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RESEARCH ARTICLE

SEROFREQUENCY OF DENGUE VIRUS AMONG FEBRILE PATIENTS ATTENDING PORTSUDAN TEACHING HOSPITAL

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ABSTRACT

Background: With more than one-third of the world's population living in areas at risk for infection, dengue virus is a leading cause of illness and death in the tropics and subtropics.
This study aimed to determine the serofrequency of IgM among Febrile patients attending portsudan hospital during May to August, 2014 at Red Sea State.
Methods: This was descriptive cross sectional study in which consenting patients with symptoms fever and joint pain) were included theirn age rang 1 to 56 with mean 27.7 . 90 plasma samples were tested by semi-quantitative enzyme linked immune sorbent assay for presence or absence of anti dengue virus (IgM) and relation of serofrequency with gender,age, platelets counts and pain joints were also detected .
Results: The frequency of IgM among 90 febrile patients attending portsudan teaching hospital was 97.7(n=88), seropositivity was 55.5%(n=50) of males and 42.2%(n=38) of females , the highest results among age groups was obseved among 1-18 years age range (43.3%). All patients had thrombocytopenia with platelets count ranged from 80, 000 to 150,000cell/mm3.
Conclusion: In the present study about 97.7% of febrile patients in portsudan had positives anti-dengue virus (IgM).

Keywords: anti-dengue virus, port sudan teaching hospital, serofrequnecy, febrile, ELISA.

INTRODUCTION:

Dengue viruses (DENV) are RNA viruses with a positive RNA strand, which belongs to the family *Flaviviridae.* Dengue fever (DF) is a vector-borne virus transmitted to humans by infected *Aedes* mosquitoes. It is an endemic disease of tropical and sub-tropical areas[(1)].

There are four distinct dengue virus serotypes, all of which originate from the family Flaviviridae and genus *Flavivirus*[(2,3,4)].

Dengue Fever/Dengue Hemorrhagic fever (DF/DHF) is a major public health problem that is responsible for millions of cases of illness and thousands of deaths in tropical countries worldwide every year[(5,6)].

The disease varies in presentation from asymptomatic infections to Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), which are the most serious forms of the disease [(7)].

The World Health Organization (WHO) estimates that two-fifths of the world's population is at risk of DF infection and that DF is now endemic in more than 100 countries [(8,9)].DF was first reported in Sudan, South Kordofan in 1967. DEN-2 was first reported in Port Sudan in 1986 (WHO) .

Dengue serotype 3 was found in outbreak among children in Port Sudan in 2005 [(10)]. Suspious of dengue in all cases with fever in Port Sudan was an important finding and conclusion of the study [(11)]. Moreover, presence of DEN-1, DEN-2 and DEN-3 was confirmed in the portsudan city and Jeddah just across the Red Sea with connections with Port Sudan [(12)]. Lately, in 2011 an outbreak of non-specific symptoms was detected through

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the National surveillance system in Lagawa locality within South Kordofan state. There was a great concern of the disease in *Terai* region of Nepal after the outbreak in Indian[13,14]DENV infection was recorded since 90's in Nepal and the first case of dengue was reported in 2004[15].

In Nepal, there have been only few studies of the serofrequnecy to DENV. DENV infection can be serologically inferred by detecting immunoglobulin M (IgM). IgM-capture enzyme linked Immune Sorbent Assay (ELISA) is used as a standard method for detecting (IgM)[16].

Their study was contributed to determine the epidemiological situation of dengue and to estimating the accuracy of dengue diagnosis in Nepal.

DF is a self limited febrile illness, while DHF is a life-threatening, often fatal illness(1).

Our study done to determine the serofrequency of anti dengue virus among febrile patients (fever and pain joints symptoms and thrombocytopenia).

MATERIALS AND METHODS

Design

The present study was descriptive cross sectional study in which consenting febrile patients with symptoms and signs dengue fever attending portsudan teaching hospital and out patients during May to August 2014, 90 patients were allowed .

Subject selection

Symptomatic and febrile patients suspected having dengue fever attending portsudan teaching hospital and outpatients during May to August, 2014, were included in this study. Asymptomatic patients and healthy person were excluded from the study.

Experimental work:

Collection of spceimens :

Before collecting blood specimen, a structural interviewing questionnaire was used to collect sociodemographic and clinical data. also informed consent was obtained from each patient or guardian. The study was carried out in Alneelain University.

Blood samples were collected from a total of 90 individuals experiencing a febrile illness clinically consistent with DENV infection and drawn in container contain EDTA anticoagulant, centrifuged. and the samples were then kept on the ice compartment of the refrigerator, and immediately were stored at -20°C refrigerator.

Processing of spceimens :

Plasma samples were analyzed using euroimmun Enzyme-linked immune sorbent assay (indirect ELISA) kits for anti-dengue virus (IgM). Positive and -negative control were used, and the ELISA kits tested (euroimmun, germany) within analyzing the plasma samples.

The reagent and samples were allowed to reach the room temperature for at least 15-30 minutes. The washer buffer concentrate was checked for the presence of salt crystals. The washer buffer diluted 1 to 9 with distilled water. The strips needed were set in strip-holder and numbered sufficient number of wells including one negative control one positive control and one calibrator .

The specimens were diluted 1:101 with sample buffer and incubated for 10 minutes at room temperature . The controls are ready to use as supplied.

100µl of samples were added in to each well and 100µl positive and negative controls in to their respective wells. A separated disposal pipette tip was used for each specimen, Negative and positive controls as to avoid cross-contamination.

The plate was covered with the plate cover and incubated for 30 minutes at 25°C.

At the ends of the incubation the plate cover was removed and discard, then washed each well automatically 3 times with 400µl working strength wash buffer. Each time the micro wells were allowed to soaked for 30-60 seconds. After the final washing cycle, the plate was blotted on to a clean towel, to remove any remaining buffer.

100µl of peroxidase –labelled anti- IgM human (goat) were added in to each well except the calibrator.

The plate was covered with the plate cover and incubated for 30 minutes at 25°C.

Then the plate cover was removed and washed each well 3 times with 400µl working strength wash buffer. After the final washing cycle, the plate was blotted on to a clean towel, to remove any remaining buffer.

100µl of chromogen/substrate solution were added in to each well including the Blank, then was Incubated at 25°C for 15 minutes.

100µl of Stop solution was added in to each wells in the same order and the same speed as the chromogen/substrate solution was introduced.

The plate reader was calibrated with the calibrator well and read the absorbance at 450nm.

Measurement:

Photometric measurement of colour intensity was be made at 450 wavelength within 30 minutes of added stop solution and the samples were shaked before measuring.

Calculation of results and interpretation:

Results were elevated semi quantitatively by calculated a ratio of the extinction value of the calibrator. use the following formula to calculate the ratio:

Extinction of control or patients samples / extinction of calibrator = Ratio

EUROIMMUN recommends interpreted results as follows:

Ratio <0.8: negative
Ratio ≥ 0.8 to 1.1 broder line
Ratio ≥ 1.1 positive

Platelets counts results were obtained from hematology lab.

Analyzing

The data retrieved from the questionnaires were analyzed using the Statistical Package for Social Sciences (SPSS) version 11.5 and the Microsoft Excel (MS) software program.

The serofrequncey of anti-dengue virus (IgM) , gender, age, platelets count and joints pain distribution were analysed among symptomatic patients.

The degree of association of serofrequnecy (IgM) with gender ,age ,platelets count and joints pain and were determined using Chi square test. Statistical significance was set at p-value of less than or equal to 0.05 (p-value ≤ 0.05).

Results

A total of 90 febrile patients who attending portsudan teaching hospital were enrolled in this study to detect anti-dengue virus (IgM) , out of them 88 were positive (97.7%) For anti-Dengue virus (IgM) was showed in figure (1).

A positive results of anti-dengue virus (IgM),were recorded based on the gender, age, joints pain and platelets count. The highest seropositivity was observed among males 55.5% (n=50) as compared to female 42.2% (n=38), and among age range from 1 to18 years, the statistically analysis show no significant relation between IgM seropositivity and gender or age(p-value=0.110 and 0.258 respectively), see figures (2) and (3).

All patients had thrombocytopenia with platelets count ranged from 80, 000 to 150,000 cell/mm3,and suffered from joints pain.

However there it were significant association between sreopositivity and both platelets count and joint pain (were set at a p-value 0 .00).

Discussion:

Dengue fever it is currently regarded as the most important arboviral disease internationally as over 50% of the world's population live in areas where they are at risk of the disease, and approximately 50% live in dengue endemic countries, Up to 3.6 billion people are estimated to now live in tropical and subtropical areas where the dengue viruses have the potential to be transmitted. Global estimates vary, but regularly approximate 50 million to 200 million dengue infections, 500,000 episodes of severe dengue (DHF/DSS), and over 20,000 dengue related deaths occur annually [17,18,19,20]. there are not yet vaccines to prevent infection the most effective protective measures are those that avoid mosquito bites . IgM antibodies are the first to appear and are detectable in 50% of patients by days 3 – 5 after onset of illness, increasing to 80% by day 5.

This was the first study carried in portsudan estimating the ant- dengue virus (IgM) among febrile patients or clinically consistent with dengue virus infection.

The present study showed slightly higher serofrequency of anti-dengue virus (IgM) 97.7% when compared with study carried out in portsudan (2005), their results was 90% dengue IgM positive by RAPID-cassette test. Of 40 serum samples 23 had enough volume for further confirmation and all were confirmed by IgM -capture ELISA kits. Nine of 23 were PCR positive for DEN virus serotype 3.

The highest serofrequnecy of anti-dengue virus (IgM) indicate recent infection by dengue virus which may be due to that the samples on our study collected during outbreak, from febrile patients and clinically diagnosed infected by dengue virus. regarding platelets count, the present study results agreed above mentioned studies, all patients had Thrombocytopenia.

Out of 88 positive patients in this study, 50 were males patients who constitute 55.5% of the total of males patients and 38 were female patients which comprise 42.2 % of the total females patients. Statistically there was no significant relationship between sex and the occurrence of dengue fever (p= 0.110). In present study the numbers of positive were higher in males because males are more likely to be exposed to mosquitoes during their outdoor activities. The study it dissimilar a results dengue fever infection among apparently healthy people in the boarder state between Sudan and the new republic of South Sudan carried to determine seroprevalence of anti-dengue virus (IgG), our study carried to determine anti-dengue virus (IgM) among febrile patients. The age wise distribution of positive dengue fever were highest in the age group 1 to 18 years i.e. pediatric age group 39(43.3% from a total 97.7%). Statistically, there is no significant relationship between age groups for the occurrence of disease (p=0.258).

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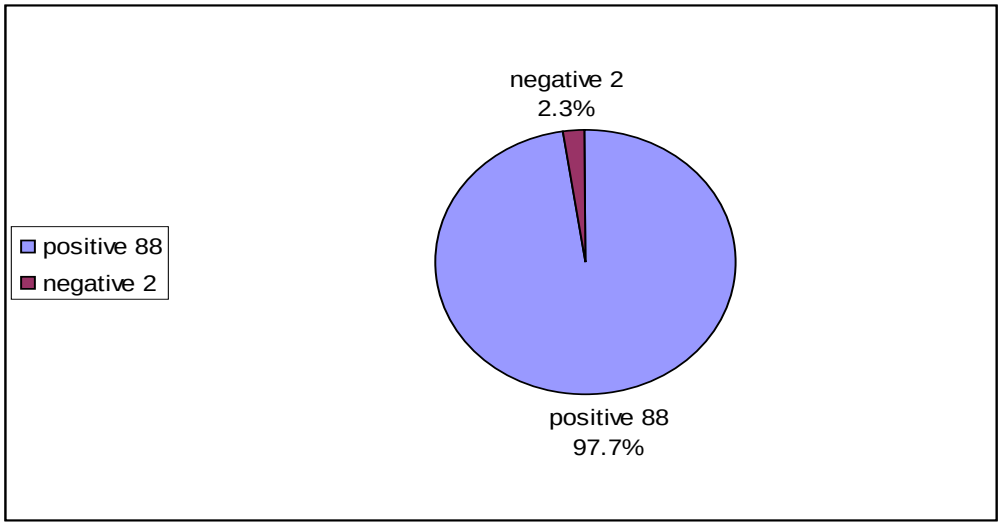

Figure 1: frequency of IgM seropositivity among 90 fibrile patients

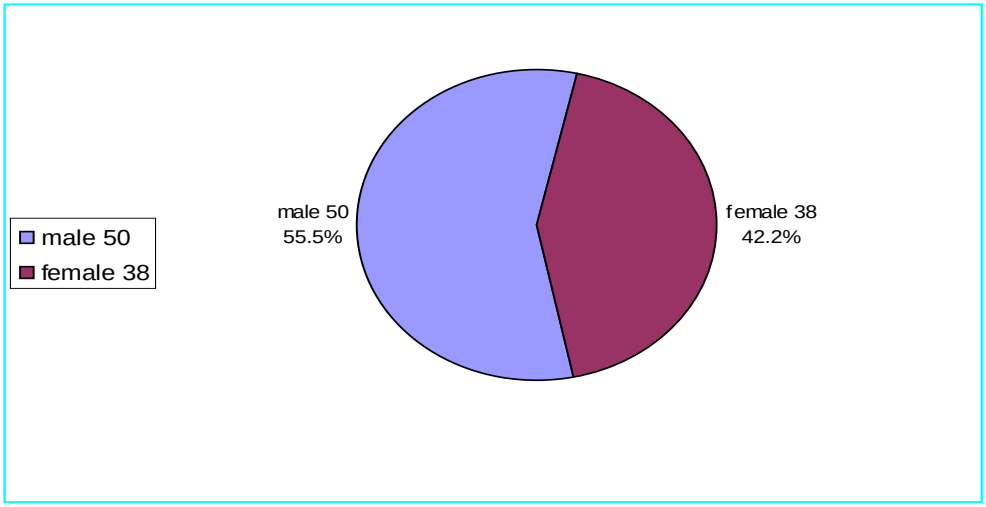

Figure 2: Relation between a gender and seropositivity

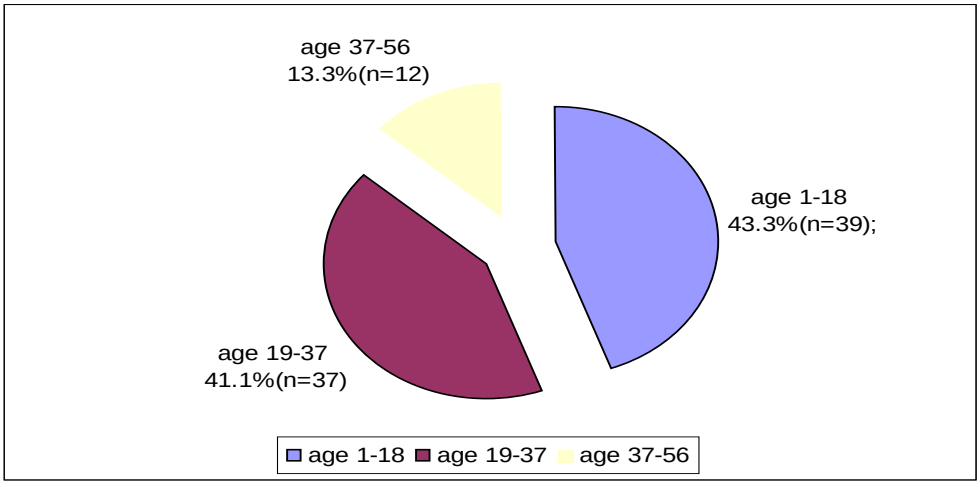

Figure 3: frequency of IgM positivty according to age :

n: number of patients show positive result.

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