Sero-Prevalence of Dengue Fever Virus Antibodies in Red Sea State, Sudan- A Cross-Sectional Study

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Background: Dengue fever (DF) is caused by dengue virus (DENV), a positive-sense single stranded RNA virus of the genus Flavivirus in the family Flaviviridae. The DF has been reported as one of the most important arboviral diseases in many parts of the world including Sudan. DENV is a widely spreading disease that has resulted in an emerging infectious disease world-wide.

Aim: The aim of this study was to estimate the prevalence of dengue virus in Red sea state, Sudan.

Materials and Methods: This study is a cross sectional study, carried out in Eastern part of Sudan with an impartial method to determine the DENV antibodies status in Red Sea state, Sudan. Dengue Virus (DENV) antibody test was done for each sample. Blood samples were collected from each patient in a plain container, and then serum was separated and tested for (DENV) IgM by using ELISA.

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Dengue fever; dengue virus; red sea; Sudan.

1. INTRODUCTION

Dengue is an acute febrile disease caused by the mosquito-borne dengue viruses (DENVs), consisting of four serotypes (DENV 1 to 4). Dengue virus is a member of the family Flaviviridae and of the genus Flavivirus. It is a spherical virus of about 50 nanometers in diameter consisting of three structural proteins; the capsid (C), the pre membrane/membrane (prM/M) and envelope (E) [1]. The 10.7 kilo base positive sense single stranded ribonucleic acid (RNA) genome also encodes for 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) [2]. The order of gene is in 5-CprM(M)-E-NS1-NS2A-NS2b-NS3-NS4A-NS4B-NS5-3[3].

Once the human being has been infected by the virus, it activates the interferon signaling pathway, but the virus develops resistance by NS2A, NS4A and NS4B that blocks the interferon cascade to escape the immune response[2]. There are 4 known serotypes of the Dengue virus circulating in the tropical and subtropical region in the world. These serotypes (Dengue virus 1 -4) differ from one another by 25-40% amino acid level [3]. The co-circulation of diverse serotypes is common in hyper endemic regions and mostly an outbreak arises from a dominant serotype in a 2-4-year cycle[4].

Additionally, the serotypes are further separated into subtypes that differ by 3%. There are 3 subtypes of Dengue virus 1, 6 for Dengue virus 2 (one of which is found in non-human primates), 4 for Dengue virus 3 and 4 for the serotype 4 [5]. Phylogenetic studies have shown that subtypes normally circulate within a defined area [5,6]. However, the DENV is presently the most common reason behind arboviral disease globally, and all four serotypes of DENV is found worldwide[6]. Over the past century hundred countries are endemic, primarily affecting 2.5 billion people within the tropical and climatic zone regions in addition as a hundred and twenty million travelers to those regions a year[7]. The world Health Organization (WHO) estimates annual incidence of roughly a hundred million infections, with or so 500,000 people with dengue hemorrhagic fever (DHF) requiring hospitalization, an oversized proportion being youngsters. The DHF might turn into dengue shock syndrome (DSS) whereof the fatality rate is approximately 1–2.5%. Successful treatment of patients with DHF and DSS is labor intensive and expensive, however while not correct treatment, fatality rates might exceed 20%. [8]. The four DENV serotypes can cause a wide range of diseases in humans even though DENV infections may also be asymptomatic. The diseases range in severity from undifferentiated acute febrile illness, classical dengue fever (DF), to the life-threatening conditions DHF/DSS [9]. Dengue illness was previously categorized on a I–IV grade scale, but a simplified categorization for dengue case classification has been proposed by WHO’s Special Program for Research and Training in Tropical Diseases (TDR) in 2009 where DHF and DSS cases are grouped together as ‘severe dengue’ (group C) to avoid false-negative DHF/DSS diagnosis[10].

A systematically review the published data on dengue virus (DENV) seroprevalence in Sudan, they searched, reviewed, and extracted online available reports on DENV in Sudan. Among 168 identified records, 19 were selected. Dengue infections were documented in 11/18 states. The overall seroprevalence of DENV in Sudan was estimated to be 27%, while the prevalence of dengue IgM was 22% and IgG was 38%. The prevalence of dengue estimated from community and hospital-based cross-sectional studies were...
26% and 30% respectively. Additionally, one cohort study and a single PCR-based study reported a prevalence of 1% and 4%, respectively. Regional analysis revealed that the variation in seroprevalence in East, North, West, and Central Sudan was 23%, 24%, 36% and 43%, respectively. Interestingly, they found that DENV is circulating countrywide with a significant spatiotemporal variation in the disease seroprevalence[11]. Furthermore, publications on dengue prevalence are temporally and geographically fragmented, perhaps due to limited resources. However, this gap in data and knowledge highlights the urgent need for a country-wide surveillance system and continued study of dengue burden in Sudan to accurately estimate the disease prevalence and determine the associated risk factors.

2. MATERIALS AND METHODS

This was cross sectional study, carried out to determine DENV antibodies status in Red Sea state, Sudan. A total of 380 Patients were included in this study. Dengue Virus (DENV) antibody test was done for each sample. Blood sample was collected from each patient in plain container, and then serum was separated and tested for (DENV) IgM by using ELISA.

2.1 ELISA Procedure

DENV IgM was tested using ELISA EUROIMMUN (Germany), semiquantitative analysis was done according to manufacturer instruction as:

1- 100µl of the calibrators, positive control, negative controls and diluted patient’s samples were transferred to microplate wells and incubated for 30 min at 32°C.
2- 300µl of working strength wash buffer was used to Wash microplate wells. And this process repeated three times.
3- 100µl of enzyme conjugate was pipetted into each of the microplate wells and incubated for 30 min at room temperature.
4- The microplate wells were washed again as previously described in step two.
5- 100µl of chromogen / substrate was pipetted into each microplate wells and incubated for 30 min at 32oC.
6- 100µl of stop solution was pipetted into each microplate wells and incubated for 5 min.
7- Results was recorded at 450 nm wavelength filter's and also at 650 nm for reference reading by ELISA reader.
8- Calibrator 2 was measured to evaluate the results according to following formula:

\[ \text{Ratio} = \frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} \]

Specimens with antibody concentrations >18 U/ml above the mean concentrations of negative controls were considered as positive.

2.2 Data Analysis

Data were entered and analyzed by SPSS program version (21). All demographic data of the study population were presented as mean ± SD in the text and Odds Ratio was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated.

3. RESULTS

Of the total 380 dengue cases tested 106 (27.9%) were identified as anti-dengue IgM positive (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>106</td>
<td>27.9</td>
</tr>
<tr>
<td>Negative</td>
<td>274</td>
<td>72.1</td>
</tr>
<tr>
<td>Total</td>
<td>380</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 1. Serodiagnosis test results by IgM-ELISA

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>163</td>
<td>42.9</td>
</tr>
<tr>
<td>Female</td>
<td>217</td>
<td>57.1</td>
</tr>
<tr>
<td>Total</td>
<td>380</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2. Distribution of gender among study population
Table 3. Correlation between results by IgM-ELISA and gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>IgM result</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>negative</td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td>112</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>162</td>
</tr>
</tbody>
</table>

Table 4. Correlation between results by IgM-ELISA and age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>IgM result</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Less than 10 years</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>10-19 years</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>20-29 years</td>
<td>27</td>
<td>46</td>
</tr>
<tr>
<td>30-39 years</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>More than 40 years</td>
<td>37</td>
<td>115</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Dengue fever (DF) is an arthropod-borne viral disease (arbovirus) caused by dengue virus (DENV) which circulates in tropical and subtropical areas where the environment is suitable for vector breeding [12]. Humans are the main carrier of the virus and the amplifying host with non-human primates play a considerable role in sylvatic cycle. A total of 380 serum samples were collected from patients suspected of DVI, among them, 57.1% (n=217) were females and 42.9% (n=163) were males. The result contrasts with most of the studies in Nepal which have reported male to female ratios of 1.2:1 (Pun, 2009; Shah, 2010). Another agreement was observed in study done by Pant .2107 [13] which found 50.6% (n=185) were females and 49.1% (n=179) were males. Male to female ratio was 1:1.41. there was no significant variation between gender and the occurrence of the disease (p=0.123).

The result was not in harmony with the previous studies done in Nepal in which the number of DV cases was more in males [14,16]. However, the result agreed with study by Shah et al. [14] and Neupane [17]. The number of cases was more in females than in males because females are engaged more in household activities than males. A.aegypti, the principal mosquito vectors of dengue are peri-domestic, day-biting species that lives and breeds in and around the home. A. aegypti prefers to lay eggs in artificial water containers such as flower vases, old automobile tires, buckets that collect rainwater, cement cisterns, drums and barrels and trash in general. This makes females more prone to being bitten and infected by the mosquito during daytime.

5. CONCLUSION

We can conclude that serotypes (IgM) of dengue virus prevalence is 27.9% in Red Sea State, Sudan and this finding indicate that the residents of the Red State are at risk of developing the disease. Also, there was significant variation between age and the occurrence of the disease. The Ministry of health should initiate dengue surveillance and commence an integrated vector control programme.

CONSENT AND ETHICAL APPROVAL

Ethical approval from University and Red Sea state ministry of health research department. Research purpose and objectives was explained to participant in clear simple words. Participants had right to voluntary informed consent. Participants had right to withdraw at any time without any deprivation. Participants had a right to no harm (privacy, confidentiality by using coded questionnaire) Participants had right to benefit from researcher knowledge and skills. Verbal consents were provided for all patients of
the study; they were free to decide whether to participate or not.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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