a result of the strategic locations of the three different hospitals. The hospital with the highest rate is strategically located in the heart of Enugu town were the largest market (Ogbete main Market) is located. This could also explain the reason while traders were the highest recruited for the study and also had the highest seropositivity to both IgM and IgG.

In this study, varying proportions of dengue fever immunoglobulins were found among individuals with Plasmodium infection. The prevalence of 55.4% reported in this present study is higher than the reported prevalence of 13.02% in South Eastern Nigeria (Onochie-Igbinedion *et al.,* 2022). Our findings confirm the presence of *Aedes* species in Enugu State and the circulation of the dengue fever virus among the population. This supports the notion that dengue fever is more prevalent in densely populated areas of tropical countries (CDC, 2016; Otu *et al.,* 2019). However, our IgG prevalence of 39.2% and IgM prevalence of 16.2% are lower than the IgG prevalence of 77% reported in Osogbo, Southwestern Nigeria, by Adeleke *et al.* (2016), and the IgM prevalence of 77.1% reported in Southeastern Nigeria by Chukwuma *et al.* (2018). These differences in observations could be attributed to variations in environmental conditions, sample size, and the presence and utilization of malaria preventive interventions. Additionally, the location of the study and the distribution of subjects seem to influence the incidence of dengue fever (Gamil *et al.,* 2014; Afolabi *et al.,* 2016). The study also demonstrated a high prevalence of dengue and *Plasmodium falciparum* co-infection of 12.2%. This could be as a result of the presence of *Anopheles* and *Aedes* mosquitoes in Enugu State. The high prevalence rate of recent dengue virus infection in Enugu State shows that most complications and recurrent cases of pyrexia in

patients could be as a result of co-existence of *Plasmodium* and Dengue virus of different strains. Though determining the most prevalent strain of dengue viruses (D1, D2, D3, D4 and the most recent strain D5) was beyond the scope of this present study. Over time, dengue fever occurrence among Nigerians has received less attention. The higher prevalence of IgG among our subjects indicates past exposure and potential recovery. The presence of IgM in some subjects suggests current infection, and the detection of both IgG and IgM suggests fresh infection in sensitized individuals. This highlights the need for improved intervention measures in vector control and entomology studies in Enugu and Nigeria as a whole, to determine the circulating mosquito species. The prevalence of dengue fever immunoglobulin (54.3%) was lower than the prevalence of *P. falciparum* (62%) among our subjects. This finding contradicts the results of previous studies conducted by Adeleke *et al*. (2015), Mustapha *et al*. (2017), and Chukwuma *et al*. (2018) in Southwestern, North central, and Southeastern Nigeria, respectively, where higher dengue fever prevalence was reported. Variations in observations may be attributed to differences in the distribution of dengue fever vectors, environmental conditions, sample size, and the presence and utilization of malaria preventive interventions. The lower prevalence of dengue fever found in our study aligns with the findings of other studies in Southwestern (Ayolabi *et al*., 2019) and Southeastern Nigeria (Otu *et al*., 2019).

Some subjects in our study were found to have both malaria parasites and dengue fever immunoglobulins. This finding is consistent with reports of dengue fever-malaria co-infections by Adeleke *et al*. (2016), Chukwuma *et al*. (2018), and Akaninyene *et al*. (2019) in different regions of Nigeria. This confirms the existence of dengue fever-*P. falciparum* co-infection among febrile patients in Enugu, Nigeria. Malaria remains a disease with significant morbidity and mortality in tropical countries, while the true burden of dengue fever infection remains underestimated. Therefore, it is crucial to initiate dengue fever surveillance to enhance the effectiveness of malaria control interventions and reduce misclassification of patients with fever of unknown origin.

However, our study indicates that gender, occupation, and age are sufficient predictors of dengue fever immunoglobulin, suggesting that there may be other unexplored factors at play. The lack of statistically significant differences in dengue fever seroprevalence among the assessed factors may suggest that everyone within the study area, regardless of ocupation, age, and sex, is at risk of infection. Our findings align with previous studies conducted by Ayukekbong (2014), Adeleke *et al*. (2016), and Ahmadu *et al.* (2020), which reported high dengue fever seroprevalence rates among subjects with febrile illness in Southwest Nigeria and a separate study on the prevalence and determinants of dengue virus immunoglobulin among febrile patients in Naval Medical Hospital at Victoria Island Lagos, Nigeria, respectively. However, our findings differ from a study conducted by Obaidat and Roess (2018) in Jordan, which reported significant associations between age, socioeconomic status, travel history, and dengue fever seropositivity. The discrepancies observed may be attributed to differences in lifestyle, subject sampling, diagnostic methods, and the period of sample selection.

Additionally, the location of the study and the distribution of subjects seem to influence dengue fever seroprevalence (Gamil *et al*., 2014). Various factors have been described globally to predict dengue fever seroprevalence in humans, particularly in tropical countries. Residing in endemic areas of the tropics is a major contributing factor. The presence of dengue fever immunoglobulins among our subjects strongly confirms the existence of *Aedes* spp. in our study area (Ahmadu *et al.,* 2020). Dengue fever is a viral infection that manifests in diverse ways, with clinical symptoms ranging from mild febrile illness to severe plasma leakage with hemorrhagic manifestations. Common symptoms include nonspecific fever, along with headache, retro-orbital pain, arthralgia, muscle pain, and rash without localized signs or symptoms (Gamil *et al*., 2014).

CONCLUSION AND RECOMMENDATIONS

A significant proportion of our subjects were found to have dengue fever immunoglobulins, confirming the occurrence of dengue fever-malaria co-infection among the citizens of Enugu State. This poses a threat to rational drug use and may contribute to antimalarial

resistance, as dengue fever is not routinely detected among febrile individuals in Nigeria. Based on our findings, we recommend the inclusion of dengue fever screening in patients presenting with febrile illness in Enugu State. Furthermore, future entomological studies in Enugu State are recommended to verify the presence of *Aedes* species as potential vectors of the dengue virus. To better understand the circulating viral strains, a study on dengue fever strain classification would be necessary to identify the specific species in circulation.

STUDY LIMITATION

This study has several limitations that should be taken into account. Firstly, it is important to note that this study is based solely on hospital data and the sample collection period was limited. Therefore, caution must be exercised when generalizing the findings. The prevalence of dengue fever immunoglobulin may be higher in the population than what was assessed in our study, as we focused only on subjects presenting with febrile illness to the missionary hospitals. To obtain a more accurate estimate of prevalence and determine the circulating strain(s) in Enugu State, an extensive year-round study and a surveillance system targeting the population at risk are necessary. Additionally, it is crucial to investigate the most prevalent strain of the dengue virus in circulation in Enugu State and evaluate the extent of damage in patients with co-infection of dengue virus and malaria.

ACKNOWLEDGEMENT

The authors wish the express their gratitude to the management of the mission hospitals used in this study for their undiluted support during the process of sample collection. Also, we acknowledge the commitment of the molecular laboratory section of National Arbovirus and Vectors Research Centre (NAVRC), Enugu, for the thorough analysis of the samples upto the molecular level.

REFERENCES

[1] Adeleke, MA., Muhibi M.A., Ajayi E.I., Idowu OA., Famodimu M.I., Olaniyan, S.O., & Hassan A.N. (2016). Dengue virus specific immunoglobulin G antibodies among patients with febrile conditions in Osogbo, Southwestern Nigeria. *Tropical Biomedicine,* 33(1): 1–7.

[2] Arya C.S. Mehta K.L., Agarwal N., Argawal B.K., Mathai G., & Moondhara A. (2016). Episodes of concurrent dengue and malaria. *Dengue Bulletin.* 120(29): 208-209.

[3] Afolabi, L. O., Sani, M. & Okunowo, W. O. (2016). A Review on the Incidence, Interaction, and Future Perspective on Zika Virus. *Journal of Basic and Clinical Reproductive Sciences.* 5(2): 57–110.

[4] Ahmadu, S. M., Odekunle, B. O., Igwe, C., Nurudeen, A. A., Biobelu, A., & Usman, O. A. (2020). Prevalence and Determinants of Dengue Virus Immunoglobulin among Febrile Patients Attending Naval Medical Centre Victoria Island, Lagos State. *Global Biosecurity*, 3(1): 1-12.

[5] Ayorinde, A. F., Oboh, B. O. & Otubanjo, O. A. (2009). A survey of yellow fever vectors-Aedes mosquitoes in Lagos State. 4th University of Lagos conference book of proceedings. 63-73.

[6] Ayukekbong, J. A. (2014). Mini-Review Dengue Virus in Nigeria: Current Status and Future Perspective. *British Journal of Virology*. 1(3): 106–111.

[7] Baba, M., Logue, C. H., Oderinde, B., Abduimaleek, H., Williams, J., Lewis, J., Laws, T. R., Hewson, R., Marcello, A. D. & Agaro, P. (2013). Evidence of Arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. *Journal of Infections in Developing Countries.* 1(7): 51-59.

[8] Carme, B., Matheus, S., Donutil, G., Raulin, O., Nacher, M. & Morvan, J. (2009). Concurrent dengue and malaria in Cayenne Hospital, French Guiana. *Emerging Infectious Diseases.* 15: 668-671

[9] Center for Disease Control and Prevention (2016).*DengueCDC*.Available at:https://www.cdc.gov/dengue/clinicallab/clinic (Accessed: 21 July 2018).

[10] Center for Disease Control and Prevention (2010). "Chapter 5 – dengue fever (DF) and dengue hemorrhagic fever (DHF)". 2010 Yellow Book. Archived from the original on 29 December 2010. Retrieved 23 December 2010.

[11] Centre for Disease Control and Prevention (2011), "*NeglecteTropical Diseases"*. Archived from the original on 4 December 2014. Retrieved 28 November 2014.

[12] Charrel, R.N., Brouqui, P., Foucault, C., & De Lamballerie, X. (2005). Concurrent dengue and malaria. *Emerging Infectious Diseases.* 7 *(*11): 1153 - 1154.

[13] Chen, L. H. & Wilson M. E. (2010). Travel associated dengue infection in the United State 1996 to 2005. *Journal of Travel Medicine*. 17(4): 285.

[14] Chen, L. H. & Wilson, M. E. (2010). Dengue and chikungunya infections in travelers. *Current Opinion in Infectious Diseases.* 23(5): 438 - 44.

[15] Chukwuma, G. O., Audu, J. S., Chukwu, O. M., Manafa, P. O., Ebugasi, R. S., Akulue, J. C., Ahaneku, G. I., Nchidnda, G. W. & Esimole, C. O. (2018). Seroprevalence of dengue virus among children with febrile illness in Nnewi, Nigeria. *The Journal of Medical Research.* 4(1): 24–30.

[16] Deresinski S. (2006). Concurrent *Plasmodium vivax* Malaria and Dengue. *Emerging Infectious Diseases*. 1802:12.

[17] Domingues R.B. (2009). Treatment of viral encephalitis. *Central Nervous System Agents Medical Chempathology*. 9:56-62.

[18] Epelboin, L., Hanf, M., Dussart, P., Ouar-Epelboin, S., Djossou, F., Nacher, M. & Carme, B. (2012). Is dengue and malaria co-infection more severe than single infections? A retrospective matchec-pair study in French Guiana. *Malaria Journal*. 11:142.

[19] Gamil, M. A., Zaki, M.E., Saleh, A. E., & Basheer, A.S., (2014). Prevalence of dengue fever in Jizan area, Saudi Arabia. *Journal of Pure and Applied Microbiology* 8 (1): 225– 231.

[20] Gan V.C.H., and Leo Y.S. (2014). Current epidemiology and clinical practice in arboviral infections – implications on blood supply in South-East Asia. *ISBT Science Series.* 9: 262-267.

[21] Mohaptra, M., Patra, P. & Agrawda, R. (2012). Manifestation and outcome of concurrent malaria and dengue infection. *Journal of Vector Borne Disease*, 49: 262-265.

[22] Mustapha, J. O., Emeribe, A. U. & 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 - Research and Palliative Care.* 9: 145–151.

[23] Onochie-Igbinedion Ifeyinwa, Theresa Osarumwense, Ikegbunam Moses Nkechukwu, Ekpunobi Nzube Favour, Ibeagha Izuchukwu, Chukwuma Okechukwu George, Adikwu Micheal Umale, Esimone Charles Okechukwu (2022) "The Prevalence of Dengue Virus and Malaria Co-Infection among HIV-Infected Patients within South Eastern Nigeria *Advances in Infectious Diseases* Vol.12 No.1

[24] Otu, A., Ebenso, B., Etokidem, A., & Chukwuekezie, O. (2019). Dengue fever –an update review and implications for Nigeria, and similar ountries. *African Health Science Journal* 19 (2): 200-2007.

[25] Senn, N., Suarkia, D. L., Manong, D., Siba, P. M., McBride, W. J. H. (2011). Contribution of dengue fever to the burden of acute febrile illness in Paupa New Guinea:anage-specific prospective study. *American Journal of Tropical Medical Hygeine.* 85: 132-137.

[26] WHO (2018) Dengue vaccine: position paper. *Weekly Epidemiological Record. 36 (93): 457–76. 7.*

[27] WHO. (2011). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever – reversed and expanded edition. New Delhi: *WHO regional office for South-East Asia*. 14: 196.

[28] WHO. (2010). World malaria report 2010. Geneva. World Health Organization.

[29] WHO. 2015. World malaria report 2015. World Health Organization, Geneva

[30] WHO (2022). World Malaria Report 2022. Switzerland: World Health Organization.