Detection of IgM and IgG Dengue antibodies in febrile patients suspected of malaria attending health center in Jos, Nigeria

Nantip F. Miri*, John D. Mawak2, Chukwu O. Chukwu3, Nyam J. Chuwang4, Shadrach Y. Acheng5 and Teme Ezekiel6

1,2Institute of Human Virology Nigeria, Abuja/Department of Microbiology, Faculty of Natural Science University of Jos, Nigeria
3Department of Microbiology, Faculty of Natural Sciences, University of Jos, Nigeria
4Department of Environmental Health Technology, Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom-Jos, Nigeria.
5Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Jos, Nigeria
6Genomics and Postgraduate Research Laboratory, Department of Obstetrics and gynaecology, Faculty of Medical Science, College of Health Sciences, University of Jos, Nigeria.

*Corresponding Author: E-mail: nmiri@ihvnigeria.org or nantipmiri9@gmail.com

ABSTRACT

Background: Despite the public health importance of dengue infections, it is less investigated by clinicians and rarely considered in the differential diagnosis of febrile illnesses in Nigeria. The objective was to detect the presence of Dengue IgG/IgM antibodies and Plasmodium species in the blood of febrile patients.

Methods: This is a cross sectional study conducted among ninety-four (94) consenting febrile patients suspected of malaria in Jos. Duo detection of dengue antibodies (IgG/IgM) were determined by ELISA technique. Total DNA was extracted from patient serum and quantified to determine concentration and quality of the extraction process. Malaria was detected by Real Time Polymerase Chain Reaction.

Results: Dengue antibodies were detected in 55.3% (52/94) of the febrile patients. The mean age was 29.9±1.2. Highest dengue prevalence of 75% (39/52), 50% (26/52) and 59.6% (31/52) were recorded among females, students and non-users of mosquito nets, respectively. In all. 11.7% (11/94) of the samples tested positive for malaria. Age group 11–20 years recorded the highest prevalence of malaria, 63.6% (7/11). Dengue and Malaria co-infection was documented in 5.3% (5/94). There was a significant difference in the prevalence of dengue and malaria among febrile subjects.

Conclusion: No association of dengue infection with gender and use of Insecticide Treated Nets was found. The lower malaria prevalence compared to dengue suggests that febrile illness in this population is shown to be associated more with dengue infection. We recommend a continuous surveillance of dengue infection in this population and consideration of dengue in the differential diagnosis of febrile illnesses.

Keywords: dengue IgG/IgM antibodies, febrile illnesses, malaria, insecticide

INTRODUCTION

Dengue virus is a single stranded, non-segment, positive sense RNA virus with a genome size of about 11kb (12), and belongs to the Flaviviridae family (Pang et al., 2015). There are four (4) known serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of dengue virus and a possible fifth variant (DEN-5) that was once reported (Mustafa et al., 2015). The 4 serotypes have a 65% genetic similarity, but anti-genetically distinct (Mukhopadhyay et
An infection with one serotype does not confer long lasting immunity against another serotype (Reich et al., 2013). Some studies have considered Dengue to be the most important and widespread arboviral disease (Bhatt et al., 2013; Murray et al., 2013) that is transmitted by infected Aedes mosquitoes, primarily of the species Aedes aegypti and Aedes albopictus (Weaver and Reisen, 2010; Ayolabi et al., 2019). Globally, there are 390 million dengue infections yearly (Bhatt, 2014), with 3.9 billion people at risk of the infection in 128 countries (WHO, 2017).

Malaria is an acute disease transmitted by female anopheles’ mosquito that is infected by any of the five (5) species of Plasmodium (Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi) that are known to cause the disease in humans (Marchand et al., 2011). It’s also considered to be one of the world’s most important public health diseases (Kolawole et al., 2017). There are 219 million estimated cases of malaria worldwide, with 1.1 billion people at high risk of the infection (WHO, 2017). Most malaria cases occur in the WHO Africa region (92%) with Nigeria accounting for 25% of the burden (WHO, 2017).

Dengue and Malaria infections are responsible for high mortality and morbidity rate in the tropics and sub-tropical parts of the world, especially among high risk groups (WHO, 2017). Though these diseases are not transmitted by the same type of mosquitoes, their co-circulation is possible especially in parts of the world where the vectors are widely distributed.

The accurate diagnosis of dengue infection depends on the days of illness. In the first seven (7) days of illness, serologic, molecular or antigen detection methods can be employed to test for dengue infection. Beyond 7 days, only serologic test is suitable as dengue virus is said to disappear from the blood of febrile patients (CDD, 2014). On the other hand, laboratory diagnosis of malaria is achieved by microscopic, serologic or molecular methods. Many studies in Nigeria have reported prevalence of mono-infections of either dengue (Kingsley et al.; Dawurung et al., 2010; Adeleke et al., 2016; Onoja et al., 2016; Ayolabi et al., 2019) or malaria (Adefioye et al., 2007; Akinboro et al., 2010; Idris et al., 2013), but with paucity of data on determining concurrent infections (Mustafa et al., 2015; Kolawole et al., 2017). In Nigeria, studies conducted to detect malaria have relied on serologic and microscopic methods, which are less sensitive in comparison to the polymerase chain reaction methods (Han et al., 2017; Berzosa et al., 2018; Mfuh et al., 2019).

Dengue and Malaria diseases present with similar clinical symptoms that make differential diagnosis by clinicians difficult, especially in developing countries (Baba et al., 2009). There is an existing bias towards classifying majority of febrile illnesses as malaria, and this is evident in several studies conducted in developing countries (Amexo et al., 2004; Ndyomugenyi et al., 2007; Nankibirwa et al., 2009). The under-reporting of arbovirus diseases and an overestimation of malaria infection among febrile patients have also been reported (Thiam et al., 2011). Despite the public health importance of Dengue infection, it is less investigated by clinicians and only considered when samples test negative for malaria (Sow et al., 2016).

Immunity to dengue infection is serotype specific and subsequent re-infection by a different serotype could worsen disease progression, rather than making it less severe (Halstead et al., 1970). The need for continues surveillance is very important in this regard. This study was designed to determine the prevalence of dengue antibodies among febrile patients presumptively suspected of malaria and determine malaria infection by polymerase chain reaction. Co-infection of malaria with dengue antibodies was also determined. The association between sociodemographic and risk factors were also determined.

MATERIALS AND METHOD

Study design and Setting
This was a cross sectional study carried out among febrile patients attending Dadin Kowa Comprehensive Health Center (DKCHC) in Dadin Kowa, Jos South Local Government area of Plateau
state, Nigeria. This study spanned between April and November 2019. Jos South Local Government Area (9°46′N 8°48′E/ 9.767°N 8.800°E) has a population of 306,716 according to the last census conducted in 2006. DKCHC is a thirty bed capacity government hospital. There are only three (3) Doctors and 30 to 50 patients are attended to by a doctor per day.

**Ethical Consideration**
This study was approved and ethical clearance letter (HMB/ADM/423/T/97) obtained from the ethical review committee of the Plateau State Hospital Management Board in accordance with the Helsinki declaration on ethical considerations involving human subjects. The purpose, risk and benefits of the study were clearly explained to participants in a language they best understood. Consent was obtained from participants or guardians and all tests were carried out at no cost to the participants.

**Study Population and sampling**
This study was carried out among febrile patients who were suspected of malaria and attending Dadin kowa comprehensive health center. Patients who provided consent and met two or more of the following criteria; body temperature $\geq 37.5^\circ C$, joint or muscle pain, headache, pain behind eyes, nausea or vomiting, skin rash and bleeding from nose/gums were included in this study. Patients suspected of other illnesses apart from malaria and did not give consent, were not included in this study.

The minimum sample size (at 95% confidence level) was determined to be thirty-three (33) as described by (Naing, 2006), based on local prevalence rate of 2.2% (Dawurung, 2010). In all, ninety-four (94). Patients were recruited randomly.

**Sample collection**
Following aseptic procedure, sterile needle was used to draw 5ml of venous blood into a plain vacutainer tube, from consenting patients and allowed to clot. Blood was centrifuged at 5000rpm for 10 minutes and serum separated into sterile 2ml tubes. Sera were immediately transported in cold chain to the Institute of Human Virology Nigeria (IHVN) reference Laboratory (Plateau State Virology Research Center, Jos), an ISO 15189:2012 accredited laboratory and stored at -20°C for further use.

**Laboratory methods**
Laboratory diagnosis was carried out on sera of patients for the duo detection of Dengue antibodies (IgG/IgM) by ELISA technique and malaria by real time PCR technique.

**Detection of Dengue antibodies**
Simultaneous detection of IgG/IgM was carried out using the AccuDiag™ ELISA Dengue IgG/IgM kit (Diagnostic Automation/Cortez Diagnostics, Inc, 21250 Califa St. Suite 102 and 116, Woodland Hills, CA 91367, USA) and read at 450nm. Optical densities were taken and positive results were interpreted according to manufacturer’s instruction.

**Nucleic acid extraction and quantification**
DNA was extracted from patient serum using the Viasure RNA-DNA extraction kit (CerTest Biotec, SPAIN), following manufacture’s procedures. DNA was quantified with the Invitrogen Qubit 4 Fluorometer (ThermoFisher Scientific, USA) to determine concentration and quality of the extraction process.

**Molecular Detection of Malaria Parasite**
The presence or absence of Malaria parasite was detected by real time PCR technique, with the VIASURE Real Time PCR Malaria Detection kit (CerTest Biotec, SL, Pol. Industrial Rio Gallego, Zaragoza, SPAIN) and results interpreted following manufacturer’s instructions. The PCR master mix contained specific primers/probes, dNTPS, buffer, polymerase and reverse-transcriptase in a lyophilized/stabilized format, as well as an internal control to discard the inhibition of the polymerase activity. 15µl of rehydration buffer was added to all the wells containing lyophilized master mix. 5µl of DNA samples, negative control and malaria positive control were added each to respective wells and centrifuged. A one step real time PCR reaction was programmed on the Quantsstudio™ 3 (Applied Biosystems™) Real Time PCR system to incubate for 2 minutes at 95°C (1 cycle), 10 seconds at 95°C...
and 50 seconds at 60°C (45 cycles) to allow for polymerase activation, denaturation and annealing/extension respectively. Specific Primers and fluorescent-labeled probe amplified the conserved region of 18S rRNA gene for Plasmodium spp. Fluorescent data were collected at the extension step through the FAM (Plasmodium spp) and VIC channels (Internal control) and analyzed for presence or absence of Plasmodium spp.

**Data analysis**
Data obtained was captured on Microsoft Excel 2007 and transferred to the IBM Statistical Package for the Social Sciences software (SPSS Version 22), for analysis of mean, standard deviation and chi-square test for relationship of dengue antibodies and other variables, P<0.05 was considered statistically significant.

**RESULTS**
There was no record of decline. The mean age was 29.9 (1.2). Female group had a participation rate of 76.6% (72/94) and 23.4% (22/94) were male. Dengue seroprevalence was detected in all age ranges, with the highest participants between the ages of 20-29, 26 (27.7%) years and the least range 0-9, 2 (2.1%) years (Table 1). Out of the 94 sera, 55.3% (52/94) tested positive for anti-dengue IgG/IgM with or without malaria co-infection, 11.7% (11/94) tested positive for Malaria by real time PCR with or without dengue co-infection and 5.3% (5/94) tested positive for both Dengue and Malaria (Table 2).

The female group recorded the highest prevalence of Dengue and Malaria, 75% (39/52) and 81.8% (9/11)

**Table 1: Frequency distribution according to Age and Sex**

| Age Group (Years) | Males (n) | Females (n) | Total (%) |
|-------------------|-----------|-------------|-----------|
| 0-9               | 01        | 01          | 02 (2.1)  |
| 10-19             | 08        | 13          | 21 (22.3) |
| 20-29             | 05        | 21          | 26 (27.7)|
| 30-39             | 05        | 20          | 25 (26.6)|
| 40-49             | 04        | 11          | 15 (16.0)|
| 50+               | 01        | 04          | 05 (5.3)  |

Data presented as number and percent

**DISCUSSION**
A Dengue positive in this study was defined as the presence of either IgM and/or IgG. Our findings showed high dengue prevalence in the study population. Several dengue seroprevalence studies have been carried out within the Jos area (Kingsley et al.; Dawurung et al., 2010; Onyedibe, 2018), that also detected different dengue prevalence rates in the population and other parts of Nigeria (Adesina and Adeniji, 2016; Bello et al., 2016; Kolawole et al., 2017). The high prevalence recorded, could be due to the fact that this study coincided with the warm temperatures and rainy season (April - October) in this region, a suitable condition for the breeding of mosquito that transmits the virus.

The lower prevalence of Malaria compared to Dengue suggests that, febrile illnesses in our study population are more associated with dengue infection than malaria infection. Malaria infection respectively (Table 3). Prevalence of Dengue and Malaria according to age ranges is described in Table 4.

Although there was no significant difference in dengue infection between Nets users and Non-users (P=0.315, df=1), dengue prevalence was highest (29/52:55.8%) among Non-users (Table 5). There was no association in malaria infection between Net users and Non-users (P=0.375, df=1). There was no association of dengue with Sex (P=0.68, df=1, \(\chi^2=0.165\)) and Age (P=0.188, df=5, \(\chi^2=7.472\)). Dengue was associated (P=0.002, df=1, \(\chi^2=9.175\)) with patients who had pain behind the eyes. There was a significant difference in the prevalence of dengue and malaria infections (P=0.008, df=1, \(\chi^2=6.951\)) among febrile subjects (Table 5).

**Table 2: Prevalence of Dengue and Malaria, with or without co-infection**

| Category                  | Number | (%) |
|---------------------------|--------|-----|
| Dengue Positive/Malaria Positive | 5/94   | 5.3 |
| Dengue Positive/Malaria Negative | 52/94  | 55.3 |
| Dengue Negative/ Malaria Positive | 11/94  | 11.7 |

Data presented as number and percent
Table 3: Prevalence of Dengue and Malaria according to Sex

| Variables | Male       | Female     | Total  | χ² | df | P-value |
|-----------|------------|------------|--------|----|----|---------|
| IgG/IgM   | 9(21.4%)   | 33(78.6%)  | 42(100)| 0.16 | 1  | 0.68    |
| negative  | 13(25%)    | 39(75%)    | 52(100)|     |    |         |
| positive  | 20(24.1%)  | 63(75.9%)  | 83(100)| 0.19 | 1  | 0.66    |
| Malaria   | 2(18.2%)   | 9(81.8%)   | 11(100)|     |    |         |

Categorical variable compared using Chi-Square (χ²) test, p-value < 0.05 considered statistically significant

Table 4: Prevalence of Dengue and Malaria according to Age

| Variables | 0-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51+   | TOTAL  |
|-----------|------|-------|-------|-------|-------|-------|--------|
| IgG/IgM   |      |       |       |       |       |       |        |
| negative  | 0(0%)| 6(14.3%)| 14(33.3%)| 12(28.6%)| 9(21.4%)| 1(2.4%)| 42(100)|
| positive  | 2(3.7%)| 15(28.9%)| 12(23%)| 13(25%)| 6(11.5%)| 4(7.7%)| 52(100)|
| Malaria   |      |       |       |       |       |       |        |
| negative  | 2(2.4%)| 10(16.9%)| 24(28.9%)| 23(27.7%)| 15(18.1%)| 5(6.0%)| 83(100)|
| positive  | 0(0%)| 7(13.3%)| 2(18.2%)| 2(18.2%)| 0(0%)| 0(0%)| 11(100)|

Data presented as number (percent)

Table 5: Dengue Prevalence according to Risk Factors

| Risk Factors     | Number Examined | Number Positive (%) |
|------------------|-----------------|---------------------|
| Use of Nets      |                 |                     |
| Yes              | 46              | 24(52.2)            |
| No               | 48              | 29(55.8)            |
| History of Travel|                 |                     |
| Yes              | 0               | 0(0)                |
| No               | 94              | 52(55.3)            |
| Blood Transfusion|                 |                     |
| Yes              | 0               | 0(0)                |
| No               | 94              | 52(55.3)            |

Dengue infection was highest among Non-users of nets, even though no significant difference was established between users and non-users of nets. Use of Nets was not a risk factor for dengue infection in this study.

Respondents who had pain behind the eyes were significantly associated with dengue infection. This agrees with studies that implicated this clinical symptom to be associated to dengue and shows that visual symptoms could be an indication of possible dengue infection (Yip et al., 2012; Yudhishdran et al., 2019).

The Dengue and Malaria co-infection observed in this study is of clinical importance, because mixed infection has been shown to cause more severe illness than monoinfection as reported by Epelboin and colleagues (2012). Concurrent infection is a rare occurrence that has been reported by a few international studies (Charrel et al., 2005; Deresinski, 2006; Thangaratham et al., 2006; Ward, 2006; Epelboin et al., 2012). Co-infection prevalence is one of few reported cases in Nigeria and similar to studies recently conducted by...
Kolawole et al. (2017) and Moses et al. (2016) who reported Dengue/Malaria co-infections as 5(2.8%) and 1(10.7%) respectively. Where vectors of both malaria and dengue are endemic, mixed infections are possible.

Our study to determine the prevalence of malaria by molecular detection is the first to be carried out in Jos. The method used for the simultaneous detection of dengue antibodies did not distinguish recent and past infections, and cross reactivity may occur with other flaviviruses to give false positive results. We did not carry out a PCR to determine if there exists a cross reactivity with other flaviviruses. So far, no study has been carried out in Jos to detect dengue infection by molecular methods, and to determine the circulating serotypes and genotypes in the population. We recommend a molecular method to determine dengue and the circulating serotypes in further research, as there is paucity of data on the molecular epidemiology of the disease in Nigeria.

CONCLUSION
In this study, dengue has been shown to be an infection that is causing febrile illnesses in our study population. We have also reported concurrent dengue and malaria infections. The inclusion of dengue diagnosis in the differential diagnosis of febrile patients by clinicians should be given consideration. We also recommend continuous dengue surveillance in Nigeria.

COMPETING INTEREST
Authors declare that they have no competing interests.

FUNDING DISCLOSURE
This study was partially funded by CerTest Biotec SL (Spain) by supplies of Real Time PCR testing kits and Nucleic acid extraction kits. All other funding came from the authors.

ACKNOWLEDGEMENT
We acknowledge the Staff of Comprehensive Health Center Dadin Kowa and the patients who consented to participate in this study. The Plateau State Virology Research Center (ISO15189 accredited), and the Genomics and Postgraduate Research Laboratory of the department of obstetrics and gynecology, College of Health Sciences University of Jos, have been of great support by providing the Laboratory in which this study was carried out.

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