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Dengue virus specific Immunoglobulin G antibodies among patients with febrile conditions in Osogbo, Southwestern Nigeria

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Abstract. Dengue fever is becoming one of the major public health problems in the world and its distribution has been premised on the migration of people from infected regions. This study was carried out to determine the prevalence of dengue virus IgG antibody among the patients with febrile conditions attending health facilities in Osogbo metropolis, Southwestern Nigeria. The blood samples collected between July and September, 2014 were tested for *Plasmodium falciparum* and the sera were subsequently subjected to Enzyme Linked Immunosorbent Assay (ELISA) to detect the dengue virus IgG antibody. Of the hundred consented participants screened, 77% were sero-positive for dengue virus IgG antibody while 41% were positive for *P. falciparum*. Thirty-three (33%) of the participants were positive for both dengue virus IgG antibody and *P. falciparum*. No significant difference was found in the prevalence of dengue virus IgG antibody and malaria among the participants (P>0.05). The high prevalence of dengue virus IgG and malaria signifies the need by the government of Osun State to sensitize residents and institute urgent measures to mitigate the resultant effects of morbidity and mortality due to dengue fever and dengue hemorrhagic fever which has hitherto appeared to be alien to the area.

INTRODUCTION

Dengue is an illness caused by infection with any one of the four related dengue viruses (DENVs) serotypes (DENV- 1, -2, -3 and - 4) which are transmitted mainly by *Aedes* mosquitoes (WHO, 2010). Dengue affects an estimated 50 million people in approximately 100 countries annually (WHO, 2009). The viruses (DENV) are the etiologic agents for both dengue fever (DF) and dengue hemorrhagic fever (DHF). Dengue fever (DF) is an acute febrile viral disease frequently presenting with headaches, bone, joint and muscular pains, rash and leucopenia (Gubler, 2010). Dengue haemorrhagic fever (DHF) is characterized by four major clinical symptoms; high grade fever, haemorrhagic phenomena, signs of blood flow failure and plasma leakage known as dengue shock syndrome (DSS) (WHO, 2009).

Dengue virus serotypes are immunologically related, and it is a positive-stranded RNA virus of the genus *Flavivirus*, family Flaviviridae. Infection with one dengue virus possibly provides lifelong immunity against other serotypes (Raja *et al*., 2009). The geographic spread of dengue is similar to that of malaria, but unlike malaria, dengue is often found in populated urban and residential areas of tropical nations (WHO, 2010).

In tropical Africa, a renowned malaria endemic zone; on several occasions, most cases of fever are assumed to be, and treated empirically as malaria infection - thus considering any fever as malaria (Amexo *et al.*, 2005). Therefore, dengue is likely underreported in Africa mostly because of low awareness by health care providers, presence of other prevalent febrile illnesses, and lack of diagnostic testing and systematic surveillance. In the 1960s, Dengue Virus 1 (DEN-1), Dengue Virus 2 (DEN-2) and Dengue Virus 3 (DEN-3) were isolated for the first time from samples taken from humans in Nigeria (Fagbami, 1977, Fagbami *et al.*, 1977). Sub-sequently, dengue has been found to occur in Senegal and Burkina Faso (predominantly being transmitted in sylvatic cycles), and possibly in other tropical rainforests in West Africa (Baba *et al.*, 2011). Recent studies have also affirmed the continued transmission of the infection (Baba *et al.*, 2009; Baba *et al.*, 2013; Faneye *et al.*, 2013).

Up to this moment, there is paucity of information on the prevalence of dengue fever in Southwestern Nigeria as few existing reports on the infection were documented in 1960s through 1970's (Fagbami, 1977; Fagbami, *et al.*, 1977). Owing to the global trend of dramatic increase in the prevalence of dengue fever, it becomes imperative to have coherent data on the prevalence of the infection to stem the morbidity and mortality due to the DF and DHF and in formulating appropriate control strategies regionally and countrywide.

Osogbo is one of the tourist attraction cities in Nigeria due to the Osun-Osogbo groove which hosts foreigners of diverse origins on a daily basis. This therefore predisposes the town to imported cases of dengue fever coupled with the predominance of *A. aegypti* and *A. albopictus* in the town (Adeleke *et al.*, 2013). The present study was therefore designed to determine the prevalence of dengue virus IgG antibody among the patients with febrile conditions attending health facilities in Osogbo metropolis, Southwestern Nigeria with the view of sensitizing the government and residents of the area on the impeding danger owing to the complications associated with dengue fever and instituting responsive strategies to combat the complications.

MATERIALS AND METHODS

Study Area

The study was conducted in Osogbo, the state capital of Osun State. Osogbo lies between longitude 4° 34'E and latitude 7° 46'N. The town's land mass is approximately 47 km^2 with a population of 156,694. The predominant ethnic group occupying the town is the Yourbas with the admixture of few other ethnic groups. The town also plays host to foreigners on daily basis due to the location of Osun-Osogbo groove, an international tourist centre. The town has tertiary, comprehensive and primary health facilities (Adeleke *et al.*, 2013).

Study design and sample size

Owing to the lack of previous data on prevalence of dengue fever in Osogbo in particular, and Osun State in general, which in turn made it difficult to calculate precise sample size, this study utilized cross-sectional approach wherein the blood samples of consented patients with febrile conditions (temperature $\geq$38.0°C) were collected from various health facilities in the town. The health facilities are namely: Ladoke Akintola University of Technology (LAUTECH), Akogun Primary Health Centre, Atelewo Primary Health Care Centre and Group Diagnostica Laboratory, all located in Osogbo metropolis, Osun State, Nigeria.

Ethical clearance

The ethical approval to conduct the study was given by the ethical committee of the College of Health Sciences, Osun State University, Osogbo. Permission was sought from the Managements of the health facilities and laboratories used for the study, while informed consent was sought and obtained from the participants.

Sample and screening for *P. falciparum*

Blood samples were collected aseptically from the study participants by venipuncture.

Samples were clearly identified with codes to avoid misinterpretation of results.Thick and thin blood films were prepared. The slides were stained with Giemsa stain and examined using the 100X (oil immersion) objective lens as described by Cheesebrough (2005).

Detection of Dengue virus IgG Antibody
The serum samples of the participants were tested for the presence of dengue virus IgG antibody using MONOLISA (DENG. CE/DENM.CE) from DIAPRO, Italy. The procedures adopted followed standard protocol as detailed in the manufacturer's instruction that accompanied the kit. The kit instructions were adhered to, for the interpretation, and cut off point in determining the positive and negative samples. Briefly, serum samples of the participants were diluted in 1:101 (i.e 1000µl sample diluent + 10µl sample) as recommended by the kit manufacturer. A volume of 100µl of negative control in duplicate and 100µl of positive control and 100µl of diluted samples were dispensed into respective wells except for microwell designated as blank (BLK). The microplate was sealed and incubated at 37ºC for 60 minutes. After incubation, the microwells were washed with prepared buffer by dispensing about 350µl per well as recommended. The washing was done five times. A volume of 100µl of enzyme conjugate was added into each well, except the well designated as BLK. The microplate was incubated again at 37ºC for 60 minutes and the plates were washed accordingly. A volume of 100µl of Chromogen/substrate was pipetted into each well including the BLK well and then incubated at room temperature (18-24ºC) for 20 minutes in a dark cupboard to prevent generation of high background. At the end of the 20 minutes incubation, 100µl of Sulphuric acid (stop solution) was pipetted into the wells. The optical density (OD) of the solution was measured at 450nm using a Biotek Multi ELISA plate reader (Labsystems, MA, USA). The cut-off was taken as negative control+0.250. Therefore, serum with OD < cut-off were considered non-reactive and hence negative while samples with OD > cut-off were considered positive.

Data Analysis
The data of the study was analyzed using Statistical Package for Social Sciences (SPSS) software (version 14.0). The chi-square test was used to determine association between categorical variables and the significant difference in the parameters was determined at $p < 0.05$.

RESULTS

Demographic data of the participants
A total of 100 consented participants were enrolled for the study. The demographic data of the study participants are presented in Table 1. Out of the 100 participants, 54 were males while 46 were females. Most of the study participants were within the age group of 21-30 years (33%), followed by 31-40 years (32%) while age group 61-70 years (1%) constituted the least.

Table 1. Demographic data of the study participants

Age groups	Male	Female	Total
0-10	2	2	4
11-20	5	8	13
21-30	23	10	33
31-40	13	19	32
41-50	8	2	10
51-60	3	4	7
61-70	0	1	1
Total	54	46	100

Prevalence of dengue virus IgG antibody and malaria among the participants
Of the hundred participants screened for dengue virus IgG antibody and *P. falciparum*, 77% were sero-positive for dengue IgG antibody while 41% were positive for *P. falciparum*.

Thirty-three (33%) of the participants were positive for both dengue virus IgG antibody and *P. falciparum* (Table 2). However, there was no significant difference in the prevalence of dengue virus antibody and *P. falciparum* among the participants ($p>0.05$). The Pearson co-efficient also revealed positive correlation between the prevalence of *P. falciparum* and dengue IgG among the participants ($r=0.117$), even though the association was not significant ($P<0.05$). The results further showed that the participants within the age group 41-50 had the highest prevalence, as all participants within this age group were positive for dengue virus antibody (100%), followed by age group 51-60 (85.7%) (Table 2). The prevalence of dengue virus IgG antibody and *P. falciparum*was higher among the male participants (81.48% for dengue IgG; 46.3% for *P. falciparum*) as compared with female participants (71.70% for dengue IgG; 34.8% for *P. falciparum*) (Table 3) but the difference was not statistically significant ($p>0.05$).

DISCUSSION

Dengue fever is becoming an emerging public health problem in Africa, and at least 22 countries in the continent are at the risk of the infection (Shepard *et al.*, 2013; Amarasinghe *et al.*, 2011). The 77% sero-prevalence of dengue-specific IgG antibody among the participants plausibly showed high circulation of dengue virus in Osogbo metropolis. The circulation of dengue infection in the study area could have been on-going for long period of time, albeit, with little attention since most of the febrile conditions in Nigeria are presumptively diagnosed as malaria (Balla *et al.*, 2013; Idris *et al.*, 2013). The wide distribution of dengue fever across the world has been premised on several factors which include among many others: the proliferation in breeding of *Aedes* mosquitoes, and movement of travellers from endemic countries (mostly Asian and American continents) (Mbanugo and Okpalaonuju, 2003; Abassi *et al.*, 2009;

Table 2. Prevalence of malaria, dengue virus IgG antibodyand the co-infection in relation to age among the participants

Age Group	Number screened	No positive for MP (%)	No positive for DENV IgG (%)	No positive for MP-DENV IgG (%)
0-10	4	2 (50.0)	1 (25.0)	1 (25.0)
11-20	13	5 (38.5)	11 (84.6)	5 (38.5)
21-30	33	16 (48.5)	25 (75.8)	13 (39.4)
31-40	32	11 (34.4)	24 (75.0)	8 (25.0)
41-50	10	3 (30.0)	10 (100.0)	3 (30.0)
51-60	7	3 (42.9)	6 (85.7)	3 (42.9)
61-70	1	1 (100.0)	0 (0.0)	0 (0.0)
Total	100	41 (41.0)	77 (77.0)	33 (33.0)

Legend: MP- Malaria Parasite; DENV - Dengue virus

Table 3. Prevalence of malaria and dengue virus IgG antibody infection by sex

Parameters	Number screened	No positive for Malaria (%)	No positive for DENV IgG (%)	No positive for Malaria–DENV IgG (%)
Male	54	25 (46.3)	44 (81.48)	22 (40.7)
Female	46	16 (34.8)	33 (71.70)	11 (23.9)

Legend: DENV - Dengue virus

Adeleke *et al.*, 2013). These factors could have also promoted the high circulation of dengue virus in Osogbo, which has been established using specific antibody marker. The town has an international tourist attraction centre (Osun groove) which plays host to people of different cultural background across the globe, coupled with the prolific breeding of *Ae. aegypti* in the area (Adeleke *et al*., 2013). The non-significant differences in the prevalence of the infection between male and female as revealed by statistical analysis may suggest that everyone at the study area, irrespective of the age and sex is at the risk of the infections.

We observed 33% prevalence of co-occurrence of dengue virus IgG antibody and malaria, while 44 participants were seropositive to dengue virus IgG antibody only. Earlier studies had also documented co-occurrence of dengue fever and malaria in different parts of the world (Charrel *et al*., 2005; Deresinski, 2006; Abbasi *et al*., 2009; Caraballo, 2014). For example, Abbasi *et al.* (2009) reported concurrent of dengue fever and malaria infection in 26 out of 112 (23%) febrile patients evaluated for dengue at a hospital in Karachi, Pakistan. Carme *et al.* (2009) evaluated 1723 consecutive febrile patients in Cayenne Hospital, French Guiana, and found dengue in 238 (13.8%), malaria in 393 (22.8%), and mixed infection of dengue and malaria infection in 17 (1%) patients (Carme *et al*., 2009). Mohapatra *et al.* (2012) found dengue-malaria co-infection in 27 of 469 (5.7%) febrile patients tested for dengue and malaria in Orissa, India.

Thirty-three percent (33%) prevalence reported in co-occurrence of both dengue virus IgG antibody and *P. falciparum* in the present study was higher than the prevalence recorded by researchers in Nigeria in recent time. Dawurung *et al.* (2010) reported 2.2% prevalence in Jos, Plateau state, while Idris *et al.* (2013) reported a prevalence of 10.1% co-infection of malaria and Dengue type-3 virus infection in Maiduguri, Northern Nigeria. However, the difference (perhaps high prevalence in the present study) could be explained in two folds. Firstly, the difference could be as a result of variation in dengue fever kit used. Unlike previous study that utilized IgM, the present study used IgG. IgG usually gives account of the previous and present exposure to dengue virus antibody while IgM only accounts for current exposure. IgG is an important antibody which appears 5-7 days of the dengue infection and reach the highest titres three weeks post-infection. It would thereafter decrease, but without total disappearance, leaving the host body with immunological evidence of previous exposure (Halstead, 1988). The re-infection could however lead to abnormal increase of the IgG titre, causing severe state of the disease such as dengue haemorrhagic, or dengue shock syndrome (Halstead, 1988; WHO, 2009). The other possible factor could be associated with the small sample size (100 samples) used in the present study. This limitation is noted and would be addressed during state-wide surveillance in our future studies.

Despite this limitation, the results of the present study have significant epidemiological implications, most importantly that dengue fever is probably alien to the study area where all febrile conditions are presumptively diagnosed as malaria (Adeleke *et al*., 2013). Most of the health facilities in the study area lack diagnostic tools for the detection of dengue viruses. Many patients presumptively diagnosed for malaria could have been misdiagnosed, while those with co-infection with dengue fever would have been mishandled; as the feverish conditions likely to persist after successful malaria treatment due to the presence of an 'undetected infection'. In most cases, these unresolved treatments are considered as 'fever of unknown origin' or malaria treatment failure. This could culminate in drug resistance by malaria parasite due to the drug abuse/ pressure or fatal casualties in patients' sequel to misdiagnosis.

In conclusion, findings from the present study have shown the circulation of dengue virus antibodies and co-occurrence with malaria among febrile patients in Osogbo, Southwest Nigeria; thus providing baseline data on the level of dengue antibody circulation in the study area. These observations justify the need for policy

makers in health sector to institute measures to mitigate the resultant effects of morbidity and mortality due to dengue fever and dengue hemorrhagic fever in the study area which could have been ongoing without notice. The impacts of the dengue virus infection in febrile and immune-compromised patients are thrust of our further studies in the area.

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