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Detection of Dengue Virus IgM Antibodies in Febrile Hospitalized Patients in Khartoum State (Sudan)

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Abstract

Background: Dengue virus disease is a major public health problem in developing and tropical countries, and has been continuously spreading to new geographical areas. Among travelers returning from low and middle-income countries dengue is the second most diagnosed cause of fever after malaria.

Objective: To detect dengue virus IgM (DENV-IgM) antibodies in febrile hospitalized patients in Khartoum State (Sudan).

Materials and methods: This was a descriptive, cross-sectional study investigating 90 febrile patients attending some Khartoum State hospitals. The study was conducted during the period from October to December 2019. The enzyme linked immuno-sorbent assay (ELISA) method was used for detection of IgM antibodies of dengue virus in all serum specimens collected.

Results: From the 90 febrile patients investigated, 53 (58.9%) were males and 37 (41.1%) were females. 8 febrile patients (8.9%) were found positive for dengue virus IgM antibodies. The age incidence of all patients ranged between 1-75 years.

Conclusion: In this study a low seroprevalence rate (8.9%) of dengue fever virus IgM antibodies was detected. There was a significant correlation between malaria and dengue fever infection.

Keywords: Dengue virus IgM antibodies, Sudanese febrile hospitalized patients, ELISA.

Introduction

Dengue virus is an arthropod borne virus belonging to the genus *Flavivirus* in the family *Flaviviridae.* The arboviruses that infect humans are classified into different families: *Nairoviridae (*Crimean-Congo hemorrhagic fever virus, etc), *Peribunyaviridae (*Bunyamwera virus, Bwamba virus, etc.), *Phenuiviridae* (Rift Valley fever virus, etc.), *Flaviviridae* (dengue virus, zika virus, etc.) and *Togaviridae* (chikungunya virus, equine encephalitis viruses, etc.)[1]. Dengue virus is an enveloped virus with a single-stranded positive sense RNA genome, and contains a single open reading frame encoding three structural proteins including (capsid protein, premembrane/ membrane protein and envelope protein) and seven non-structural proteins (NS1,

NS2A, NS2B, NS3, NS4A, NS4B and NS5). Infection with dengue virus may produce a spectrum of clinical illness, ranging from asymptomatic infection, undifferentiated fever to dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).It comprises mainly four serotypes (DENV-1, 2, 3, 4)]. Dengue virus is transmitted among humans by the mosquitoes *Aedes Aegypti*[2].

Dengue virus disease is a major public health problem in developing tropical countries and has been continuously spreading to new geographical areas. Most of the time the actual number of dengue cases remains under reported and/or are misclassified. However, the World Health Organization (WHO) estimated about 50-100 million dengue cases in more than 100 countries per year, with upward trend of severe infections in Southeast Asia, Africa, South America and Western Pacific countries. Frequent international travels to dengue endemic or epidemic regions has contributed to the escalating numbers of imported dengue cases in temperate regions. Outbreaks of the four DENV serotypes had been increasing and reported in the tropics and sub-tropics mainly in Asia, South America, and the Caribbean. Multiple virus serotypes have been found circulating in the hyper-endemic regions in Southeast Asia and Pacific countries[3].

Among travelers returning from low and middle-income countries, dengue is the second most diagnosed cause of fever after malaria. Dengue fever is a severe, flu-like illness that affects infants, young children and adult, but seldom causes death. Dengue fever should be suspected when a high fever is accompanied by the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, or rash. Symptoms usually last for 2-7 days, after an incubation period of 4-10 days after the bite from an infected mosquito. Anti-DENV IgM can be detected as early as 3-5 days and reaches its peak around 12-14 days after symptoms onset. Detection of IgM is a preferred diagnostic test when the specimen is collected 5 days after disease onset[4].

Materials and methods

This was a descriptive, cross-sectional study investigating 90 febrile patients attending Khartoum Teaching Hospital (Sudan), during the period from October to December 2019. This study was approved by the Ethical Committee of Al Neelain University. Permission to collect the specimens was obtained from authorities of Khartoum Teaching Hospital (Sudan). Informed consent was obtained from each patient for the purposes of the current study. The specimens and information collected from all participants had not been used for any purpose other than this study. Data were collected through an interview using a self administered questionnaire. Confidentiality of information obtained from participants investigated was maintained. Sampling was a non-probability purposive sampling type, and sample strategy was convenience where participants were chosen on the basis of accessibility. The sample frame was febrile patients. Complete information regarding risk factors, if any, was handed to all participants under the study without concealment.

Blood samples were collected from 90 febrile patients, through vein puncture technique using 5 ml syringe, a volume of 5ml blood specimens transferred into plain containers, allowed to clot, to obtain serum; containers were centrifuged at 5000 rpm for 10 min. Serum was preserved at (- 20°C) till serological analysis was performed. The samples were processed by the 3rd

generation enzyme-linked immunosorbent assay (ELISA) technique (Euroimmun, Germany) to detect IgM antibodies of dengue virus (DENV). All reagents and samples were allowed to reach room temperature for 15 minutes before use. Washing buffer was prepared 1:9 from buffer concentrate with distilled water. The patients samples for analysis were diluted 1:101 with sample buffer (10µl sample to 1.0 ml sample buffer) and was mixed well by vortexing then the mixture was incubated for 10 minutes at room temperature, transfer 100 µl of the calibrator, positive and negative controls or diluted patients samples into the individual microplate wells then incubated for 30 minutes at room temperature (25°C). After incubation, the microplate wells were washed 3times using 300µl of working wash buffer for each wash leaved the wash buffer in each well for 30 seconds per washing cycle, then empty the wells. 100 µl of enzyme conjugate (peroxidase-labeled anti-human IgM) Reagent was then added in to each well; and the microplate was incubated for 30 minutes at room temperature. Empty the wells and each well was rinsed using 300µl of working wash buffer. This step was repeated 3 times until each well becomes dry.Then100 µl of chromogen/substrate solution was added into each of the microplate wells then was incubated for 15 minutes at room temperature(was protected from direct sunlight). 100µl of Stop solution was added into each of the microplate wells.
Measuring absorbance: The plate reader was measured colour intensity at wavelength 450 nm. The results were calculated by relating each sample optical density (OD) value to the Cut off value of the plate.
Calculation of results**:** The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by Euroimmun Company. Values above the indicated cut-off were considered positive; and those below the indicated cut-off were considered negative.
Results can be evaluated semi-quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator.
Euroimmun recommends interpreting results as follows:
*Ratio < 0.8: negative
*Ratio ≥ 1.1: positive
Negative result: Samples giving absorbance less than cut-off value.
Positive result: Sample giving absorbance equal to or greater than cut-off value.
Statistical analysis: Frequencies, mean values, standard deviations (SD) and ranges were used as descriptive statistics. The positivity of antibodies was tested by means of the Mantel-Haenszel chi-square test for linear association. The statistical analysis was performed by running the SPSS/PC+ statistical package version 21, on a personal computer. A two-tailed (p of 0.05) was chosen as the cut-off for detecting statistically significant values.

Results

A total of 90 febrile patients were enrolled in this study. 53 (58.9%) of participants were males and 37 (41.1%) were females. The participants were aged between 1-75 years. 75 of the participants (83.3%) were resident inside Khartoum State and 15 (16.6%) were resident out of Khartoum State. 33 (36.7%) of them had a travelling history, and 57 (63.3%) of them had no travelling history. Also, 53 (58.9%) of them had a 1-3 days fever, and 37 (41.1%) had a 4-7 days

fever. On the other hand, 19 (21.1%) patients were positive for malaria, and 71 (78.9%) were negative for malaria. 8 febrile patients (8.9%) were found positive for dengue virus IgM antibodies among all febrile patients (Table (1).

No significant correlation was found between dengue virus IgM antibodies and the following parameters:

* Age (p = 0.569). * Gender (p = 0.828). * Residence (p = 0.789). * Travel history (p = 0.959). * Duration of fever (p = 0.198).

As shown in Table (1), a significant correlation was detected between dengue virus (DENV) IgM antibodies and malaria (p = 0.036).

Table (1): Correlation of dengue virus IgM antibodies with age, gender, residence, travel history, duration of fever, and malaria.

Parameter	Positive IgM	Negative IgM	Total	p - value
Age:				
1-25 years	3 (6.8%)	41 (93.2 %)	44	
26-50 years	3 (8.8%)	31 (91.2 %)	34	0.569
51-75 years	2 (16.7%)	10 (83.3 %)	12	
Gender:				
Male	3 (8.1%)	48 (90.6 %)	51	0.828
Female	1 (5.3%)	34 (91.9 %)	35	
Residence:				
Khartoum Middle	2 (14.3%)	12 (85.7 %)	14	
Khartoum East	4 (11.1%)	32 (88.9 %)	36	
Khartoum South	0 (0%)	6 (100 %)	6	0.789
Khartoum North	1 (6.7%)	14 (93.3 %)	15	
Out of Khartoum	3 (9%)	30 (91 %)	33	
Travel history				
Travel	5 (8.8%)	52 (91.2 %)	57	0.959
No travel	3 (5.6%)	30 (91%)	33	
Duration of fever				
1-3 days	3 (13.6%)	50 (94.4%)	53	0.198
4-7 days	5(21%)	32 (86.5%)	37	
Malaria				
Positive	4 (21%)	15 (79%)	19	0.036
Negative	4 (5.6%)	67 (94.4%)	71	

Discussion

This study has revealed that 8.8% of patients attending three selected hospitals in Khartoum State (Sudan) had been exposed to dengue fever virus as indicated by the presence of IgM antibodies in their serum. The seropositivity rate of dengue virus IgM antibodies was 8.9%

(8/90). This finding agrees with that reported by Otu and his colleagues (2019) in Cross River State (Nigeria)[5] who found (6%) of his patients positive for DENV antibodies, 2% of them had co-existing primary dengue and malaria infections. While in this study there was a higher (21%) co-existing dengue virus and malaria infections.

Also, John and his co-workers (2012) in Northern Tanzania[6] reported (9.5%) of their patients were positive for acute DENV infection, 36 (9.8%) of were adults and adolescents, and 35 (9.2%) were infants and children.

On the other hand, Obonyo and his co-authors (2011)[7] conducted a study in North-Eastern Kenya and reported a lower seropositivity rate (13%) of dengue virus IgM antibodies in malaria infected patients.

Furthermore, the findings of the present study disagrees with the findings of the study conducted by Bello and his colleagues (2016)[8] in some hospitals of Kaduna State(Nigeria). They reported higher seropositivity rates out of the 366 patients investigated: 51.9% (190/366) were found positive and 48.1% (176/190) were found negative ($x^2 = 0.536$, df =1, p = 0.464).

While in the current study there was a lower positive prevalence rate (8.9% - 8/90) and a higher negative prevalence rate (91.1% - 82/90) of dengue IgM antibodies.

Antibodies to the virus were detected with the highest prevalence rate (72.95% - 89/122) in Kafanchan and with the lowest prevalence rate (34.42% - 42/122) in Birnin Gwari[9].

Thus the prevalence rate differs and depends on the geographical location of infection.

In the present context there was no statistically significant correlation between place of residence and prevalence rate of dengue infection. Also there was no statistically significant difference between age incidence (p = 0. 569), gender (p = 0.828), travel history (p = 0.959), duration of fever (p = 0.198), and presence of Dengue fever[IgM antibodies.

The lower prevalence rate obtained in this study as compared with other studies could be due to the low sample size, and the season of the year the study was conducted.

Recommendations: This study was conducted during the dry season between October and December 2019 where there was no rainfall and no stagnant water. These factors may reduce the breeding of *Aedes spp* mosquito which is the vector of dengue virus. Furthermore patients should be thoroughly screened for dengue fever virus alongside the screening of malaria/typhoid fever to avoid misdiagnosis of dengue with malaria and maintain effective treatment. Further large sample size studies are needed to establish the trend of infection if affected by age incidence, gender, residence, and seasonal variation of the virus.

Conclusion: In this study a low seroprevalence rate (8.9%) of dengue fever virus IgM antibodies was detected. There was a significant correlation between malaria and dengue fever infection.

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