Original Article

Risk Modelling and Seroprevalence of Dengue Virus Non-structural protein-1 Antigenemia and Antibodies among Febrile Patients in Ilorin, Nigeria

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Abstract

Background
Dengue fever is one of the global arboviral diseases posing significant health challenges, particularly in tropical and subtropical regions. However, there is limited data on the seroprevalence and risk factors of dengue virus infection among febrile patients in this region, and no studies have combined risk modelling and serological analysis for its disease surveillance.

Objectives
This study determines the seroprevalence and risk analysis of Dengue Virus Non-structural protein 1 antigen and Immunoglobulin G and M antibodies among febrile patients in Ilorin, Nigeria.

Methods
A cross-sectional study using a finite population correction (FPC) approach, where 182 blood samples were collected from consenting febrile patients and screened for DNV immunological markers. A structured questionnaire was used for socio-demographic and risk factors collation, while data analysis and Generalized Linear Model (GLM) were used for risk impact assessment on DNV outcome.

Results
Prevalence of DNV NS1 antigen, IgG and IgM antibodies was 36% (66), 32% (62) and 16% (29), respectively. At 26 years mean age, the highest prevalence was recorded among female subjects, the employed groups and participants with formal education. The effect of age and gender was statistically significant at $P<0.05$, while occupation was not. The GLM presented the combined risk factors as strong predictors of DNV infection outcome at p (Slope=0) = $2.9259\times10-9$.

Conclusions
High prevalence of dengue virus and significant association of risk factors was determined. Inclusion of DNV screening for febrile patients to reduce anti-malaria resistance is advocated.
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Keywords: Aedes Mosquito, Dengue, Malaria, Modelling, Resistance

Introduction

Dengue fever, a widely dispersed arboviral disease with 100 to 400 million infections per year, is transmitted by infected mosquitoes of the Aedes species (Aedes aegypti or Aedes albopictus).[1,2] Classical dengue fever, the most prevalent form of the disease, is characterized by a sudden onset of fever, headache, anorexia, malaise, muscle and joint pains, rash, and lymphadenopathy. [3] Dengue shock, which is characterized by severe organ dysfunction, plasma leakage, haemorrhaging, and symptoms like noticeably elevated liver enzymes and impaired consciousness, is a complication of severe dengue.[4,5]

Dengue virus poses a risk to about 2.5 to 3.6 billion people in over 125 endemic countries annually, which extends to a significant proportion of travellers to such countries. The increasing global prevalence in the past five years is pronounced in developed countries such as Asia, Bangladesh, Malaysia, Thailand and Vietnam, which may be attributed to proper reporting and diagnosis. As of 30 April 2024, over 7.6 million dengue cases have been reported to the WHO in 2024, with 3.4 million confirmed cases, over 16,000 severe cases, and over 3000 deaths. In Africa, earliest report was in South Africa in 1926, with initial isolation in West Africa in the 1960s. [6] Dengue fever is endemic in almost all of the states of Nigeria and may be the main factor in non-classifiable febrile illnesses. [7] A prevalence of subtype 3 of dengue virus was reported in Maiduguri (10.1%) and Ilorin (30.8%),[8,9] while among febrile patients, reports of 17.2% and 30.8% were recorded in southwestern Nigeria.[10,11] Further prevalence, such as 77% DNV IgG in Osogbo,[12] 77.1% DNV IgM in southeastern Nigeria[13] and 24.9% in Lagos[14] indicates the localized transmission of the virus in Nigeria. Recent data also supports this as evidenced in 44.2% reported in Oyo and 20.3% in Enugu state.[15,16]

Despite evidence of dengue's endemicity in Nigeria, there remain gaps in standardized seroprevalence data combining detection of NS1 antigen and antibodies. Additionally, no studies have explored risk prediction models for its surveillance or for mitigating misdiagnosis in febrile illness management. This study addresses these gaps by integrating serological testing with computational risk modelling in Ilorin, Nigeria.

Materials and Methods

Study Design and Sample Size

This was a purposeful cross-sectional study involving consenting febrile patients attending selected hospitals in Ilorin, Kwara State, Nigeria. These included both primary and secondary healthcare facilities within the metropolis, which are primarily assessed by residents seeking medical attention. An adjusted sample size of 182 was achieved via the finite population correction (FPC) approach.[17]

Study Population and Eligibility Criteria

Consenting febrile patients attending the selected health facilities were enrolled. These included patients with a body temperature of 37.5 °C, joint or muscle pain, headache, pain behind the eyes, nausea or vomiting, skin rash, and bleeding from the nose or gums. Excluded from the study were non-febrile patients, those who met the inclusion criteria but did not give consent, and patients with acute illness or cognitive impairment.

Sample Collection and Serological Assay for DNV Immunological Markers

About 5 ml of venous blood was collected from consenting participants into a vacutainer tube and allowed to clot. This was centrifuged at 5000 rpm for 10 minutes for serum separation into prelabeled plain tubes and stored at -20 °C for serological assay. The serological assay for dengue virus NS1 antigenemia, IgG and IgM antibodies was done, calculated and interpreted according to the instruction manual of SunLong Biotech Company Limited.[20]

Data Collection and Analysis

A pre-tested structured questionnaire was administered to obtain information on the socio-demography and risk factors.[18,19] The duly completed questionnaire was checked for response errors and processed for appropriate presentation in tables and charts using IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, NY, USA), R-studio and Microsoft packages. Associations between demographic/clinical factors and dengue markers (NS1, IgG, IgM) was analyzed using univariate logistic regression for categorical variables (gender, age groups, education, occupation) and chi-square/Fisher's exact tests for symptom associations. All models used female gender, oldest age cohort (51-70 years), no formal education, and unemployed status as reference categories, with odds ratios (ORs) and 95% confidence intervals calculated through maximum likelihood estimation. Statistical significance was set at $p<0.05$ and all analyses were conducted in SPSS 20.0 and replicated in R 4.0 for consistency.

Risk Factor Modelling for DNV infection

An extension of traditional linear model was employed to allow different distributions of dependent variables with a link function that can relate linear predictor to the distributions mean. Using Past4 version 4.14, set of risk factors from the questionnaire such as blood transfusion history, malaria parasite, residential area, typhoid fever, proximity to bushes and stagnant or uncovered water was used to examine the outcome of DNV infection in the location on Generalized Linear Model (GLM).

Ethical Consideration

Approval was granted by the Kwara State Ministry of Health (ERC/MOH/2023/02/091). Only patients (or guardians) who met the inclusion criteria and consented were enrolled after explanation of the study purpose.

Results

Prevalence of DNV Markers and Malaria Co-infection Patterns in Respondents

A total of 182 subjects participated in this hospital-based study, of which a significant prevalence of the viral protein was recorded. The prevalence of NS1 antigen, IgG and IgM antibodies was 36.7% (66), 34.4% (62) and 15.6% (28), respectively (Figure 1). Furthermore, 24 of the respondents were recorded to possess DNV IgG and IgM, accounting for a prevalence of 13.3%.

Figure 2 presents the co-infection of malaria and NS1 antigenemia, IgM and IgG immunoglobulin. Females recorded a prevalence of 21% (39/182) while males had 14.8% (27/182) for NS1 antigenemia (Table 1) while DNV IgG and IgM were 22.5% (41/182), 11.5% (21/182) and 7.1% (13/182), 8.2% (15/182) respectively (Table 2).

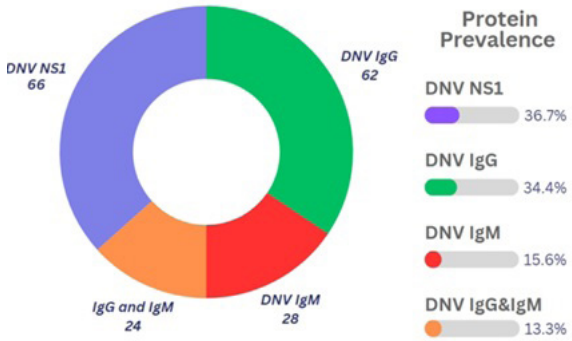

Figure 1. Prevalence of Dengue virus Seromarkers among the Participants

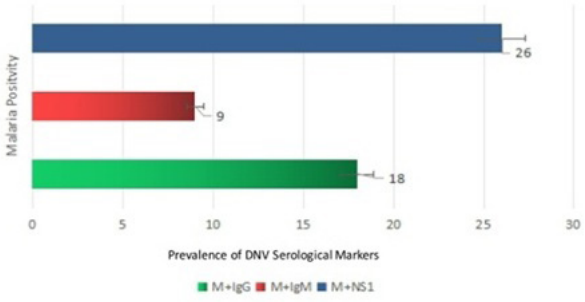

Figure 2. Prevalence of Dengue Virus Seromarkers with Malaria Positivity

Table 1. Demographic Characteristics and Their Association with DNV NS1-Antigen Prevalence

Demographic Characteristics	n	NS1 (%)	P Value	Odds Ratio	CI: 95%
Gender					
Male	66	27(14.8)	0.290	1.28	0.67-2.44
Female	116	39(21.4)			
Total	**182**	**66(36.2)**			
Age group (years)					
<10	39	9(4.9)	<*0.001	0.66	0.50-0.90
11-20	83	31(17)			
21-30	44	14(7.7)			
31-40	4	4(2.2)			
41-50	8	8(4.4)			
51-60	3	1(0.5)			
61-70	1	0(0)			
Total	**182**	**67(36.8)**			
Formal education					
Yes	114	38(20.9)	0.407	2.33	0.32-16.96
No	68	28(15.4)			
Total	**182**	**66(36.3)**			
Occupation					
Employed	116	38(20.9)	0.423	0.52	0.19-1.47
Unemployed	9	4(2.2)			
Student	57	24(13.2)			
Total	**187**	**66(36.3)**			

*Statistical significance; n, number of participants; CI, 95%confidence interval

DNV Infection in Correlation to Demographic Factors

The mean age of the study subjects was 26 years, as shown in Table 1, prevalence was highest (17%) in the age group 11-20 years, while subject aged 61-70 years had the lowest prevalence of (0%) for DNV NS1. For DNV IgG and IgM, respectively (Table 2),the 11-20 and <10 years' age groups had the highest prevalence of 12.6% and 5%.

Subjects with formal education had a high prevalence of 20.9% (38/182) for NS1, while respondents without formal education had a higher prevalence of 8.8% (16/182) for DNV IgM, respectively (Tables 1 and 2). Concerning occupational status, prevalence was high for the employed group followed by the student group (Tables 1 and 2).

Table 2. Relationship between Socio-Demographic Characteristics and Distribution Pattern of Dengue Virus Antibodies Among Febrile Patients

Demographic Characteristics	DNV IgG+				DNV IgM+			
	n (%)	p-value	OR	CI:95%	*n* (%)	p-value	Odd Ratio	CI:95%
Gender (n)								
Male (66)	21(11.5)	0.69	0.66	0.33-1.40	15(8.2)	0.04*	1.96	0.81-4.75
Female (116)	41(22.5)				13(7.1)			
Total (182)	**62(34)**				**28(15.3)**			
Age group (years-n)								
<10 (39)	20(11)	0.01	0.10	0.38-0.70	11(6)	0.03*	0.46	0.32-0.65
11-20 (83)	23(12.6)				9(5)			
21-30 (44)	12(6.6)				4(2.2)			
31-40 (4)	1(0.5)				1(0.5)			
41-50 (8)	4(2.2)				1(0.5)			
51-60 (3)	1(0.5)				1(0.5)			
61-70 (1)	1(0.5)				1(0.5)			
Total (182)	**62(33.9)**				**28(15.2)**			
Formal Education (n)								
Yes (114)	31(17)	0.11	1.91	0.20-18.3	12(6.6)	0.03*	0.46	0.32-0.65
No (68)	31(17)				18(8.8)			
Total (182)	**62(34)**				**28(15.4)**			
Occupation (n)								
Employed (116)	34(18.7)	0.13	022	0.07-0.70	17(9.3)	0.31	0.00	
Unemployed (9)	5(2.7)				3(1.6)			
Student (57)	23(12.6)				8(4.4)			
Total (182)	**62(34)**				**28(15.3)**			

*Statistical significance at P<0.05; CI, 95% confidence interval

Association of DNV Infection Prevalence with Risk Factors in Study Participants

The evaluated risk factors for the model about DNV infection presented a higher prevalence among subjects with no history of blood transfusion at 25.8% (47/182) for NS1 and 23.6% (43/182) for DNV-IgG, while subjects with a blood transfusion history were low. Subjects with no malaria parasite had the highest prevalence for NS1 at 16% (29/182), and for IgG at 19.8% (36/182) while those with malaria parasite had the lowest prevalence. Based on residential distribution, participants from urban area had prevalence of 27% (49/182) for NS1 and 11% (20/182) for IgG compared to rural area having 9.3% and 2.2% respectively. Subjects with no typhoid fever presented prevalence of 31.9% (58/182) for NS1 and 22% (40/182) for IgG. Subjects who live far away from the bush had prevalence of 19.23% (35/182) and 7.1% (13/182) for NS1 and IgG while those nearer 17.0% (31/182) and 2.2% (4/182) respectively. NS1 and IgG prevalence was 28.6% (51/182) and 7.7% (14/182) for participants who reside closer to stagnant water while 11% (20/182) and 2.2% (4/182) were recorded for those farther. Figure 3 presents the GLM for the risk factor analysis using NS1 and IgG for outcome prediction. The “y” which represents the outcome of interest i.e. probability or presence of DNV infection in the populace is “0.23805x-0.16232”, the “x” represents

the linear combination of the presented risk factors while the intercepts recorded as "-0.16232" and slope at "0.23805" depicts the expected value of "y" when all risk factors are at "0" and the rate of change in "y" with respect to changes in the combined risk factors respectively. Thus, for every unit increase in combined risk factors of DNV infection, the "y" increases by "0.23805". The model also presents a highly significant statistical correlation of the risk factors to the outcome of infection, denoted by the small P value at p (Slope=0) = 2.9259×10−9.

Association of Clinical Symptoms with DNV Infection Prevalence

The analysis of clinical symptoms and dengue virus (DNV) positivity among febrile patients showed varying prevalence rates (Table 3). Patients with headaches had a DNV prevalence of 10.4%, but there was no significant association between the symptom and DNV positivity. Similarly, fever had a prevalence of 13.2% among those affected, with no statistically significant link to DNV positivity. Fatigue showed a prevalence of 4.4% while dizziness was 3.3% without a significant association with DNV positivity.

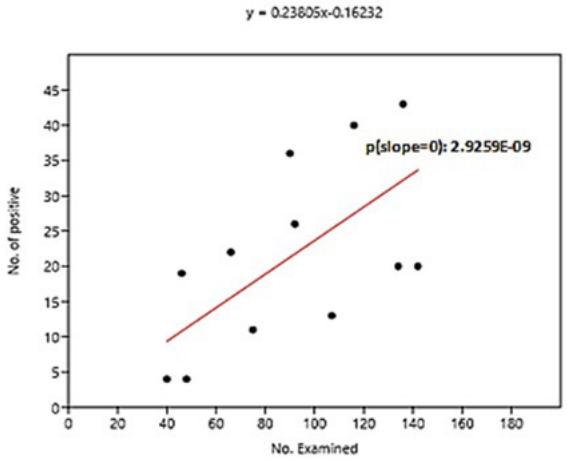

Figure 3. Generalized Linear Model for Selected DNV Risk Factors

Table 3. Seroprevalence of Dengue Virus NS1 antigenemia about Some Clinical Symptoms among Febrile Patients Attending Some Selected Hospitals in Ilorin

Clinical symptoms	n	No. of positive DNV (%)	X^2	p-value	OR	CI: 95%
Headache						
Yes	59	19 (10.4)	0.69	0.41	0.78	0.14-4.41
No	122	47 (25.8)				
Fever						
Yes	66	24 (13.2)	0.01	0.92	3.13	0.69-14.23
No	116	42 (23.1)				
Fatigue						
Yes	36	8 (4.4)	3.83	0.50	0.29	0.49-1.80
No	146	58 (31.9)				
Dizziness						
Yes	27	6 (3.3)	2.7	0.1	0.81	0.13-5.12
No	155	60 (33)				

CI- confidence Interval: OR- odd ratio

Discussion

This study determines the seroprevalence and risk analysis of DNV NS1 antigen, IgG and IgM antibodies among febrile patients in Ilorin, Nigeria, while using modelling technique for predictive analysis. Of the 182 respondents, prevalence of DNV NS1 antigen was the highest (66) while the least was IgM seropositivity (28) where females had higher prevalence. The mean age was 26years where 11 to 20 years had the highest prevalence, and individuals with formal education and urban residency exhibited increased rates. Participants without blood transfusion history and those living near stagnant water or bushes were more affected. Clinical symptoms such as fever, headache, fatigue, and dizziness showed varying DNV prevalence but lacked significant statistical associations. A GLM model confirmed a strong correlation between combined risk factors and DNV infection.

The prevalence of dengue virus reported in this study at 36.7% (66), 34.4% (62) and 15.6% (29) for NS1, IgG and IgM is lower than the previously reported prevalence at Osogbo; [12] 78.3% in Kano Metropolis, Nigeria,[21] and the 77.1% in Nnewi, Nigeria.[13] It is, however, higher than another report of 30.8% prevalence.[22] The reported prevalence differences may be adduced to the difference in sample size, varying environmental conditions and geographical location of study. [23] The significant prevalence of the dengue-specific NS1 antigen indicates a continuous infection with viral replication that is active, where signs of acute dengue fever could appear during this time. The presence of IgM in the blood of these patients suggests recent viral infection or re-infection that could result from contact with its vector through bite, while IgG indicates previous infection.[24]

The most virus-positive based on past exposure (IgG) evaluation, were female, 22.5% compared to the male counterpart (11.5%), while based on current exposure (IgM), males had higher seroprevalence (8.2%) than female (7.1%), this observation agrees with an earlier report,[10] but disagrees with another[24] that observed higher IgM in females than males which may be due to the masculinity of men that tends to wait until they have serious health issues before visiting the hospital.
Despite the insignificant statistical correlation between age and dengue fever virus, it was found that participants under the age of 10 years (6%) had a higher prevalence and patients above the age of 31 years had the lowest frequency. This observation is in consonance with a report that observed that children aged 0 to 15 years had the highest prevalence of anti-dengue virus IgM antibodies.[21] Although, other studies reported that the frequency of dengue virus was higher among adults aged 26 to 35 years. [25,26,27]

In relation to occupation, the employed had the highest prevalence of dengue virus IgM antibody (9.3%), while the unemployed had a prevalence of 1.6% which agrees with another report.[10] The difference between DNV prevalence of employed and unemployed group of respondents could result from increased probability of exposure to vector or any other route of transmission during their daily job activities. A higher incidence was detected among urban than rural residents and this aligns with a report that urban inhabitants had the greatest incidence of anti-dengue IgM antibodies.[10] This may be the result of poor urban planning, which provides mosquitoes with more locations to breed or other transmission route yet to be documented. Industrialization has been posited to significantly affect vector control and speed up the transmission of the virus in urban areas. Rapid urbanisation in Africa has increased vector density because of human activities that promote mosquito breeding.[28] This also correlates with model prediction where residential area was posited to be a significant factor due to varying levels of risk to vector exposure and public health infrastructure. It further presents the proximity to breeding site evaluated as bushes and stagnant water in the model. Residing close to bushes or stagnant water would increase exposure to mosquito bite and thus increases the risk of DNV infection.

The outcome of this model evaluation also aligns with report that highlighted the relationship between arboviral infection to breeding site.[29]

The findings of this study indicated that 16% of participants registered at the selected hospital in Kwara State, Nigeria had been exposed to the dengue fever virus, as shown by the presence of IgM antibodies in their serum. According to another study the presence of IgM in the blood of these individuals indicates a recent virus infection and a high frequency of vector mosquito bites.[24] Malaria and dengue co-infection may be risk factors for dengue infections because Malaria and dengue are transmitted by mosquitoes that multiply rapidly during rainy seasons and thus may co-occur temporally.[30] Anopheles and Aedes mosquitoes have been repeatedly documented to exist in the southeastern region.[31] Thus, exposure to both dengue and malaria can occur simultaneously, leading to co-infection in the same person and one being a risk factor for the other. This can pose a public health challenge due to the symptom overlap, diagnostic bias and resource constraints for proper screening of the infectious agent.

The presence of dengue and malaria is predicted which also aligns with the study's findings. The model outcome showed a highly significant correlation where the p-value is extremely small suggesting that as linear correlation, the risk factors have strong association with outcome of infection. Deducing from the model outcome, blood transfusion is a significant predictor of DNV infection but can be influenced if the transfusion is not within area of high DNV prevalence.The model also supports that; the presence of malaria parasite can exacerbate the outcome of DNV or be mistaken based on symptoms similarity. Similar misinterpretation is also posited from the model outcome where significant prevalence of typhoid fever as a measured factor could indicate marker of poor sanitation which can highly increase risk of DNV infection.

This finding is significant since Nigeria is one of the few African nations that only examines typhoid and malaria in clinical studies of febrile diseases, completely ignoring viral infections. A technical approach to the prevention and management of dengue and malaria is sustainable vector control. Because these illnesses are endemic in Nigeria, management of mosquito breeding places and/or bites must be consistently emphasized at all levels of the population.

Limitation and Strength of the Study

Although the sample size was adequate as it provides valuable insight into DNV prevalence, it may not be large enough to generalize findings across the entire population of Ilorin and the geographical scope was also limited to selected health facilities in Ilorin, which may not fully capture the epidemiology of dengue in other regions. Additionally, the cross-sectional design only allowed for the identification of associations between risk factors and dengue virus infection, not causation. The information through questionnaires may have been subject to recall bias, particularly regarding socio-demographic and risk factor data. The combination of NS1 and the IgG/IgM antibodies ensures a comprehensive immunological profile, but reliance on serological markers alone may not be sufficient for deeper analysis of the virus, use of molecular techniques could provide other insights on strain distribution. Data-driven insight through model-based analysis presented an application of predictive tool in public health.

Conclusion

Data obtained from this study indicates that dengue fever virus is in circulation among people in Ilorin, Nigeria with higher prevalence in female. This prevalence poses important public health implications where majority of participants had detectable dengue antigen and immunoglobulins. Additionally, co-infection with malaria was confirmed posing medication risk that may encourage anti-malarial resistance since

there exist no DENV screening for febrile patients in Nigeria. Furthermore, The GLM presents a significant relationship between the combined effect of the selected risk factors and the outcome of DENV infection where the highly significant p-value indicates that these factors, collectively, are strong predictors of the outcome. Although, the model presented data-based insights for the public health, casualty cannot solely be inferred as it shows the association and not the causation. There is need for replication the larger cohorts to address potential confounding factors and borderline association of statistical significance. These findings demand urgent policy changes such as routine dengue testing for febrile patients, clinician training on differential diagnosis, and public health campaigns addressing co-circulating diseases. Nigeria's healthcare system must adapt to this dual disease burden to reduce preventable morbidity and antimicrobial misuse.

Disclosure of conflicting interest
The authors have none to declare.

Authors' contribution
MBO conceptualized this research and put up the original draft of the manuscript. SMM curated the data, reviewed and edited the manuscript. AOU supervised the research and proof read the manuscript. OMO enrolled and collected samples from participants and conducted the laboratory research under supervision and OSO administered questionnaires and involved in the laboratory research under supervision. Final version of the manuscript was reviewed and approved by all the authors.

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