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A cross sectional study of dengue virus infection in febrile patients presumptively diagnosed of malaria in Maiduguri and Jos plateau, Nigeria

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Abstract

Background

In Nigeria, where malaria is endemic, greater than 70% of febrile illnesses are treated presumptively as malaria, often without a laboratory evaluation for other possible causes of fever. This cross-sectional study evaluated the presence of dengue virus infection in febrile patients, presumptively diagnosed of malaria infections in the clinic.

Methodology

Blood samples were collected from 529 febrile patients (246 in Jos and 283 in Maiduguri) attending the general outpatient clinics of the Jos University Teaching Hospital (JUTH) and the University of Maiduguri Teaching Hospital (UMTH) and tested for anti-dengue immunoglobulin M (IgM) and immunoglobulin G (IgG), as well as anti-non-structural protein (NS1) by ELISA. The samples were also evaluated for presence of P. falciparum malaria parasites by microscopic examination of Giemsa-stained blood smears.

Results

The prevalence of confirmed, highly suggestive and probable dengue virus infections categorized in relation to duration of illness since onset of fever were 2.3%, 5.5% and 1.5% respectively, while the prevalence of anti-flavivirus IgG and IgM seropositivity was 11.7%. In a total of 117 (22.1%) patients (32 in Jos, 85 in Maiduguri), malaria parasites were detected by blood smear microscopy, out of which 7 (6%) also had a positively confirmed, highly suggestive or probable dengue test result.

Conclusion

Although the high cross-reactivity of anti-flavivirus antibodies should be taken into account in the interpretation of the seroprevalence data, our findings suggest a significant presence of dengue virus in this environment, some of which may otherwise be misdiagnosed as malaria. These findings are strong enough to recommend serological screening for anti-dengue virus titer and NS1 antigen for all febrile patients, as part of fever diagnostic protocols in tropical regions. Given the prevalence of dengue virus infections, there is also a need for a dengue control program and public education to prevent outbreaks and occurrence of severe dengue complications.

Key words: Dengue, Malaria, Febrile illness, Jos, Maiduguri, Nigeria

Introduction

Dengue virus is a ribonucleic acid (RNA) virus belonging to the genus Flavivirus in the family of Flaviviridae. The virus, which has four serotypes designated Den-1, Den-2, Den-3 and Den-4, is transmitted by the mosquito *Aedesaegypti* and causes a disease which manifests as dengue, with or without warning signs (classical dengue fever) or severe dengue.[1] Classical dengue fever, the most common type of dengue illness, is characterized by sudden onset of fever, headache, anorexia, malaise, muscle and joint pains, rash and lymphadenopathy.[1,2] Severe dengue is associated with dengue shock, evidenced by plasma leakage, haemorrhage and features of severe organ dysfunction, such as markedly elevated liver enzymes and impaired consciousness[1,2]. Case fatality rate of severe dengue may be in excess of 5% and figures as high as 44% have been documented[1-3]. The worldwide incidence of dengue has risen 30-fold in the last 50 years[4]. More countries are reporting their first outbreaks and these outbreaks severely disrupt communities and drain economies[4,5]. Today, dengue ranks as one of the most important mosquito-borne viral diseases in the world, making the human and economic costs substantial[4,6]. Fifty million dengue infections are estimated to occur annually and approximately 2.5 billion people live in dengue endemic countries[4]. The first documented case of dengue in man was in Nigeria in the 1960s[7]. From August, 1964, to December, 1968, 32 strains of dengue virus were recovered from febrile patients seen at the University College Hospital, Ibadan, Nigeria[7]. Since then, several other studies have identified dengue virus infections in different geographical regions in the country[8-13]. A 1977 serosurvey in the general population reported a 30% prevalence of dengue

neutralizing antibody on the Jos Plateau and 33% in the Sudan savanna zone in northern Nigeria[8]. In the rainforest zone, prevalence of dengue neutralizing antibodies was reported to be 45%[8]. There have been recent reports of dengue and its co-infection with malaria in patients in northern Nigeria[14-17]. The emergence of severe dengue in Nassarawa state of north central Nigeria and Kaduna state of North West, Nigeria, in 2014 (Directorate of Public Health, Kaduna and Nassarawa State Ministries of Health, 2014, personal communication), brings new focus to the problem posed by dengue in our environment. Despite these reports of dengue infections and severe dengue, it is still not a reportable disease in Nigeria and in most African countries with most cases left undiagnosed or misdiagnosed as malaria[18,19]. In many healthcare facilities in Africa, malaria is recognized as the main cause of acute febrile illness, with enteric fever coming a distant second[14-17]. Most fever screening protocols in these institutions test for only malaria and sometimes enteric fever, while overlooking other causes of acute febrile illnesses, such as dengue fever virus and many other bacterial and viral causes of fever[14-17]. Therefore, the objective of this study was to determine the proportion of febrile patients with dengue fever infection and the proportion of dengue and malaria co-infection in patients clinically suspected to be infected with *Plasmodium* in two northern Nigerian teaching hospitals.

Methods

Study design and recruitment of participants

This was a prospective cross-sectional study conducted at the Jos University Teaching Hospital (JUTH) in North CentralNigeria and the University of Maiduguri Teaching Hospital (UMTH) in North East Nigeria between March and August, 2014, during the rainy season. Informed consent was sought from patients who met the inclusion criteria of fever (axillary temperature >37.8°C) and were clinically diagnosed or suspected to have malaria infection at the general outpatient clinics of the two hospitals. Patients who met the inclusion criteria and gave consent to participate were recruited into the study. Ethical clearance was obtained from the ethics review boards of both institutions. Relevant clinical and demographic data were collected from patients by means of a questionnaire.

Laboratory procedures for malaria diagnosis

Blood samples were collected from all consenting participants by venipuncture. Malaria parasitemia was determined by Giemsa stained thick and thin film microscopy[20]. Thick and thin blood films were prepared using clean grease-free non-silicate glass slides. The films were air dried without convection, and stained with 10% freshly prepared Giemsa stain maintained at a PH of 7.2. Thin blood films were fixed with 100% methanol prior to staining. The stained blood films were viewed under a light microscope at x1000 magnification (X100 oil immersion lens). The diagnosis of malaria was based on the identification of asexual stages of *Plasmodium* on the thick blood smears, while thin blood smears were used to identify species of *Plasmodium*. If no parasite was seen, blood films were declared negative. Each slide was read independently by two trained microscopists. In the event of discordant results, the slide was examined by a third microscopist.

Laboratory procedure for dengue virus diagnoses

Serum was separated from the blood samples by centrifugation at 1500g for 4 minutes and tested for NS1 antigens and presence of antibodies to dengue virus in the laboratory by standard ELISA techniques[1,21,22]. Dengue NS1 antigen, IgM and IgG assays were performed using a sandwich format microplate enzyme immunoassay for the detection of dengue virus NS1 antigen, IgM and IgG antibodies in human serum or plasma. Panbio dengue virus NS1, IgM and IgG ELISA kits were used and tests performed as described by the manufacturers[23,24]. In this semi-quantitative microassay, a positive ELISA result was defined as having an index value >1.1[23,24]. All samples were processed in the medical microbiology laboratories of both hospitals. Aseptic techniques, universal precautions and quality control measures were strictly observed from sample collection to processing in the laboratory.

Diagnostic criteria for categorization of patients

Patients were categorized into the following classes taking into account the serology result and the duration of illness since onset of fever: (i) Confirmed dengue (positive anti-dengue IgM + positive for NS1 antigen within 7 days of fever onset, (ii) highly suggestive of dengue (positive anti-dengue IgM or positive for NS1 antigen after 7 days of fever onset or as described in table 2, (iii) probable dengue (either positive anti-dengue IgM or positive for NS1 and as described in table 2), and (iv) positive anti-flavivirus IgG. A detailed description of this categorization is shown in table 2.

Data analyses

All data generated were collated, processed and analyzed with EPI info version 3.5.2 statistical software (CDC, Atlanta, GA, USA). Continuous variables were expressed as means ± standard deviation (SD), while categorical variables were expressed as proportions. Chi-square test was used to compare categorical variables while mean values of two groups were compared using Student "t" test. P-value of <0.05 was considered significant.

Results

The demographic characteristics of all participants in both study sites are presented in table 1. A total of 529 febrile patients were recruited for the study; 246 were from Jos and 283 were from Maiduguri; 179 (33.8%) were men and 350 (66.2%) were women, with a male: female ratio of 1:2. The participants in Jos were younger than those in Maiduguri (21.4 ± 18.0 years vs. 33.4 ± 13.8 years, p<0.001). The IgM antibody and NS1 antigen results of individual patients were interpreted in correlation to the duration of febrile illness at the time of sample collection. This interpretation was to account for the expected immunological response to dengue virus infection from the time of onset of illness (positive NS1 antigen within the first 2 to 7 days of onset of illness, positive IgM within 3 to 10 days from onset of illness and positive IgG after 10 days of onset or during a secondary infection). The proportions of confirmed dengue, highly suggestive of dengue, probable dengue and anti-flavivirus IgG seropositivity in the entire population were 2.3%, 5.5%, 1.5% and 11.7%, respectively (figure 1a).

Table 1: Demographic characteristics of all the patients studied for dengue virus and malaria infection in Jos and Maiduguri, Nigeria

	Study Location				
Demographic	Jos (n = 246)	Maiduguri (n = 283)	df	χ^2	p-value
Sex					
Male	81 (32.9)	98 (34.6)	0.17	1	0.680
Female	165 (67.1)	185 (65.4)			
Location					
Rural	174 (70.7)	78 (27.6)	98.32	1	<0.001
Urban	72 (29.3)	205 (72.4)			
Age (mean ± SD)	21.4 ± 18.0	33.4 ± 13.8			<0.001
Age categorization					
Children (< 18yrs)	130 (52.9)	42 (14.8)	86.63	1	< 0.001
Adult (≥18yrs)	116 (47.1)	241 (85.2)			
Age group (yrs)					
< 18	130 (58.9)	42 (14.8)			
18 – 27	38 (15.4)	65 (23.0)	95.74	5	< 0.001
28 – 37	32 (13.0)	86 (30.4)			
38 – 47	25 (10.2)	62 (21.9)			
48 – 57	16 (6.5)	13 (4.6)			
58 and above	5 (2.0)	15 (5.3)			

As shown in table 2, we categorized study participants into the following classes taking into account the serologic finding and the duration of illness since onset of fever: (i) confirmed dengue, (ii) highly suggestive of dengue, (iii) probable dengue (iv) anti-flavivirus IgG and IgM positive, and (v) negative serology (See table 2 for details).

Table 2: Proportions of dengue virus infection in febrile patients studied in Jos and Maiduguri, Nigeria using both serology and duration of fever

		Study location		
		Total (n=529)	Jos(n=246)	Maiduguri (n=283)
Patient Classification		Freq (%)	Freq (%)	Freq (%)
	Serologic finding			
Confirmed Dengue	Positive IgM + PositiveNS1 Ag within 7 days of fever onset	12 (2.3)	7 (2.9)	5 (1.8)
Highly Suggestive of Dengue	Negative IgM + Positive NS1 Ag within 7 days of fever onset	6 (1.1)	4 (1.6)	2 (0.7)
	Positive IgM + Positive NS1 Ag after 7 days of fever onset	1 (0.002)	1 (0.004)	0 (0.0)
	Positive IgM + Negative NS1 Ag after 7 days of fever onset	22 (4.2)	16 (6.5)	6 (2.1)
Probable Dengue	Positive IgM + Negative NS1 Ag within 7 days of fever onset	7 (0.01)	5 (0.02)	2 (0.007)
	Negative IgM + Positive NS1 Ag after 7 days of fever onset	1 (0.002)	1 (0.004)	0 (0.0)
Anti-flavirus IgG Positive	Positive IgG only	23 (4.4)	10 (4.1)	13 (4.6)
Anti-flavivirus IgM And IgG Positive	Positive IgM and IgG	39 (7.4)	22 (8.9)	17 (6.0)
No Dengue Infection	Negative for all dengue serologic markers including IgM, IgG and NS1	418 (79.0)	180 (73.2)	238 (84.1)

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Compared to patients in Maiduguri, the patients in Jos had higher seroprevalence for confirmed (2.9% vs. 1.8%, p=0.41), highly suggestive (8.5% vs. 2.8%, p=0.004) and probable dengue (2.4% vs. 0.7%, p=0.10), while patients in Maiduguri had higher seroprevalence of anti-flavivirus IgG seropositivity (4.6% vs. 4.1%, p=0.77), although this finding was only statistically significant for highly suggestive dengue serology.

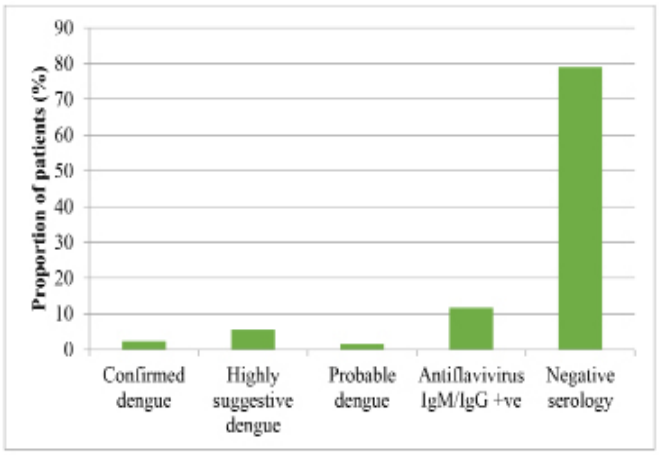

Figure 1 (a): Distribution of dengue serology among febrile patients (entire study population, N=529)

Out of the 529 blood samples, 117 (22.1%) were positive for malaria parasites(13% (32/246) in Jos and 30% (85/283) in Maiduguri). All cases of malaria parasitemia were caused by *Plasmodium falciparum*. However, 5.3% of patients were positive for both malaria parasite and anti-flavivirus/anti-dengue serology (28 out of 529 samples; 13 in Jos, 15 in Maiduguri). Only 6% (7/117) of patients who had a malaria infection also had confirmed, highly suggestive or probable dengue virus infection. The distribution of dengue virus serology among the 412 (77.9 %) malaria negative febrile patients and the distribution of malaria positive and negative parasitemia across various dengue virus/antiflavivirus categories are shown in figures 1b and 1c respectively. The proportions of confirmed, highly suggestive or probable dengue were much higher in patients with negative malaria blood smear negative compared to patients with a positive malaria blood smear.

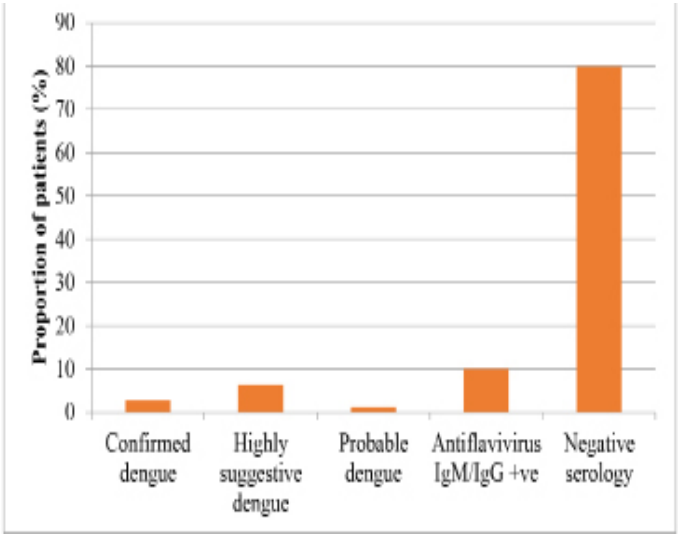

Figure 1b: Distribution of dengue serology among malaria smear negative febrile patients, N=412

Figure 1b shows that from the 77.9% (412/529) clinically misdiagnosed for malaria, 10.2%(42/412) either had a confirmed, highly suggestive or probable dengue serology and a total antiflavivirus + dengue serology of 20.1% (83/412) amongst the malaria smear negative patients

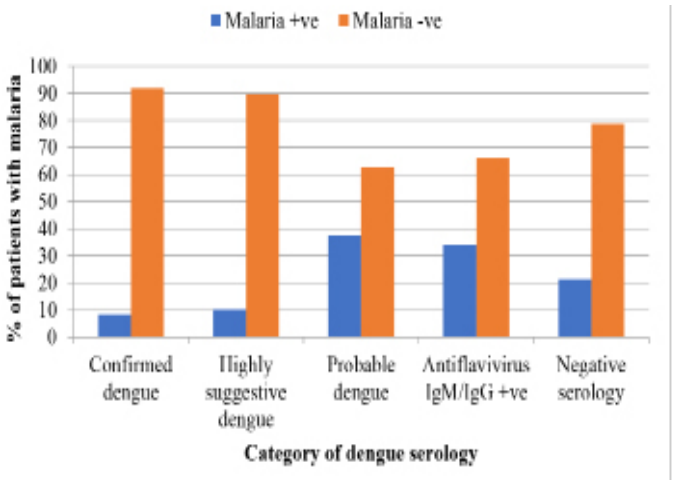

Figure 1 (c): Distribution of dengue fever/antiflavivirus categories in relation to malaria parasitemia

Fig 1c shows that the percentages of confirmed, highly suggestive or probabledengue were much higher in patients that were malaria blood smear negative (red) compared to patients with a positive malaria blood smear (blue).

Discussion

Reported cases of dengue have increased in a number of regions in recent decades, but the epidemiology of dengue in Africa is not clear[19]. Findings in the current study, using dengue serological tests (IgM and IgG antibodies and NS1 antigen), suggest that transmission of flaviviruses, such as dengue, is still occurring at a considerable proportion in Africa. Considering that antibody cross-reactivity between dengue and other flaviviruses often occurs[4], we classified our patients using the combined IgM antibody and NS1 antigen results, while factoring in the duration of illness since the onset of fever.

Variable seroprevalence rates of dengue virus infection have been reported in different studies in Nigeria[7-17,25]. Incidentally, most of these studies were either based on anti-dengue IgM seropositivity or positive NS1 antigenaemia in febrile patients, without necessarily interpreting the two serological markers together or relating the serologic findings to the onset of febrile illness. This makes comparison in dengue seroprevalence somewhat difficult. Based on anti-dengue IgM seropositivity, Idoko*et al*[15] documented seroprevalence of dengue infection of 1.8% among febrile patients in Kaduna, North West Nigeria while Oladipo*et al*[25] found that 17.2% of apparently healthy individuals in Ogbomosho, South West Nigeria, had anti-dengue IgM seropositivity, and Adesina*et al*[13] reported a much higher anti-dengue IgM seroprevalence of 25.7% among febrile patients in Ile-Ife, South West Nigeria. Using positive NS1 antigenaemia, 2.2% of febrile patients were found to have evidence of dengue virus infection in Jos, North Central Nigeria[14], compared to a much higher dengue NS1 antigenaemia of 35%, reported in febrile patients in Ibadan, South West Nigeria.[12] Our finding was similar to reports from other parts of sub-Saharan Africa which found a combined anti-flavi virus seroprevalence of 21-26.3%[26,27]. However, Kuniholm*et al*[28], using plaque-reduction neutralization tests, found 12.5% anti-dengue seropositivity in Cameroonian adults undergoing infectious diseases surveys.

Furthermore, climatic conditions in the rain forest region

of Southern Nigeria which supports increased mosquito breeding could possibly influence the transmission of dengue fever virus more than in the dry Sahel region of Northern Nigeria accounting for the variable seroprevalence of dengue virus infection reported in Nigeria and other parts of Africa[10-15,25,29]. Other factors such as presence of the virus in the environment, sufficient numbers of susceptible population and mosquito vectors are indeed critical to dengue transmission[29]. With demographic changes and observed increase in *Aedes spp*. populations in our environment[30], increased dengue transmission is likely to occur in many parts of Africa.

The diagnostic method used is an important factor that could have influenced the reported seroprevalence of dengue virus infection in available studies. Compared to the study of antibody titers, dengue virus isolation or PCR tests are much more specific and reliable[31]. Molecular detection by PCR was not carried out in this study due to cost limitations. However, dengue NS1 antigenaemia has proven to be a useful test for early diagnosis of dengue virus infection and in combination with anti-dengue IgM antibody tests, it can increase the diagnostic efficiency for dengue infection especially in the first few days of illness[31]. On the other hand, the anti-dengue IgG antibody test is not specific for dengue virus infection due to frequent cross reactions with other flaviviruses[31]. Although anti-dengue IgM is more specific for dengue compared to IgG, there is also residual cross-reactivity.[31]In many communities in Nigeria, several flaviviruses co-circulate with dengue viruses including yellow fever, West Nile, Usutu, Wesselsbron, Uganda S, Zika, Dakar Bat, Potiskum and Banzi and cannot be quite reliably differentiated from dengue using only antibody assays[32-36]. In addition, our study showed that the percentages of confirmed or highly suggestive dengue serology were much higher in patients with a malaria negative blood smear compared to those with a positive blood smear. This implies that febrile patients who were categorized as either confirmed or highly suggestive of dengue were also more likely to be malaria negative and suggests that dengue fever virus was the actual cause of their febrile illness.

In both study sites, we found twice the number of female versus male patients. This was a random occurrence as our sampling method was of consecutive patients who presented at the outpatient department of both hospitals with a febrile illness. Such a demographic occurrence might have been due to the higher tendency of females seeking medical care compared to their male counterparts. Additionally, the terrorist insurgency in Maiduguri at the time of this study could also explain why there were more women recruited, as men were either fighting in the insurgency or already victims of the insurgency. The observed disparity in age distribution between the two study sites and the rural–urban disparity between patients recruited in Jos and those from Maiduguri seem to have stemmed from the terrorist insurgency as well. The insurgency may have prevented patients, including children, from rural suburbs of Maiduguri and other parts of the state from coming to the teaching hospital to access care.

Historical data shows that epidemics of yellow fever had occurred in 1952, 1953 and 1969 in Okwoga district, Benue-Plateau State[8,32], which is close to Jos. Yellow fever and dengue fever are transmitted by the same mosquito vector of the *Aedes* species, which has been found in large numbers by some researchers in Jos and other parts of Northern Nigeria, and constituted the most prevalent population (72.6%) among the total mosquito population in a zoological survey in 2008[30]. Unplanned urbanization is a major factor in facilitating the increase of these mosquito populations.[33] Accumulation of non-biodegradable, human-made containers in and around living areas has provided the aquatic environment required by these mosquitoes[34,35], which is commonly seen in and around Jos and Maiduguri, where most of the patients in this study came from, and an obvious potential cause for dengue fever outbreaks. Public enlightenment and environmental sanitation will be very important to limit the number of aquatic environments that can harbor mosquitoes. Generally, severe dengue is reported to be infrequent in sub-Saharan Africa[37,38]. Although there are hypotheses regarding possible immunoprotective capacity of the cross-reactivity among flaviviruses[33,36], the role of such a phenomenon in the observed low rates of severe forms of dengue in sub-Saharan Africa is unknown[19]. Further studies may be required to probe this possibility.

One may wonder why dengue fever infection is perceived to be uncommon in this environment despite serological and ecological evidence suggesting otherwise. This might be because febrile illnesses such as malaria, tuberculosis, and HIV are endemic in many parts of sub-Saharan Africa and are more easily recognized and diagnosed. Most clinicians diagnose and treat for malaria once a patient presents with a febrile illness in our setting[18]. Dengue virus infection is hardly considered in the differential diagnosis of febrile patients in most parts of Africa. This study showed that only one fifth of febrile patients in the entire study population had evidence of malaria infection while others had dengue or remained undiagnosed of the cause of their febrile illness. It is also noteworthy that only about 5% of all febrile patients had evidence of both malaria parasitaemia and anti-flavi virus seropositivity. Other causes of febrile illnesses such as enteric fever, septicaemia, urinary tract infections, Lassa fever and other viral fevers, which were not the focus of the present study, are frequently missed in the evaluation of febrile patients in the African setting[11,16-18]. The implication of continued misdiagnoses and lack of any sense of clinical suspicion for other causes of febrile illnesses is imminent outbreaks of dengue and other febrile illnesses without warning or public health preparedness. Such an occurrence would impose a very heavy burden on the already overstretched health care systems in many African countries. Suffice to say that malaria misdiagnosis or over-diagnosis in the tropics would continue to have serious public health and economic consequences if unchecked[18]. The consequences of overtreatment and unnecessary prescription of antimalarial therapy to a large proportion of the population (77.9 % in this study) who are mostly poor and vulnerable and who in most cases do not have any health insurance, include: spending finances on unnecessary antimalarial medications, unwarranted adverse drug reactions and toxicities and increased risk for selection of antimalarial-resistant parasites, which have the potential to cross borders to other countries.

While our findings elucidate the possible role of dengue as an important cause of fever in our environment, the limitations of our study deserve to be mentioned. Molecular detection of viral proteins of Den 1 – 4 in the blood of participant, which would have differentiated secondary from past dengue infections and confirmed primary infections, was not carried out due to cost limitations. Some of the IgG, IgM or antigen results may not have been true positives due to rheumatoid

factor or cross-reactivity in the assay procedure. In particular, cross-reactivity of dengue with other flaviviruses was a major limitation for the assays. The absence of malaria parasite density is also acknowledged as a study limitation.

Conclusions

Our study findings suggest that dengue transmission is ongoing in northern Nigeria and are strong enough to recommend serological screening for dengue virus using NS1 antigen and anti-dengue IgM for all febrile patients, as part of fever diagnostic protocols in Africa. In addition, adequate training of clinicians and laboratory workers on laboratory diagnosis and case management of dengue should be ensured. Community-based public enlightenment programs for preventive measures will also help in reducing the transmission of dengue and other mosquito-borne diseases in our environment.

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Conflict of Interest

None to declare

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