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INCIDENCE OF DENGUE VIRUS INFECTIONS IN FEBRILE EPISODES IN ILE-IFE, NIGERIA

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Abstract

Background: Dengue viruses have been identified as the most important arboviral pathogen in the world. They are transmitted by mosquitoes of *Aedes* species. While dengue infection is accompanied by little or no subclinical signs in many, about 1-2% may produce clinically severe Dengue Haemorrhagic Fever/Dengue Shock Syndrome. Early recognition, appropriate treatment and elimination of mosquito vectors will help control it. The study is aimed at determining the incidence of dengue infections in Ile-Ife.
Materials and Methods: Three millilitres venous blood was collected from each of one hundred and seventy nine patients presenting with fever in the last two weeks, and analyzed for the presence of anti-dengue IgM antibodies using Dengue Virus IgM ELISA kit (DIA.PRO, Italy) according to the manufacturer's instructions while the results and demographic data were analyzed using SPSS version 16.
Results: It was observed that 46 (25.7%) of the 179 had detectable IgM antibodies to dengue virus with 9 of them having no detectable malaria parasite. The incidence was 26.5% and 25% in male and female respectively. Further studies will be necessary to confirm the relatedness of blood transfusion as an important risk factor to the transmission of dengue virus.
Conclusion: The study established the presence of fresh dengue infections for the first time in Ile-Ife among different groups of people. Clinicians are advised to prioritize laboratory diagnosis, especially of fever.

Key words: Dengue, Dengue shock, Flaviviridae, ELISA, IgM

Introduction

Dengue, a mosquito borne viral infection caused by Dengue virus, is endemic in urban, peri-urban and rural areas of more than one hundred countries in Africa, the Americas, the eastern Mediterranean, Southeast Asia and the Western Pacific threatening the health of more than 2.5 billion people worldwide (Dengue, 2010). Dengue is highly prevalent in tropical Asia, especially Southeast Asia (Ooi et al., 2009). The infection can progress from dengue fever to the more serious dengue hemorrhagic fever or dengue shock syndrome (DHF or DSS). Annually, the virus causes about 50-100 million infections with cases of DHF/DSS estimated as close to 500,000 (Webster et al., 2009) and resulting in approximately 12,500 to 25,000 deaths (Dengue, 2010).

Dengue is caused by four serotypes (DEN 1-4) of Dengue Virus (DEN V) all of them sharing common transmission cycles with the mosquitoes *Aedes aegypti* and *Aedes albopictus* being the main vectors (but *Aedes polynesiensis* and *Aedes scutellaris* have also been implicated) most of which are widely distributed in tropical and subtropical areas of the world. Dengue Virus is a positive strand RNA virus of the family *Flaviviridae* closely related to Japanese encephalitis virus (JEV) and West Nile Virus (WNV). Humans serve as the reservoir and amplification hosts.

Dengue infections may be asymptomatic or give rise to undifferentiated fever (clinically indistinguishable from other viral infections) called dengue fever which occurs either during primary or secondary infections. DHF usually follows secondary infections but may sometimes follow primary infections especially in infants in whom maternally acquired antibodies are presumed to enhance primary infections (a phenomenon in human infections described only in dengue) (Martinez et al., 1993, Halstead et al., 2002) and/or DSS associated with very high mortality (Kabra et al., 1999) as a result of severe plasma leakage.

Management of dengue infections is mainly symptomatic, as there are no specific drugs effective against the virus and proper maintenance of fluid balance has been described as cornerstone in this. In addition to this, early identification of the leakage phase with prompt resuscitation help to reduce complications and improve outcome. It has been found out that mortality rates have been low in patients admitted early to hospital before the onset of shock (Tripahi et al., 1998). Since there is no effective antiviral therapy and no licensed vaccines yet, vector control, advances in clinical care and early laboratory diagnosis of dengue virus infection is important to reduce the morbidity and hence mortality rates.

Reports on dengue infections is scanty in Nigeria and indeed Africa probably because there has not been any reported epidemic of DHF (Nimmannitya, 2002; CDC, 2008) possibly because individuals of African origin have been said to have a degree of inherent resistance to the disease. Although Nigeria is regarded as one of the countries in which dengue is endemic, for ten years now, no study has demonstrated the incidence or circulating serotypes of dengue virus in Osun State, Nigeria. To contribute to effective control of dengue infection due to surveillance data, this study was embarked upon to determine the incidence of dengue virus infection by screening for Anti-dengue IgM antibodies in patients presenting with fever.

Materials and Methods

One hundred and seventy-nine consenting febrile patients including eighty three males and ninety six females at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) and the Health Centre (HC), Obafemi Awolowo University, Ile-Ife were used for this study.

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Sample collection commenced after obtaining the informed consent of the patients and in cases involving juveniles, parent's consent was obtained as well as the ethical approval from the Medical Advisory Committee of the Teaching Hospital. Some of these patients were asked to go for malaria parasite test at the hospitals of study. The malaria parasite results were obtained from the laboratories of the hospitals to determine the origin of their febrile conditions and so determine if Dengue virus was involved in their clinical conditions. The studied population is a mixture of people of different financial and academic backgrounds visiting the laboratories for malaria parasite test. Socio-demographic data were collected via administered questionnaires and analysed using SPSS version 16. The study was expected to give the incidence of dengue infections in the OAU Community and Ile-Ife in general and give an idea of the effect of different economic, academic and social statuses on dengue distribution.

Three milliliters (3 mL) of each patient's venous blood was collected into plain bottles, separated into serum and packed cells and stored at –20^{o}C in appropriately labeled bottles until analyzed. The serum samples were screened for dengue IgM antibodies using Dengue IgM ELISA kit (DIA-PRO, Italy) according to the manufacturer's instructions.

Results

Forty six (25.7%) out of the one hundred and seventy nine serum samples screened had detectable IgM antibody to dengue virus in them implying that at least one in every four individuals had been infected with dengue in the study area in Ile-Ife. Table 1 shows the distribution of anti-dengue IgM distribution with age. It is worth noting that 9 of the patients presenting with fever had no detectable malaria parasite but detectable Anti-dengue IgM suggesting the possible role of Dengue virus in pyrexia.

Forty six percent {(46%) n=82} of the patients screened were students of Obafemi Awolowo University, Ile-Ife out of which 27 (33%), most of whom were resident in their various hostels, had fresh Dengue infection. Civil servants were the next group of people recently infected with dengue as 25% (n=6) had the dengue IgM antibody. The ratio of dengue IgM positivity between male and females can be approximated to 1:1 as 26.5% of males and 25.0% of the females had the anti-dengue antibody in them. The details of the socio-demographic data collected and the dengue IgM antibodies are as shown in Table 2. Table 3 gives the summary of the various risk factors and the distribution of anti-dengue IgM using SPSS version 16 and their levels of significance at 95% confidence interval. The Chi-Square Tests (computed for a 2x2 table) of the blood transfusion and dengue virus IgM revealed an exact significance (2-sided) value of 0.029 while the 1-sided exact significance was 0.021. (This happens to be the only statistically significant value obtained in the study).

Table 1: The age distribution of dengue IgM antibody

Age categories (years)	Positive (%)	Negative	Total
< 1	0 (%)	2	2
1-10	5 (38.5)	8	13
11-20	5(23.8)	16	21
21-30	20(31.3)	44	64
31-40	6(17.6)	28	34
41-50	4(16.7)	20	24
51-60	3(30)	7	10
61-70	1(16.7)	5	6
71-80	1(25)	3	4
81-90	1(100)	0	1

Table 2: Socio-demographic data and dengue IgM antibody distribution

Socio-demographic data		Dengue IgM Ab	
		Positive (%)	Negative (%)
Sex	Male	22(22.65)	61(77.35)
	Female	24 (25)	72(75.0)
Marital Status	Married	20 (21.5)	73(78.5)
	Single	26 (30.2)	60(69.8)
Academic Qualification	None	7 (41.2)	10(58.8)
	Primary school	3 (16.7)	15(83.3)
	Secondary school	19 (30.6)	43(69.4)
	Tertiary	17 (20.7)	65(79.3)
Occupation	Student	27 (32.9)	55(67.1)
	Health worker	1 (11.1)	8(88.9)
	Teaching	3 (13.6)	19(86.4)
	Other civil servants	6 (25.0)	18(75.0)
	Retiree	4 (25.0)	12(75.0)
	Self-employed	5 (22.7)	17(77.3)
	Artisans	0 (0.0)	4(100.0)

Table 3: Risk factors associated with dengue infections

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Risk factors		Dengue IgM Ab		
		Positive (%)	Negative (%)	p Value
Travel history	Within Osun state	17 (28.3)	43(71.7)	0.549
	Outside Osun state	7 (17.1)	34(82.9)	
	Within Ile-Ife	21 (8.0)	54(92.0)	
	Outside Nigeria	1 (33.3)	2(66.7)	
Tattoo	Yes	1 (11.1)	8(88.9)	
	No	45 (26.5)	125(73.5)	
Blood transfusion	Yes	1 (5.3)	18(94.7)	0.029
	No	45 (28.1)	115(71.9)	
Hospitalization	Yes	14 (22.6)	48(77.4)	0.590
	No	32 (27.4)	85(72.6)	
Dental extraction	Yes	4 (26.7)	11(73.3)	
	No	42 (25.6)	122(74.4)	
Mosquito exposure	Yes	25 (26.0)	71(74.0)	1.000
	No	21 (25.3)	62(74.7)	
Malaria parasite	Yes	37 (27.4)	98(72.6)	0.430
	No	9 (20.5)	35(79.5)	

Discussion

The incidence of dengue has grown dramatically around the world in recent decades with over 2.5 billion people – over 40% of the world's population – now at risk from dengue. World Health Organisation currently estimates there may be 50–100 million dengue infections worldwide every year. Dengue is spreading fast across the globe as it is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific contrary to what it used to be in the 1970's when it was found only in nine countries of the world. The American, South-east Asia and the Western Pacific regions are the most seriously affected, http://www.who.int/mediacentre/-factsheets/fs117/en).

This study revealed the occurrence of recent dengue virus infections in Ile-Ife, Osun State Nigeria with the likely predisposing factors. This will be the first reported study on the incidence of dengue infections in Ile-Ife and in Osun State, Nigeria. This makes it impossible to compare the results obtained in this study with any previous study in the area. However, it corroborates the endemicity of dengue in subtropical and tropical regions to which Ile-Ife belongs showing 25.7% of the patients screened to be serologically positive for dengue infection. Analysis based on gender showed that 22 (n =83) males and 24 (n = 96) females had detectable dengue IgM antibody in them implying an approximate 1:1 ratio of infection. It also reveals that in Ile-Ife, at least one in every four of all the people screened has dengue infection and could develop any of the complications of severe dengue i.e. DHF or DSS were it not for the purported inherent resistance to dengue, an advantage that is lacking in other continents where dengue virus ravages. Nine (20.4%) out of the forty four patients who tested negative to malaria parasite test had detectable anti dengue IgM in their sera. This suggests that dengue virus can be implicated in their febrile conditions. It is necessary, therefore, that clinicians watch out for dengue in febrile conditions and not just assume it to be malaria. Laboratory diagnosis should also be encouraged to reduce wrong diagnosis and hence management to avoid deleterious effects not only on the patient but by extension on the world at large.

The designation of dengue virus as a blood pathogen has prompted many studies on transfusion-related transmission of the virus. In Puerto Rico, Mohammed and colleagues (2008) observed that nearly 1 in 1000 blood donations contained dengue virus RNA and virus could be cultured from transcription-mediated amplification positive donations suggesting a transfusion transmission risk similar to that which existed in the United States for West Nile virus before universal donation screening. In Hong Kong, the prevalence of this mode of transmission stands at one in 126 (Chuang et al., 2008). Also, viral isolation was confirmed in 6.7% of a studied population in Colombia with majority of them being asymptomatic (Mendez et al., 2006). This study showed that those without blood transfusion had a statistically significant value of 0.029 at 95% CI and anti-dengue IgM antibody in them. Having no blood transfusion probably reduced the exposure of such to DenV. In spite of this statistical significance, it may not be possible to conclusively implicate blood transfusion as the source of transmission in this study. The link between the date of transmission and onset of dengue infections could not be established. This does not rule out the fact that dengue virus can be transmitted through blood being a blood pathogen but calls for a closer attention and proactive measures to control the spread of dengue via transfusion of blood and blood products. The fact that the transmission of dengue virus by blood transfusion had been documented in humans (Tambyah et al., 2008) coupled with blood transfusion as a possible route of transmission of dengue virus demands greater caution among clinicians and haematologists to prevent the spread of this virus through their various procedures. Inter-human dengue transmission has been said to be highly efficient due to the relatively high viraemia titres found in many infected persons. More importantly, the behaviour and ecology of the vector-*Aedes aegypti* aid the spread of the virus.

Although, the presence of Dengue viruses has not been able to produce an epidemic of DHF in Africa, infected dwellers of the region travel to the susceptible areas thereby spreading the virus to areas where there is no "inherent resistance". The possibility of transmission via travel has been documented by Gautret and others (2010) where two cases of dengue fever in travelers from Benin to France were reported. The detection of anti-dengue IgM antibodies in about one third (32.9%) of the students may be connected to the densely populated, mosquito infested and unkempt environment many of them live in. One in every four females and 26.5% (n = 83) of the males screened had detectable dengue IgM antibody in them indicating an almost equal chance of infection in both sexes. Although the role of individual immune status was not considered in this study, it only shows a slight deviation from a male-to-female ratio of 2:1 obtained by Gupta and others (2005), Ukey and others (2010) and Kumar A and others (2010).The incidence observed in this study could be said to be high probably because of the various routes of transmission of the virus which could be from humans who are the only vertebrate hosts for most dengue strains. However, ancestral dengue viruses are also represented by primitive non-

human primate-borne strains that continue to circulate in the forests of West Africa and Southeast Asia (Gubler, 1997; Wang et al., 2000). The detection of dengue IgM antibodies in these patients support the claim that dengue viruses (like other arboviruses) are a frequent cause of febrile disease in Ile-Ife as in many other tropical and subtropical regions of Africa. Molecular detection of the viral RNA would have helped to confirm this. However, clinicians in these regions should note that dengue virus, a blood pathogen, could also be implicated in such conditions and to exclude its involvement, appropriate laboratory tests should be carried out. Laboratory capacity in sub-Saharan Africa and Nigeria in particular needs to be improved for the diagnosis of dengue infections. More intensive routine surveillance for the detection and characterization of the virus should also be promoted to enhance understanding of the serotype diversity which will in turn aid the production of vaccine against the virus.

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Authors' contributions: Adesina Olufisayo conceived the idea of and designed the study, recruited subjects, collected and processed the samples, performed the statistical analysis and prepared the manuscript. Adeniji Johnson supervised the study and made useful contributions to the manuscript. All authors read and approved the final manuscript.

Nomenclature: DEN V = Dengue virus; DHF = Dengue haemorrhagic fever; DSS = Dengue shock syndrome; JEV = Japanese encephalitis virus WNV = West Nile virus; IgM = Immunoglobulin M; ELISA = Enzyme-linked immunosorbent assay

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