A survey of malaria and some arboviral infections among suspected febrile patients visiting a health centre in Simawa, Ogun State, Nigeria

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KEYWORDS
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Chikungunya;
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*Plasmodium vivax*;
Febrile

Summary Most febrile patients are often misdiagnosed with malaria due to similar symptoms, such as fever shared by malaria and certain arboviral infections. This study surveyed the incidence of malaria, chikungunya and dengue infections among a number of suspected febrile patients visiting Simawa Health Centre, Ogun State, Nigeria.

Venous blood samples were obtained from 60 febrile patients (age 3–70 years) visiting the centre between April and May 2014. The rapid diagnostic test (RDT) was used to detect the presence of chikungunya (CHK) antibodies (IgM), dengue (DEN) virus and antibodies (NS1, IgM and IgG) and malaria parasites (*Plasmodium falciparum* and *Plasmodium vivax*). Malarial confirmatory tests were by microscopy and nested polymerase chain reaction (PCR) using the polymorphic region of Glutamate-Rich Protein (GLURP) gene. The complexity of *P. falciparum* infection in the community also determined by the use of nested PCR. These three mosquito-borne infections were observed in 63% (38) of the patients. The prevalence of CHK, DEN and malarial infections singularly were 11%, 0% and 63%, respectively, whereas malaria with either CHK or DEN infections were 24% (9) and 3% (1), respectively. No subjects were positive for CHK and DEN co-infection. Malarial microscopic confirmation was in 94% (32) of the malaria RDT-positive samples, 50% (17) were successfully analysed by nested PCR and the mean multiplicity of infection was 1.6 (1–3 clones). One patient sample harboured both *P. falciparum* and *P. vivax*. The study reports the presence of some arboviral infections having similar symptoms with malaria at Simawa, Ogun State. The proper diagnosis of infectious diseases is important for controlling them. © 2015 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier Limited. All rights reserved.

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Introduction

Mosquitoes are carriers of various pathogens that cause disease in humans. These diseases include malaria, yellow fever (YF), dengue fever, chikungunya, and some filarial diseases [1]. It is estimated that over two billion people worldwide live in regions where these diseases are rampant [2].

Malaria parasites are transmitted by female *Anopheles* mosquitoes, whereas DEN and CHK viruses are transmitted by female *Aedes* mosquitoes [3]. Co-infection among the three diseases is possible in geographical locations where the respective vectors co-exist [4]. As the species of mosquitoes responsible for transmission of these infections are present in Nigeria [5], co-infection is expected to occur.

Malaria, DEN and CHK share common symptoms such as sudden high fever, headache and joint pain among others. In most Sub-Saharan countries including Nigeria, malaria is commonly attributed to all febrile illnesses [6]. Due to this similarity and lack of specificity of symptoms, misdiagnosis is often common among clinicians. Misdiagnosis is more probable when these infections occur simultaneously [7].

*Plasmodium falciparum* is the most common species of malarial infection and is more prevalent in Sub-Saharan Africa than in many other regions of the world [8]. *Plasmodium vivax* is estimated to cause 20% of malaria infections and is commonly found in tropical areas outside of Africa [9]. The absence of the Duffy binding protein, which is required for invasion of the human host by *P. vivax* in individuals from West and Central Africa, is responsible for the low prevalence in these regions. However, cases of *P. vivax* infections are now being reported daily in areas where the parasite has never been described before [10].

The aim of this study was to determine the incidence and types of malaria parasites (*P. falciparum* and *P. vivax*), the prevalence of co-infection by malaria parasites with dengue virus and chikungunya virus, the prevalence of present, past dengue and chikungunya infections among suspected febrile patients visiting Simawa Health Centre, Ogun State Nigeria. The relationship between age and sex to these three mosquito-borne diseases was also determined.

Materials and methods

Study site

The study was conducted among in-patients and out-patients visiting the Simawa Health Centre (3°29'E, 6°45'N). Simawa is a rural community and one of the 15 wards in Sagamu Local Government area, Ogun State, Nigeria. Two types of health services exist in this community: modern healthcare services (Primary Health Centre) and the traditional health services (use of herbs), which are more commonly patronised than the former. Simawa Primary Health Centre is one of the 3 health centres in Sagamu local government area of Ogun State, Nigeria. Simple curative services in these health centres include antimalarial treatment. The Sagamu local government area has a total population of 255,885 [11]. There is one doctor per 2992 people [12] with only 3% of these doctors in the Primary Health Centres [13].

Patients selection

Patients presenting to the health centre with some signs and symptoms compatible with the diagnosis of malaria, dengue and chikungunya (fever which can be recent or in evidence during the previous 2–4 days or other symptoms of febrile diseases such as chills, headache, joint, muscle and body pains), normal anatomical conditions allowing for venous blood via peripheral arm veins and only those who gave their consent were enrolled into the study (consent from adult patients directly, consent from parents/guardians of children below 9 years of age and assents from children between 9 and 17 years of age). Attitudes (customs and traditions) of certain Nigerians, especially in rural areas, have been reported to affect participation in research involving the collection of blood samples [14]. The 60 patients who gave their consent out of the suspected patients were enrolled in the study from April to May 2014. Exclusion criteria included patients with good health status and with chronic diseases.

The study protocol was approved by the Ogun state Ministry of Health, Nigeria.

Sample collection

Whole blood samples were obtained from the patients (April to May 2014) by venipuncture and transferred into plain bottles. Whole blood (3–5 drops) was used for the RDT malaria parasite detection, approximately 20 µl of which was spotted onto 3MM Whatman filter paper for malarial molecular analysis and to prepare thin and thick blood films for malaria parasite species identification and quantification. The remainder of the whole blood sample from each patient was centrifuged and the sera were used for dengue and chikungunya rapid diagnostic tests.
Rapid diagnostic tests (RDT)

Malaria

P. falciparum and P. vivax were detected in the samples using Standard Diagnostic Malaria antigen Plasmodium falciparum/Plasmodium vivax rapid test kit (SD Bio line, Standard Diagnosis INC Korea) according to the manufacturer’s instructions. The results were interpreted according to the manufacturer’s protocol.

Dengue

Serum samples were tested for the presence of anti-dengue NS1 antigen IgM and IgG antibodies using Standard Diagnostic Dengue NS1 + Ab combo rapid test kit according to the manufacturer’s instruction. The results were interpreted according to the manufacturer’s protocol.

Chikungunya

Serum samples were tested for anti-chikungunya IgM antibodies using Chikungunya IgM rapid diagnostic test kit from Standard Diagnostics according to the manufacturer’s instructions.

Microscopy

Thin and thick blood films were stained with 20% Giemsa stain for parasite species identification and quantitation. Asexual form of parasites were counted against 200 white blood cells (WBC) to determine the parasite density per microliter of blood.

Molecular analysis

Parasite genomic DNA was extracted from the blood-impregnated filter papers using Qiagen DNeasy Blood and Tissue Extraction Kit (Qiagen Sciences, Maryland 20874 USA). Further confirmatory and genotypes of the malaria parasite population from blood samples collected from patients with positive and negative rapid diagnostic test results were determined using the nested polymerase chain reaction (PCR) technique [15] with the use of primers specific for region II of Glutamate-Rich Protein (GLURP) gene.

Statistical analysis

Data entry was performed using Microsoft Excel. Analysis was performed using both Excel STAT and SPSS.

Result

The demographic profiles of enrolled patients

A total of 60 patients composed of 16 (27%) males and 44 (73%) females were enrolled in this study. The subjects ranged from young children (age 3) to adults (age 70) (Table 1).

Malaria and the 2 arboviral infections

Thirty-eight (63%) patients tested with the RDTs were found to be positive for malaria, dengue and chikungunya, or a combination of two of these infections. Among the positive patients, the 21–30-year-old group had the highest infection rate (32%), malaria and chikungunya co-infection was found in 24% (9) of patients with an age range, 20–70 years (mean: 36.3 ± 19.7), whereas malaria and dengue co-infection was found in 3% (1) of these patients (Table 1). In addition, females had a higher infection rate of 74% (28) when compared with males (Fig. 1). When the association of co-infection (malaria—dengue, malaria—chikungunya) with occupation was considered, traders had the highest rate (80%) of infection (Table 2).

Malaria infection

Among the patients positive for any one of the infections, 63% (24) were positive for malaria alone (mean age, 29.9 ± 16.9) (Fig. 2) and 26% (10) were positive for malaria and any one of the arboviral infections (chikungunya or dengue) by RDT. A mixed infection of P. falciparum and P. vivax was observed in 3% (1) of the total malaria-positive patients. Within the group infected with malaria alone, females had a higher infection rate (45%) when compared to males, (Fig. 1), 21 (55%) patients had body temperatures ≤37.5°C, whereas 3 (8%) had body temperatures above 37.5°C (Table 3).

Of the 34 samples that tested positive for malaria by RDT, 94% (32) were confirmed by microscopy. Additionally, 3 samples tested positive by microscopy from the RDT-negative samples. Of the 34 samples positive by RDT, 17 (50%) tested positive by the nested PCR and 8 of the negative samples were also positive by PCR. The highest malaria parasite count of 277,777 parasites/µl was recorded within the malaria infection alone group and the 0–10-year-old age group. The highest malaria parasite count among the
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Table 1  Age and sex distribution of positive and negative individuals to the three mosquito borne diseases.

<table>
<thead>
<tr>
<th>Age range</th>
<th>Sex</th>
<th>No. examined (60)</th>
<th>Malaria Plasmodium falciparum P N</th>
<th>Malaria Plasmodium vivax P N</th>
<th>Dengue NS1 P N</th>
<th>Dengue IgM P N</th>
<th>Dengue IgG P N</th>
<th>Chikungunya IgM P N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—10</td>
<td>M</td>
<td>4</td>
<td>3 1</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>1 3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4</td>
<td>1 3</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>4 0</td>
</tr>
<tr>
<td>11—20</td>
<td>M</td>
<td>2</td>
<td>1 1</td>
<td>0 2</td>
<td>0 2</td>
<td>0 2</td>
<td>0 2</td>
<td>1 1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3</td>
<td>3 0</td>
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<td>0 3</td>
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<td>0 3</td>
<td>3 1</td>
</tr>
<tr>
<td>21—30</td>
<td>M</td>
<td>6</td>
<td>3 3</td>
<td>1 5</td>
<td>0 6</td>
<td>6 0</td>
<td>6 0</td>
<td>6 0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>15</td>
<td>8 7</td>
<td>0 15</td>
<td>0 15</td>
<td>0 15</td>
<td>0 15</td>
<td>3 12</td>
</tr>
<tr>
<td>31—40</td>
<td>M</td>
<td>4</td>
<td>1 3</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>0 4</td>
<td>4 1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>6 2</td>
<td>0 8</td>
<td>0 8</td>
<td>1 7</td>
<td>0 8</td>
<td>2 6</td>
</tr>
<tr>
<td>41—50</td>
<td>M</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>4 4</td>
<td>0 8</td>
<td>0 8</td>
<td>0 8</td>
<td>0 8</td>
<td>2 6</td>
</tr>
<tr>
<td>51—60</td>
<td>M</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>1 1</td>
<td>0 1</td>
<td>0 1</td>
<td>0 1</td>
<td>0 1</td>
<td>1 1</td>
</tr>
<tr>
<td>61—70</td>
<td>M</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>3 2</td>
<td>0 5</td>
<td>0 5</td>
<td>5 0</td>
<td>5 0</td>
<td>1 4</td>
</tr>
</tbody>
</table>

Dengue infection

The malaria—dengue (IgM) co-infection in 3% (1) of the patients positive for all 3 infections was in a female aged 38 years and exhibiting a body temperature of 37.0 °C.

Chikungunya infection

Among the patients positive for the 3 infections, 11% (4) had anti-chikungunya IgM antibodies alone, with an age range of infection that was 20–50 years (mean: 30.8 ± 9.1) (Fig. 2) and 24% (9) had a malaria—chikungunya co-infection. Among the patients with chikungunya infection alone, no differences in infection were recorded among the two sexes and the various age groups (Figs. 1 and 3). A patient within the CHIK-malaria co-infection group had a body temperature of 39.1 °C and was 3 years of age.

Discussion

Malaria may not be the only mosquito-borne disease that causes fever in this community, as demonstrated by presence of 34% and 3% of CHK and DEN IgM antibodies, respectively. The co-infection of patients with malaria and either CHK or DEN antibodies in the study implies the possibility of being infected either by mosquitoes carrying more than one pathogen or more than one infected mosquito.

co-infection group (malaria-chikungunya) was 196,000 parasites/μl, which was among the 51—60-year-old age group (Table 4). The multiplicity of infection detected 3 clones (mean: 1.6). The highest number of clones was observed among the 31—40-year-old age group.

![Figure 1](image1.png)  Frequency of infections with gender.

![Figure 2](image2.png)  Frequency of infections with gender.
Figure 2 Malaria, chikungunya infections, malaria and chikungunya co-infections with mean ages at 95% CI.

Table 3 Temperature range among positive individuals to the three mosquito borne diseases.

<table>
<thead>
<tr>
<th>Temperature range</th>
<th>Malaria only (%)</th>
<th>Dengue only (%)</th>
<th>CHIK Only (%)</th>
<th>Mal—CHIK (%)</th>
<th>Mal—DEN (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 37.5 °C (≤37.5 °C)</td>
<td>21 (55%)</td>
<td>0 (0%)</td>
<td>4 (11%)</td>
<td>8 (21%)</td>
<td>1 (3%)</td>
<td>34 (90%)</td>
</tr>
<tr>
<td>Above 37.5 °C (&gt;37.5 °C)</td>
<td>3 (8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (63%)</td>
<td>0 (0%)</td>
<td>4 (11%)</td>
<td>9 (24%)</td>
<td>1 (3%)</td>
<td>38 (100%)</td>
</tr>
</tbody>
</table>

Table 4 Parasite counts and multiplicity of infection in malaria only and malaria—chikungunya co-infection.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age groups</th>
<th>Parasite count (parasites/μL)</th>
<th>Number of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>0—10</td>
<td>277,777</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>11—20</td>
<td>12,000—19,520</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>21—30</td>
<td>39,600—100,000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>31—40</td>
<td>12,000—79,298</td>
<td>1,2,3</td>
</tr>
<tr>
<td></td>
<td>41—50</td>
<td>133,333</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>61—70</td>
<td>68,571</td>
<td>2</td>
</tr>
<tr>
<td>Malaria and chikungunya</td>
<td>0—10</td>
<td>63,900</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>11—20</td>
<td>55,600</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>21—30</td>
<td>142,857</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>31—40</td>
<td>68,571</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>41—50</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>51—60</td>
<td>196,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>61—70</td>
<td>47,450</td>
<td>2</td>
</tr>
</tbody>
</table>

[16,17]. A survey of Aedes species in a farm settlement (Odogunyan) located in Ikorodu, a boundary of Sagamu local Government area, with a road distance of 16.4 km from Simawa, reported Aedes aegypti co-existing with Ae luteocephalus [5]. Previous studies have also reported chikungunya virus transmission in Africa involving Aedes aegypti in urban areas and a sylvatic cycle involving Aedes aegypti with sylvatic forms such as Aedes fulcifer and Aedes luteocephalus [18]. The movement of people within Ikorodu and Simawa [19] may have facilitated the movement of these vectors [20].
and/or CHIKV [21], thus, establishing a sylvatic cycle in Simawa community.

In this study, the co-incidence of malaria and anti-arboviral IgM antibodies was high among traders. This co-incidence may also be attributed to the movement of the traders from rural to urban areas. The majority of women in Simawa are involved in agriculture and visit the urban areas periodically to market their produce [20]. A high number of dengue infection cases among the working age group in urban areas has been reported [22]. It has been hypothesized that a high number of dengue cases in urban areas may be as the result of high population densities and rapid development activities, which are favourable to the development of vectors and eventually lead to arboviral transmission [22].

In this study, 34% of the patients were determined to be positive for anti-chikungunya IgM antibodies. Similar results have been reported in India [23]. The authors of that study suggested that infection must have been endemic in the study area but was undetected and likely misdiagnosed as malaria. The highest anti-chikungunya IgM antibody infection rate (CHIK only and co-infection with malaria) in this study was recorded among individuals within the age brackets of 21–40 years, echoing similar reports in France and India [24,25]. The authors of those studies have suggested age and gender are proxy-factors for specific behaviour that cause higher exposure to *Ae. albopictus* bites. These behaviours include staying outdoors during daytime when these vectors bite, without the use of any personal protection measures. It is possible that practices and lifestyle of adults whose ages range 21–40 years may make them to be susceptible to these mosquito-borne diseases [26].

The highest infection rate of the three infections was among females, rather than males. These may be due to variation in the physiological status (lactation, pregnancy) of women, which may contribute to lowered immunity among this group [27].

Although most of the patients had fever in the previous 3–4 days, when they presented at the health centre, they had body temperatures below 37.5 °C with headaches and or body/joint/muscle pains. The patients with CHIK IgM alone or co-infection with malaria can be categorized as having had an earlier CHIKV infection. The IgM specific to CHIKV, though detectable 2–3 days after the onset of symptoms, can persist for several weeks up to 3 months [28]. However, there is the possibility of the co-infection been acute. A number of outbreaks of CHIK have been reported in patients with joint pains without fever [28]. In addition, although CHIK infection has an abrupt onset of fever, the fever can be biphasic or "saddleback": fever subsides in 2–3 days and then returns [29]. Studies on the use of herbs to manage fever in Sagamu Local Government area, Ogun State have been reported [30]. The use of herbs may have resulted in the low recorded body temperatures without a reduction in the parasite and/or viral load.

One of the patients with very high body temperature (39.1 °C) was a child (3 years old) who had CHIK—malaria co-infection. This can be regarded as acute infection. Acute CHIK infection has been reported to be two times more common in infants than in adults and adolescents [31]. This is consistent with the hypothesis that CHIKV infection causes lifelong protection against re-infection.

One (1.7%) of the patients tested was anti-dengue IgM antibody-positive. Various studies have reported the detection of dengue fever virus among febrile patients in some parts of Nigeria [32–35]. The individual was also *P. falciparum* positive. The infection may be an earlier infection because NS1 antigen was not detected. It may also be acute infection. Although the body temperature was below 37.5 °C, the duration of infection in dengue infection has been reported to be gradual [29].

In this study, the malarial positive patients were determined to be infected with two types of *Plasmodium* species (*P. falciparum* and *P. vivax*). All of the malarial infected patients were positive for *P. falciparum* and one of these patients had a mixed infection of *P. falciparum* and *P. vivax*. Mixed infection of *P. falciparum* and *P. vivax* has also been reported in Abuja, Nigeria [36]. Usually, *P. vivax* is uncommon among black populations because of the absence of the Duffy antigen in their red blood cells. The Duffy antigen is important for invasion by *P. vivax* [37]. However, recent studies have reported the inability of Duffy negativity to provide complete protection against *P. vivax* infection among
the black population [10] The unusual occurrence of *Plasmodium vivax* in Nigeria might be as a result of migration [38], mutation and/or intermarriage [39].

The complexity of malaria infection reported in the study is similar to other studies in the country [40,41]. Additionally, the sensitivity of the RDT used for malaria diagnosis compared to microscopy indicate the efficiency of the former for the detection of the malaria parasite. Thus, its use in the domestic management of malaria can be encouraged.

Conclusions

Febrile patients visiting health centres in Nigeria are mostly misdiagnosed and treated for malaria infection. The observations of malaria/dengue and malaria/chikungunya co-infections in this study should be interpreted with caution considering the limited number of clinical investigations reported. Although the study has a limited number of samples, it emphasizes the importance of arboviral diagnosis. Therefore, there is a need to include the diagnosis of arboviral infections to avoid misdiagnosis and mistreatment of infections.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ogun State Ministry of Health, Nigeria.

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References

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