The Prevalence of Dengue Virus and Malaria Co-Infection among HIV-Infected Patients within South Eastern Nigeria

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Abstract

**Background:** Dengue and malaria are infections of great public health concern, especially in tropical countries like Nigeria where the burden of HIV infection is high. This study was conducted to determine the seroprevalence of dengue virus and dengue/malaria coinfection among febrile HIV-infected patients attending the University of Nigeria Teaching Hospital and Nnamdi Azikiwe University teaching university both in the southeastern region of Nigeria. **Methods:** In this cross-sectional study, blood samples from 338 consenting HIV-infected patients were collected and tested for plasmodiasis and DENV using malaria microscopy and ELISA, respectively. Interviewer-based questionnaires were used to assess subjects’ sociodemographic variables and dengue risk factors. This study was conducted within the peak period of the dry season (January-February 2016). **Results:** Of the 338 screened participants, 13.02% were seropositive for DENV, whereas 55.8% were positive for Plasmodium spp. About 2.7% were positive for both dengue virus and Plasmodium spp. The overall seroprevalence of dengue virus by NS1 antigenemia, IgM and IgG antibodies was 9.5%, 5.6% and 8%. **Conclusion:** The high prevalence of malaria and DENV indicates the need to strengthen vector control and dengue surveillance programs.

Keywords

Dengue, Malaria, Coinfection, Seroprevalence, Southeast Nigeria

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1. Introduction

The spread of vector borne diseases has drawn great concern worldwide particularly in tropical and subtropical regions because of their recurring outbreaks [1]. Some of these diseases are now endemic in certain areas causing millions of cases every year [2]. The most common of these diseases includes Malaria and Dengue spread by mosquito bites. Malaria has been long recognized as a significant public health threat with around 212 million cases reported in 2015 alone [3].

Dengue fever, also known as break bone fever, is an infectious tropical disease transmitted by Aedes mosquitoes, particularly A. aegypti. It is classified among the Neglected Tropical Diseases (NTDs). There are four strains of the virus, referred to as DENV-1, DENV-2, DENV-3, and DENV-4. All four serotypes can cause the complete spectrum of disease. Dengue hemorrhagic fever occurs when a person contracts a different strain of Dengue virus after a previous infection with a different strain [4] [5] [6] [7].

Malaria fever on the other hand is a mosquito borne parasitic infectious disease of humans caused by the bite of female Anopheles mosquito infected with a parasite of the genus Plasmodium. Among the five species of plasmodium which will infect humans, are P. falciparum, P. knowlesi, P. malariae, P. ovale and P. vivax. Severe disease in humans is essentially caused by Plasmodium falciparum. It is a significant health problem in countries of the tropics affecting over 240 million people, accounting for over 40% of the world’s population in more than 100 countries in the tropics, from South America to the Indian Peninsula [8] [9].

In Nigeria, over 95% malaria infection is due to Plasmodium falciparum with P. ovale and P. malaria playing a minor role, while P. vivax is not found among indigenous Nigerians. Malaria is responsible for 30% of childhood mortality, 11% of maternal mortality, and more than 50% of outpatient visits in hospitals. A total of 70 - 110 million clinical cases per annum are recorded. Nigeria accounts for 25% of all cases of malaria in Africa and at least 250,000 Nigerian children below 5 years die yearly from malaria [10] [11].

According to the WHO, World Malaria Report, there were 241 million cases of malaria in 2020, a 14 million increase from the previous year. In 2020, malaria is estimated to kill 627,000 people, with four African countries accounting for over half of the deaths. Nigeria ranks the highest with 31.9% of the overall death rate [12].

The dengue virus has been characterized as an important flavivirus that confers a protective role against HIV by transiently inhibiting its replication. Coincidentally, all four serotypes of DENV circulate in Nigeria. DENV and HIV coinfection would be an important factor for delayed progression to AIDS [13].

Arboviruses are widespread in Nigeria considering that the mosquito vectors responsible for the transmission of dengue, yellow fever, chikungunya (Aedes spp) and those responsible for malaria (Plasmodium spp) are well established in the country [14]. Malaria and Dengue virus fever are the most prevalent world-
wide and are found in similar places resulting in co-infections [15]. They both have similar signs and symptoms hence one can be mistaken for the other except if proper diagnosis is made. Also, Nigeria is one of the tropical countries where HIV, malaria and dengue co-exist as important infectious diseases. However, few data exist on dengue-malaria co-infection in HIV seropositive patients. Co-infections of this category could have fatal outcomes if left undiagnosed and untreated. Thus, this study aims to investigate the incidence of co-infections with DENV and malaria parasites among febrile patients attending teaching hospitals in Southeast Nigeria.

2. Materials and Methods

2.1. Study Design

This study adopted a cross-sectional design that included prospective hospital-based surveillance of cases among febrile participants attending two major health facilities within the southwest region of Nigeria. This study was conducted within the peak period of the dry season (January-February 2016). Interviewer-based questionnaires were used to assess subjects’ sociodemographic variables and dengue risk factors.

2.2. Study Area

The University of Nigeria Teaching Hospital (UNTH) located in Enugu Nigeria and Nnamdi Azikiwe University Teaching Hospital situated at Nnewi were the major health facilities the study was carried out.

The University of Nigeria Teaching Hospital (UNTH) is located in Enugu Nigeria (6.44˚N 7.5˚E 192 m). Enugu State is one of the states in the eastern part of Nigeria with a population of 3,267,837 people at the census held in 2006 (estimated at over 3.8 million in 2012). The mean temperature in Enugu State in the hottest month of February is about 87.16˚F (30.64˚C), while the lowest temperatures occur in the month of November, reaching 60.54˚F (15.86˚C).

Nnamdi Azikiwe University Teaching Hospital is situated at Nnewi. Nnewi, is the second largest city in Anambra State in Southeastern Nigeria. As of 2006, Nnewi has an estimated population of 391,227 according to the Nigerian census. The city spans over 1076.9 square miles (2789 km²) in Anambra State. Geographically, Nnewi falls within the tropical rain forest region of Nigeria. The city is located east of the Niger River, and about 22 kilometers south east of Onitsha in Anambra State, Nigeria.

2.3. Study Population

A total of 338 HIV-infected participants attending the two university teaching hospitals within the southeast region of Nigeria were recruited for this study. The inclusion criteria, which were based on their medical history, being seropositive for HIV and malaria symptomatology, included ongoing febrile illness, suspected to be malaria, pyrexia of unknown origin at the two university teach-
ing hospitals within the southeast region of Nigeria. This category of subjects was selected because the prodrome stage of Dengue and these diseases are similar. The study population included males and females from all age groups.

2.4. Sample Collection

The clinical manifestation of malaria and dengue are similar so blood samples for the diagnosis of DENV infections and for blood smears (thick and thin) and RDT for malaria diagnosis were collected from HIV seropositive patients. A total of 338 blood samples were collected and used for differential diagnosis in the study. The blood samples were collected into EDTA bottles from each participant using a needle and syringe and were immediately transported in a cold chain to the Microbiology Laboratory. Each bottle was labeled indicating their age, sex and location. Blood samples were shared into 2 EDTA bottles and one to be used for DENV ELISA and the other for malaria.

2.5. Sample Processing

The 338 blood samples were analyzed in the laboratory by rapid diagnostic test (RDT) methods and the enzyme linked immunosorbent assay (ELISA) method detection of dengue. RDT and microscopic methods were used to detect the malaria parasite and to confirm malaria presence and co-infection in the study participants too.

2.5.1. Detection of DENV

DENV diagnosis was made by the detection of an IgM, IgG antibody and NS-1 antigen in blood serum by RDT using the One step dengue ns1 antigen rapid test kit (Qingdao Hightop Biotech co. LTD, China); One step dengue AB-IgG/IgM rapid test kit (Qingdao Hightop Biotech co. LTD., China), the human dengue virus NS1 (DV NS1) ELISA kit (Biosino Biotechnology and Science Inc.) following the manufacturer’s instructions. The optical density was measured at 450 nm in the ELISA reader (Titertek Multiskan Plus, Finland, type-314).

2.5.2. Detection of Malaria Parasite

Whole blood samples were analyzed for malaria parasite using Rapid diagnostic kits (SD BIOLINE) Malaria Antigen P.f/P.v test (Standard Diagnostics Pvt. Ltd., Gurgaon, Haryana, India) CareStart Malaria HRP2 (Access Bio, Inc., USA), Partec Cyscope fluorescent microscopy (Partec GmbH, Germany) following the manufacturer’s instruction. Thin films were fixed with methanol and stained with 3% Giemsa stain at pH 7.0 for 30 min for the identification of haematozoa.

2.6. Data Analysis

Data was expressed as mean and standard deviation for continuous variables and percentage for categorical data. Chi Square test of association was used to assess the relationships between groups. Independent sample t-test was used to compare two groups of continuous data while chi square (goodness of fit) test was
used to compare categorical data. The test of significance was set at $p < 0.05$. All data were analyzed using both SPSS Software version 20.0 and Graph Pad Prism software, version 8.0.

3. Result

3.1. Study Population

A total number of 338 human blood samples of people living with HIV having malaise during the study period were examined for dengue and malaria infection as shown in Table 1.

3.2. Clinical Characteristics of the Population

Figure 1 shows that the study participants exhibited typical non-differentiable symptoms of dengue and malaria and no severe symptoms of either diseases.

3.3. Dengue Seroprevalence

The overall positive seroprevalence for DENV in the study population was 13.02% using the NS1 ELISA method as standard.

A total of forty-four (44) study participants tested positive for dengue in both study sites combined (Figure 2 and Figure 3). Figure 2 shows the distribution of DENV Prevalence among HIV-infected patients while Figure 3 points out the seropositive Prevalence of DENV in the study population stratified by study locations. Dengue prevalence in UNTH was 15 (34.09%) while that of NAUTH was 29 (65.91%).

Figure 4 shows that out of the 338 patients that were included for this project, the prevalence of Dengue virus NS1 antigenaemia, IgM and IgG seropositivity were 8.9%, 5.6% and 8.0%, respectively (Figure 4). There were no significant differences observed in dengue NS1 antigenemia vs. antidengue IgM seropositivity ($p = 0.439$); dengue NS1 antigenemia vs. antidengue IgG seropositivity ($p = 0.808$); and antidengue IgM vs. antidengue IgG seropositivity ($p = 0.593$) respectively.

Out of the 338 study participants, 55.8% of the participants tested positive for malaria parasite. Hence, as is expected, the prevalence of malaria was high because malaria is endemic in Sub-Saharan. Data indicated that 9 (2.66%) of the HIV-infected patients tested positive for malaria parasite and DENV. Thus, 35 (79.5%) of the patients who tested positive for dengue virus, tested negative for malaria. Of the 294 that tested negative for dengue virus, 115 (39.7%) tested positive for malaria parasite, while 179 (60.9%) tested negative (Table 2).

The prevalence of malaria and dengue co-infection in the two study locations in South East Nigeria was 2.66% of the study population. This confirms the endemicity of both dengue and malaria and their co-existence in HIV-infected patients. No significant association was observed between dengue IgG, NS1 and the demographic parameters while age affected IgM and malaria.
Table 1. Demographic characteristics of participants.

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>STUDY SITES</th>
<th></th>
<th></th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNTH</td>
<td>ENUGU</td>
<td>NAUTH</td>
<td>NNEWI</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>67 (31.5%)</td>
<td>69 (55.2%)</td>
<td>136 (40.2%)</td>
<td></td>
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<tr>
<td>Females</td>
<td>146 (68.5%)</td>
<td>56 (44.8%)</td>
<td>202 (59.8%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 - 19</td>
<td>2 (0.9%)</td>
<td>6 (4.8%)</td>
<td>8 (2.4%)</td>
<td></td>
</tr>
<tr>
<td>20 - 24</td>
<td>1 (0.5%)</td>
<td>6 (4.8%)</td>
<td>7 (2.1%)</td>
<td></td>
</tr>
<tr>
<td>25 - 29</td>
<td>18 (8.5%)</td>
<td>12 (9.6%)</td>
<td>30 (8.9%)</td>
<td></td>
</tr>
<tr>
<td>30 - 34</td>
<td>44 (20.9%)</td>
<td>9 (7.2%)</td>
<td>53 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>35 - 39</td>
<td>48 (22.7%)</td>
<td>17 (13.6%)</td>
<td>65 (19.3%)</td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>98 (46.4%)</td>
<td>75 (60.0%)</td>
<td>173 (51.5%)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>26 (12.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>125 (60.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separated</td>
<td>2 (1.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>4 (1.9%)</td>
<td></td>
<td></td>
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<tr>
<td>Widowed</td>
<td>51 (24.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>11 (5.3%)</td>
<td>0 (0%)</td>
<td>11 (3.3%)</td>
<td></td>
</tr>
<tr>
<td>Trader</td>
<td>105 (50.2%)</td>
<td>114 (91.2%)</td>
<td>219 (65.6%)</td>
<td></td>
</tr>
<tr>
<td>Civil Servant</td>
<td>29 (13.9%)</td>
<td>2 (1.6%)</td>
<td>31 (9.3%)</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>19 (9.1%)</td>
<td>0 (0%)</td>
<td>19 (5.7%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>27 (12.9%)</td>
<td>9 (7.2%)</td>
<td>36 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>Politician</td>
<td>18 (8.6%)</td>
<td>0 (0%)</td>
<td>18 (5.4%)</td>
<td></td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christianity</td>
<td>208 (98.1%)</td>
<td>124 (99.2%)</td>
<td>332 (98.5%)</td>
<td></td>
</tr>
<tr>
<td>Islam</td>
<td>4 (1.9%)</td>
<td>1 (0.8%)</td>
<td>5 (1.5%)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibo</td>
<td>203 (96.2%)</td>
<td>123 (98.4%)</td>
<td>326 (97.0%)</td>
<td></td>
</tr>
<tr>
<td>Hausa</td>
<td>3 (1.4%)</td>
<td>1 (0.8%)</td>
<td>4 (1.2%)</td>
<td></td>
</tr>
<tr>
<td>Yoruba</td>
<td>1 (0.5%)</td>
<td>0 (0%)</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4 (1.9%)</td>
<td>1 (0.8%)</td>
<td>5 (1.5%)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Formal</td>
<td>20 (9.5%)</td>
<td>0 (0%)</td>
<td>20 (6.0%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>51 (24.2%)</td>
<td>9 (7.2%)</td>
<td>60 (17.9%)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>91 (43.1%)</td>
<td>103 (82.4%)</td>
<td>194 (57.7%)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>49 (23.2%)</td>
<td>13 (10.4%)</td>
<td>62 (18.5%)</td>
<td></td>
</tr>
<tr>
<td>Nationality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigerian</td>
<td>209 (98.6%)</td>
<td>125 (100%)</td>
<td>334 (99.1%)</td>
<td></td>
</tr>
<tr>
<td>Non-Nigerian</td>
<td>3 (1.4%)</td>
<td>0 (0%)</td>
<td>3 (0.9%)</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2. Prevalence of malaria and dengue co-infection in the two study locations in South East, Nigeria.

<table>
<thead>
<tr>
<th>Denv Fever Test</th>
<th>Malaria Tests Outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (20.5%)</td>
<td>35 (79.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>115 (39.1%)</td>
<td>179 (60.9%)</td>
</tr>
<tr>
<td>Overall</td>
<td>124 (36.7%)</td>
<td>214 (64.8%)</td>
</tr>
</tbody>
</table>

Figure 1. Clinical characteristics of the study population.

Figure 2. Distribution of DENV Prevalence among HIV-infected patients.

Figure 3. Seropositive prevalence of DENV in the study population stratified by study location.
4. Discussion

In Nigeria, most people only associate mosquitoes with malaria. Most other arboviruses are considered alien and inconsequential thereby positioning dengue really as a neglected tropical disease indeed with very low awareness, no health policies nor significance in the health sector. Even the NCDC does not recognize dengue in its national disease listing. Dengue fever infection has always been considered an emerging public health problem in several African countries and tropical regions with risk of severe infections [16] [17]. Most febrile cases are routinely diagnosed and treated for typhoid and/or malaria without proper investigation for other conditions including viral infections.

This study was carried out to determine the prevalence of dengue fever and malaria co-infection among HIV-infected patients recruited in the teaching hospitals in the southeastern part of Nigeria. This current study reported a prevalence of 13.02% for DENV among the participants and a 2.66% co-infection rate of Malaria and DENV. The prevalence of Malaria was however found to be 55.8% which is far higher than the prevalence of DENV within the southeastern region.

The observed cumulative prevalence of dengue fever (13.02%) among our subjects is similar to findings in the north southern Nigeria where a prevalence of 18% was reported [18]. These findings confirmed the assumption that the Aedes species exists in the south eastern part of Nigeria and that the DENV virus is in circulation among Nigerians. The prevalence in this study is however lower than findings in Jordan and Lagos where a prevalence of 24.6% [19] and 24.9% [20] were reported respectively.

Over the years, there has been less attention on dengue fever occurrence among Nigerians. The observed prevalence however shows the need for improved intervention measures towards vector control and entomology studies in Nigeria to ascertain circulating mosquito species.

The prevalence of DENV was lower than the prevalence of malaria parasites among our study participants. This finding is dissimilar with findings from the
work by Adeleke et al. [21], Mustapha et al. [22] and Chukwuma et al. [23] in Southwestern, Northcentral and Southeastern Nigeria respectively where higher dengue fever prevalence was reported in their studies. Variations in the observations may be due to differences in the distribution of DENV vector, varying environmental conditions, sample size as well as presence and rate of use of malaria preventive intervention services. However, the findings of lower prevalence of DENV corroborate conclusions from other studies [24] [25].

Nine (2.66%) of the study participants had both malaria parasite and DENV immunoglobulins. This finding is similar to DENV-Malaria co-infection as reported by Adeleke et al. [21] and Chukwuma et al. [23] in different parts of Nigeria. This confirms the assumption that there is DENV-Malaria coinfection among febrile patients in Nigeria.

Globally and particularly in tropical countries (Nigeria inclusive) of the world, several factors have been ascribed to the prevalence of DENV in humans globally. Living in endemic areas of the tropics is an essential predisposing factor [26] [27].

Dengue fever infection is a viral infection that presents diversely, and clinical symptoms may range from mild febrile illness to severe plasma leakage with hemorrhagic manifestations. Common symptoms may include non-specific fever, with two or more of any of the following: headache, retro-orbital pain, arthralgia, muscle pain, rash with no localized signs or symptoms [28]. Some of these features were seen in the study participants for this study.

Viral infections are known to suppress the natural immunity of the individuals affected and this in turn, predisposes the individual to opportunistic infections [18]. Thus, coinfection with DENV, HIV, and malaria as seen in this study could be very deleterious to the immunosuppressed individuals. The inability to mount immune response by producing sufficient serum levels of IgG in the presence of increasing pathogenic burden becomes an issue for such individuals.

The transmission rate of malaria and Dengue are impacted by favorable environmental conditions for the vectors breeding (stagnant water) as both malaria and dengue are transmitted by the bite of insects (Anopheles and Aedes respectively). Malaria and HIV coinfection may be risk factors for dengue infections or vice-versa. Malaria and dengue are transmitted by mosquitoes that multiply rapidly during rainy seasons and thus may co-occur temporally. Thus, exposure to malaria can be concurrent to exposure to dengue, the reason for coinfection in the same person, and one being the risk factor for another. On the other side, HIV may be a risk factor for dengue and the reverse may apply, given that both viruses target the same cells in the body [29].

The findings in the study are not shocking, because Anopheles and Aedes mosquitoes have severally been reported to be in existence in the southeastern region, hence, the presence of dengue and malaria is expected. This result is crucial as Nigeria is one of the few African countries that limit the clinical investigation of febrile illnesses to malaria and typhoid with absolute negligence of viral
infections. Sustainable vector control is a technical strategy for malaria and dengue prevention and control. With the current endemicity of both infections in Nigeria, prevention of mosquito breeding sites and/or bites must be continually emphasized at all community levels.

5. Limitation of Study

This study is an institution-based study and the period of sample collection was not all year round. Hence, generalization of findings needs proper consideration. Dengue and Malaria prevalence could be greater among the population than assessed as our study focused on HIV-infected subjects. An all year study and a surveillance system with focus on population at risk maybe needed for the determination of actual prevalence.

6. Conclusions

The study made it clear that dengue is endemic in South East Nigeria and has been around for some time without receiving proper attention and co-infection with malaria is substantive. Despite the substantial prevalence of dengue and dengue-malaria with HIV in the very few parts of Nigeria where studies have been done, knowledge of dengue fever disease is still very low, even among health workers. There may actually be more diseases from the same mosquito vectors around. Sanitation, urban planning, government policies, public enlightenment can address some of the issues if well-articulated.

More research is also needed, into the prevalence of dengue in the entire 36 states in Nigeria, impact of climate change on dengue transmission, the true economic costs of the disease burden and the relative effectiveness of interventions, in order to provide the evidence, base for planning, implementation and advocacy.

In addition to already existing treatment and control measures, new tools are required, such as effective vaccines, new treatments and innovative preventive measures.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

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