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Investigations of dengue virus and *Plasmodium falciparum* among febrile patients receiving care at a tertiary health facility in Osogbo, south-west Nigeria

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Abstract

Both dengue and malaria are diseases of the tropical and sub-tropical regions having similar symptoms especially among the febrile subjects. A descriptive hospital-based study was carried out among randomly selected 170 consenting febrile patients ((m= 92; f=78) age ranged <1 to 55 years (mean age=27.2) attending LAUTECH teaching hospital, Osogbo, between March 2015 and February 2016. The detection of *Plasmodium* parasites was screened by RDT and confirmed by microscopy while primary dengue virus detection was investigated using ELISA technique. The overall rate of 41.7% was obtained for *P. falciparum* while 1.8% was reported for dengue infection. Analysis by age and sex showed that the rate of *P. falciparum* was higher among male (42.0%) than female with highest rate (22.2%) was peaked among age range 16-25 years and 36-45 years respectively with no statistical associations (p = 0.469; OR =1.26, 95% CI 1.21-2.31). Infection rate for dengue was higher in females (2.6%) than males (1.2%) and found among age ranged of 16-25 and 26-35 years respectively. Also, dengue virus *P. falciparum* co-infection rate (0.6%) was found among participant age ranged 26-35 years. However, there were no statistical associations between age/sex in relation to dengue and malaria infections (p = 0.359; OR = 1.16, 95% CI 1.34-2.66) in this study. This study reported high rates of dengue and malaria infections among the community dwellers. There is need to ascertain the actual seroprevalence of dengue and malaria infections among the febrile patients in rural and urban centers together with those accessing care in our healthcare facilities in Nigeria.

Keywords: Dengue/malaria infections; mosquitoes; fever; Osogbo community; Nigeria**.**

Introduction

Dengue fever and malaria are the most common arthropod-borne diseases in humans and represent major public health problems. Malaria and dengue are the two common mosquito infections that are very important and cause high morbidity and mortality for many patients around the world [1] Early diagnosis of Dengue virus (DENV) infection is important for proper treatment of Dengue haemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS) DHF and DSS to avoid fatal outcome. Mosquitoes are carriers of various infectious agents known to cause various diseases in humans which include malaria, dengue fever and so many others [2]. More than two billion people are estimated globally to live in regions where these diseases are prevalent [3].

Majority of febrile illnesses in Nigeria are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis. Therefore, many patients with fever are presumedto be having fever of unknown origin or malaria and remain without a

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laboratory diagnosis even if they fail to respond to anti-malarial drugs. Malaria parasites such as *P. falciparum, P. Vivax,* etc. are transmitted by female *Anopheles* mosquitoes, whereas dengue virus is transmitted by female *Aedes* mosquitoes [4]. Co-infection with these diseases is possible in geographical locations where the respective vectors co-exist [5]. As the species of mosquitoes responsible for transmission of these infections are present in Nigeria [6], co-infection takes place generally.

Two major factors have been shown to aid co-infection of dengue virus infection and malaria which include the unprecedented global population growth and the associated unplanned and uncontrolled urbanization, especially in tropical developing countries [7]. The sub-standard housing, crowding, and deterioration in water, sewer, and waste management systems associated with unplanned urbanization have created ideal conditions for increased transmission of mosquito-borne diseases in tropical urban centers [8]. Malaria and dengue share common presentations including sudden high fever, headache and joint pain among others. In most developing countries such as Nigeria, malaria is synonymous with all febrile conditions [9]. However, due to common similarity with fever without specific clinical conditions, misdiagnosis is often rampant among clinicians and usually more pronounced when these infections take place at the same time in affected patients [10].

Dengue fever mimics malaria and in regions where malaria is endemic, more than 70% of febrile illnesses are treated as presumptive malaria [11]. Insufficient urban piped-water supplies, necessitating the storage of water for drinking and washing, and poor sanitation, resulting in the accumulation of vast amounts of human detritus that collect rainwater, such as discarded bottles, cans, and automobile tires, have been responsible for an enormous expansion of *A. aegypti* vector populations [4]. This environmental transformation has occurred in a setting where the supply of susceptible human hosts for dengue transmission is now virtually inexhaustible.

Malaria is endemic and the most common problem of febrile illnesses occurring always in greater part of Africa than in many other regions of the world [12]. Despite the efforts of the government and non-governmental organizations in reducing the morbidity and mortality as a result of vector-borne diseases like malaria, there is still high degree of febrile illness among the reported hospital cases of infection in the developing countries. Therefore this study aimed to determine the preponderance of malaria parasite (*P. falciparum*) and the co-infection rate of malaria parasites with dengue virus among suspected febrile patients assessing care at a tertiary health facility in Osun State, Nigeria.

Materials and methods

Study site

This study was conducted at General Out-Patient Department (GOPD) and Children Out-Patient Department (COPD) in Ladoke Akintola University of Technology Teaching Hospital (LTH), Osogbo, south-west, Nigeria. LTH provide health care services to the local dwellers. Osogbo is the capital of Osun State which is one of the states located in the south-western part of Nigeria. Osogbo lies on coordinates 7°46 North, 4°34 East with an area of 47 sq. km. According to the 2006 Population and Housing Commission Census, the city has a population of 156,694 people majorly made up of Yoruba people. Osogbo shares boundary with Ikirun, Ilesa, Ede, Egbedore and Iragbiji and is easily accessible from any part of the state because of its central nature. Osogbo attracts a large number of visitors/tourists, and migrants most of whom are employed in the commercial port and the industrial zones of the country**.**

Study design

There was no official data showing the prevalence of dengue fever in Osogbo metropolis in order to determine a precise sample size, hence, a cross sectional study was carried out on randomly selected one hundred and seventy consenting patients with febrile conditions who attended the LAUTECH teaching hospital. Participants within the age-range of 1-55 years were recruited for the study. The ethical approval for the study was obtained from the ethical committee of Ladoke Akintola University of Technology(LAUTECH) Teaching Hospital, Osogbo, Nigeria while parental assents were given for the under aged participants. The ethical approval was obtained from LTH IRB committee with the number (LTH/EC/2015/11/236).

Blood sample collection and detection of dengue IgG and IgM antibody

A total of 170 blood samples were collected from consenting participants while structured questionnaire was used to capture their demography and pattern of recent febrile conditions. About 5 ml of blood samples were collected in a tube without anticoagulant through antecubital vein. Each sample was spun on a bench centrifuge at 3,000 rpm for 5 minutes to obtain serum. The sera were tested for Dengue fever virus IgG and IgM by the Enzyme-Linked Immunosorbent Assay (ELISA) test (Commercial Dengue virus IgM ELISA kit (DIA.PRO – Italy). All the specimens were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA). The presence or absence of dengue fever virus IgG and IgM were determined by comparing the sample absorbance with the absorbance of the cut-off calibrator.

Microscopy

The initial screening for *P. falciparum* was done using Malaria Rapid Diagnostic Test (RDT) kit and subsequently confirmed by microscopy. Thick blood films were prepared according to the methods of Chesbrough [13]. Air-dried thick film was stained with 10% Giemsa

solution for 15 minutes. Malaria parasites were examined using oil immersion lens objectives (x100) of a high quality microscope with an incandescent light source. Parasitaemia was expressed as the number of asexual forms of *P. falciparum* per microliter.

Statistical analysis

The data obtained was entered into a computer and analysed using Statistical Package for Social Science (SPSS) version 17.0 (SPSS Inc., Chicago, USA). The data for the IgM titers was expressed in geometric mean. A *p*-value less than 0.05 was considered statistically significant.

Results

The demographic profiles of enrolled patients

A total of 170 patients consisting of 92 (57.6%) males and 78 (48.4%) females were enrolled in this study. The subjects ranged from young children (age 7 months) to adults (age 60 years) (Figure 1). By age stratification, age range 16-25 years had highest frequency while the lowest frequency was in age range <1-5 years (Figure 2).

Figure 1. Frequency of febrile patients by sex.

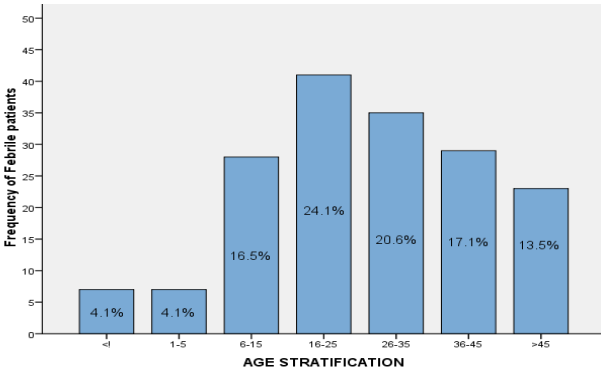

Figure 2. Frequency of febrile patients by age stratification.

Malaria and dengue infections

In this study, 72(41.7%) patients (mean age, 29.6 ± 16.1) were found positive for malaria infection by microscopy. The highest affected group vulnerable to malaria in this study were participants from age groups of 16-25(22.2%) and 36-45(22.2) years while lowest rate (4.2%) was found in age group <1 year respectively with body temperatures ranging between ≤ 37.5°C and >37.5%. However, no significant difference was recorded between malaria infection and different age groups respectively (p= 0.469; OR=1.26, 95% *CI* 1.21-2.31). Malaria infection was higher in male (42.0%) than their female (22.2%) (Table 1).

Of the total 170 blood samples collected from febrile subjects tested (Figure 1), 3 (1.8%) cases were confirmed as dengue infection while one hundred and sixty seven (98.2%) cases were found to be negative for dengue. non-dengue (Table 2). Gender differences in primary dengue infection were not significant by age groups (p>0.05). Out of the three (1.8%) Dengue IgM positive cases, which consist of one (1.2%) male and two (2.6%) females (Figure 3) and fell within the age ranges of 16-25 years, 26-35 years and >45 years respectively. Dengue IgM was found in 3(1.8%) febrile patients had mean age 31.5 ± 17.9. In addition, female had a higher dengue infection rate of 2.6% when compared with male (1.1%) counterparts (Table 2, Figure 3).

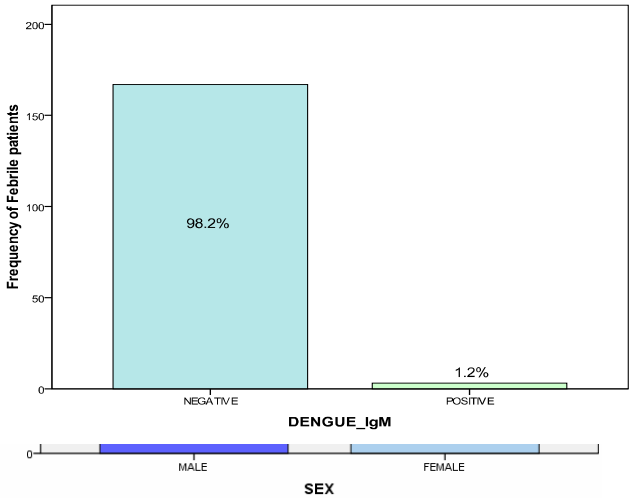

Figure 3. Distribution of dengue infection among febrile subjects.

Furthermore, dengue/malaria co-infection rate (0.6%) was found among a participant age ranged 26-35 years (Tables 3 and 4). However, there was no statistical associations between age/gender in relation to dengue and malaria infections (p = 0.359; OR = 1.16, 95% *CI* 1.34-2.66) in this study.

Table 1. Frequency of *Plasmodium* malaria among febrile subjects.

		Malaria Parasite				
		Negative		Positive		
Parameters		Count (*n*)	*n* %	Count (*n*)	*n* %	*p*-value
Sex	Male	50	51.0%	42	58.3%	
	Female	48	49.0%	30	41.7%	
	Subtotal	98	100.0%	72	100.0%	0.347
Age stratification	<1	4	4.1%	3	4.2%	
	1-5	3	3.1%	4	5.6%	
	6-15	17	17.3%	11	15.3%	
	16-25	25	25.5%	16	22.2%	
	26-35	22	22.4%	13	18.1%	
	36-45	13	13.3%	16	22.2%	
	>45	14	14.3%	9	12.5%	
	Total	**98**	**100.0%**	**72**	**100.0%**	**0.839**

Table 2. Distribution of dengue IgM infection among febrile subjects.

		Dengue IgM				
		Negative		Positive		
		Count (*n*)	*n* %	Count (*n*%)	*n* %	*p*-value
Sex	Male	91	53.5%	1(1.1)	33.3%	
	Female	76	47.5%	2(2.6)	66.7%	
	Subtotal	167	100.0%	3(1.8)	100.0%	0.469
Age stratification	<1	7	4.2%	0	0.0%	
	1-5	7	4.2%	0	0.0%	
	6-15	28	16.8%	0	0.0%	
	16-25	40	24.0%	1	33.3%	
	26-35	34	20.4%	1	33.3%	
	36-45	29	17.4%	0	0.0%	
	>45	22	13.2%	1	33.3%	
	Total	**167**	**100.0%**	**3**	**100.0%**	**0.406**

($p = 0.469$; OR = 1.26, 95%CI 1.21-2.31).

Table 3. Co-infection of dengue and malaria infections among patients with febrile condition.

		Co-Infection				
		Negative		Positive		
Parameters		Count (*n*)	*n* %	Count (*n*)	*n* %	*p*-value
SEX	Male	91	53.8%	1	100.0%	
	Female	78	46.2%	0	0.0%	
	Subtotal	169	100.0%	1	100.0%	0.359
Age stratification	<1	7	4.1%	0	0.0%	
	1-5	7	4.1%	0	0.0%	
	6-15	28	16.6%	0	0.0%	
	16-25	41	24.3%	0	0.0%	
	26-35	34	20.1%	1	100.0%	
	36-45	29	17.2%	0	0.0%	
	>45	23	13.6%	0	0.0%	
	Total	**169**	**100.0%**	**1**	**100.0%**	**0.791**

($p = 0.359$; OR = 1.16, 95%CI 1.34-2.66).

Table 4. Summary of co-infection of dengue and malaria infections among patients with febrile conditions.

Parameters		Sex		Age stratification						
		Male	Female	<1	1-5	6-15	16-25	26-35	36-45	>45
		Count	Count	Count	Count	Count	Count	Count	Count	Count
Sex	Male	92	0	5	4	14	24	16	13	16
	Female	0	78	2	3	14	17	19	16	7
	Subtotal	92	78	7	7	28	41	35	29	23
Malaria Parasite	Negative	50	48	4	3	17	25	22	13	14
	Positive	42	30	3	4	11	16	13	16	9
	Subtotal	92	78	7	7	28	41	35	29	23
Dengue IgM	Negative	91	76	7	7	28	40	34	29	22
	Positive	1	2	0	0	0	1	1	0	1
	Subtotal	92	78	7	7	28	41	35	29	23
Co-infection	Negative	91	78	7	7	28	41	34	29	23
	Positive	1	0	0	0	0	0	1	0	0
	Total	92	78	7	7	28	41	35	29	23

Discussion

This study found rates of 41.7%, 1.8% and 0.6% for *P. falciparum* infection dengue virus and co-infection of dengue/malaria respectively and this has clearly shown that malaria may not be the only mosquito-borne disease that causes febrile illnesses in this community. The co-infection of patients with malaria and dengue antibodies in this study suggests the possibility of any resident of this community being infected either by mosquitoes carrying more than one pathogen or more than one infected mosquito [14, 15]. A rate of 41.7% detected for Malaria in this study is however higher than 34% reported in a previous study carried out among similar population with febrile illness in Simawa Health Centre [16]. Although dengue/malaria co-infection rate of 0.6% found in our study was lower than 3% reported in a previous study [15], it however calls for urgent attention.

The feverish conditions associated with early symptoms of an arbovirus infection such as dengue include high grade fever, headache, fatigue, malaise, nausea, vomiting which mimic malaria, typhoid, measles and influenza are hyper-endemic in the environment, thereby rendering the diagnosis of this viral infection very confusing. In this study, a rate of 1.8% dengue infection found among febrile patients is rather low when compared with other studies carried out in different parts of Nigeria. According to Faneye [17], dengue IgM seroprevalence of 30.8% was reported in Nigeria among febrile children while another study in the northern Nigeria among healthy children revealed a rate of 17.2% [18]. Similarly, another study by Adedayo [19] revealed a prevalence of 30% of active dengue virus infection among children in Ilorin, Nigeria. Further evaluation of dengue IgG seroprevalence among malaria patients revealed that all the malaria patients in the study were positive for dengue IgG antibodies suggestive of a past dengue infection and consistent with the endemicity of dengue virus in the region [20].

This study has reported a higher rate of malaria infection among male (42%) than in female (22.2%) counterparts. It is however not the case for dengue in which the female infection rate was higher than that of the male participants. According to an earlier report, highest infection rate of the malaria and arboviral infections was detected 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 [21]. Furthermore, these infections have their peak rate among participants age ranged 16 and 45 years whose some risky behavioural patterns expose them to these infections and this is in concordance with previous report [22]. These behaviours include staying outdoors during daytime when these vectors bite, without the use of any personal protection measures among others. It is possible that practices and lifestyle of adults whose ages range from 21-40 years may make them more susceptible to these mosquito-borne diseases [22].

In this study, 1(0.6%) male patient with febrile illness was tested positive for dengue IgM antibody and malaria (*P. falciparum)*. In malaria endemic region, one may have suspected only malaria and neglecting other febrile illnesses such as dengue. Various studies have reported the detection of dengue fever virus among febrile patients in some parts of Nigeria [17, 23-25]. The condition could be an earlier infection because NS1 antigen was not detected. It may also be acute infection with the duration of infection in dengue infection reported to be gradual [26]. This co-infection rate found in this study is lower than 1.7% reported among similar population in Simawa Health Centre but comparable with other previous studies [16]. It is therefore advisable not to assume that febrile conditions in malaria endemic regions should target *P. falciparum* and salmonella typhi but include arboviruses as well in order to reduce the problem of misdiagnosis of related cases.

Conclusions

This study has reported high rates of malaria and dengue fever infections when compared with previous findings. It has also noted the rampant problems of misdiagnosis of febrile illnesses for malaria in many of our healthcare facilities in Nigeria. Arboviral infections have been found to have similar clinical presentations characteristic of Malaria fever and salmonellosis. It is therefore suggested that other pathogens such as arboviruses of which dengue is one of them should be incorporated in our laboratory investigations when handling cases of febrile conditions. Also, all forms of eradication of vectors of pathogens causing feverish illnesses and their habitations must be intensified with public awareness and sensitizations. This will help in no small measures in reducing problem of misdiagnosis, morbidity and mortality in our communities in Nigeria.

Competing interests

None has been declared as far as it was ascertained.

Ethical approval

The ethical approval was granted by the LAUTECH Teaching Hospital Ethical Review Board, Osogbo, Nigeria.

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