A STUDY TO DETERMINE THE INCREASING DENGUE INCIDENCE IN BIHAR REGION: AN OBSERVATIONAL STUDY.
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Abstract
Introduction: Dengue is one of the most serious mosquitoes borne viral infection mainly affecting tropical and subtropical countries of the world. In absence of specific treatment and vaccine for dengue fever (DF); vector control is the only method by which spread of dengue can be prevented. The present study was conducted to determine the increasing dengue incidence in Bihar region.

Material and Method: This study was done to report the sero prevalence of Dengue virus infection at Bihar, North India. The laboratory records of clinically suspected Dengue patients from Jan 2018 to Nov 2018 were analyzed retrospectively for demographic features, seasonal variations, and results of IgM and IgG anti dengue antibodies tested by Dengue IGM capture enzyme linked immune sorbent assay (MAC ELISA).

Results: A total of 1035 serum samples were analyzed. Out of which 209 samples (20.19%) were found positive for dengue virus infection. Maximum positive cases were seen in 2019 (53.11%). Seasonal trend showed that infection started appearing in August, peaked in October and slowly tapered by December.

Conclusion: The most affected age group was 11 to 15 years of age, (57.3%) followed by 6 to 10 year’s group (27.3 %) and least effective age group was 0 to 5 year (15.4%), majority of cases were found to be of secondary dengue virus infection (93.8%). Male to female ratio was 2:1. The present outbreak thus emphasizes the need for continuous sero epidemiological surveillance for the timely formulation and implementation of effective dengue control programme.

Keywords: Dengue, dengue haemorrhagic fever, IgM antibody capture enzyme linked immune sorbent assay (MAC ELISA), India, vector.

Introduction
Dengue viruses (DV) belong to family Flaviviridae and there are four serotypes of the virus referred to as DV-1, DV-2, DV-3 and DV-4. It is found mainly in areas of the tropics and sub-tropics. It is a positive stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins. It is transmitted mainly by Aedes aegypti mosquito and also by Aedes albopictus. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).

The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe, though the 1997 WHO classification is still widely used. The 1997 classification divided dengue into undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF). Four main characteristic manifestations of dengue illness are:

(i) Continuous high fever lasting 2-7 days;
(ii) Haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis;
(iii) Thrombocytopenia (platelet count ≥100×10^9) and (iv) Evidence of plasma leakage manifested by haemoconcentration (an increase in haematocrit 20% above average for age, sex and population), pleural effusion and ascites, etc.

Approximately 2.5 billion people live in dengue risk regions with about 100 million new cases each year worldwide. Dengue disease presents highly complex pathophysiological, economic and ecologic problems. In India, the first virologically proved epidemic of dengue fever (DF) occurred in Calcutta and Eastern Coast of India in 1963-1964 [7,8]. All four serotypes cause dengue fever, a severe flu-like illness; usually without cold and cough. The disease is prevalent in third world tropical countries and now spreading to sub-tropical developed countries. WHO estimated that 50-60 million cases of dengue fever occurs worldwide each year, including
serious clinical types: Dengue hemorrhagic fever and dengue shock syndrome.\(^1\)

Primary infection manifests as self-limiting mild to severe fever, lasting for 5-7 days, severe headache with pain behind eyes muscle, joint, rash and vomiting. Secondary infection is more common in Southeast Asia, Including India and South America. Primary dengue infection is detected with specific NS1 antigen in 0-9 days after the onset of symptoms; the symptoms usually persist for 15 days.\(^9,10\) The early diagnosis of dengue reduces risk of complication of severe clinical types DHF and DSS, especially in endemic countries. IgM antibodies are not detected till 5-10 days in case of primary infection and till 4-5 days in secondary infection after onset of illness.

MATERIALS AND METHODS

In this retrospective study, blood samples from the clinically suspected cases of Dengue were reviewed for a period of 11 month from Jan 2018 to Nov 2018. Samples were collected from outpatient department as well as patients admitted in different clinical wards of medical college, Bihar India. It is a tertiary care teaching hospital and provides a full range of medical, surgical and super specialty facilities.

Processing of samples was done at Department of Microbiology, Medical College, and Bihar, India. Briefly, 2 to 3 ml of blood was collected from each patient by nursing personnel, male orderlies or physicians using strict aseptic precautions and serum was collected using standard methods. All the samples were collected after obtaining the informed consent from the patients and contacts.

Methodology

Serum collected was tested for IgM and IgG anti dengue antibodies by Dengue IGM capture enzyme linked immune sorbent assay (MAC ELISA) and IgG MAC ELISA (Panbio Pty limited, Queensland, and Australia). Briefly the procedure was done as follows: 125 µl of peroxidase-labelled anti-dengue monoclonal antibody conjugate was added in the micro well containing dengue 1 to 4 antigens (antigen plate), resulting in the formation of antigen-antibody complex. Within 10 min of addition of conjugate to the antigen plate, 100 µl of 1:100 diluted serum and control were added to another plate (assay plate) containing antihuman IgM antibodies or IgM antibodies attached to micro well test strips. The assay plate was incubated at 37°C for one hour and then washed. After that, 100 µl of complexed antigen conjugate solution was transferred from the antigen plate to assay plate which was further incubated for one hour. After incubation, the micro wells were washed and 100 µl of tetramethylbenzidine/ hydrogen peroxide (TMB/H 2O 2) substrate solution was added to each well. After 10 min of incubation at room temperature, stop solution was added to each well and the colour density of the residue (optical density) was read within 30 min at the wavelength of 450 nm. Patients with positive anti dengue IgM were considered positive cases for dengue viral infection. A primary infection was indicated if IgM/IgG index ration was more than 1.2 and as secondary case if this ratio is less than above value.\(^11\)

Statistical analysis

The recorded data was compiled entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages, means and standard deviations were calculated.

Results

During the three years study period, 1035 serum samples were analyzed. Out of these, 209 samples (20.19%) were positive for dengue virus infection. From Nov 2017 to Nov 2019, dengue fever was moderately low in prevalence, but its prevalence suddenly jumped in 2019(53.11%) resulting in an epidemic in this region (Figure 1). Seasonal trend showed that there were no positive cases from January to July every year; the infection started spreading in August, peaked in October and slowly tapered by December. The most affected age group was 11 to 15 years of age (57.3%) (Pediatric population), followed by 6 to 10 years age group and least effective age group is 0-5 year(15.4%) (Table: 1). The youngest age showing positive result was 6 months and oldest case was 15 years old. Male to female ratio was 2:1 (Table: 2) and majority of cases were of secondary dengue virus infection (93.8%) (Table: 3).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>32</td>
<td>15.4</td>
</tr>
<tr>
<td>6-10</td>
<td>57</td>
<td>27.3</td>
</tr>
<tr>
<td>11-15</td>
<td>120</td>
<td>57.3</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>9.10±2.18</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Showing different age group of dengue cases
Table 2: Showing gender based dengue cases

<table>
<thead>
<tr>
<th>Sex distribution</th>
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<tbody>
<tr>
<td>Male</td>
<td>143</td>
<td>68.4</td>
</tr>
<tr>
<td>Female</td>
<td>66</td>
<td>31.6</td>
</tr>
</tbody>
</table>

Table 3: Showing immunological characteristics of dengue cases

<table>
<thead>
<tr>
<th>Immunological type</th>
<th>N</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infection</td>
<td>13</td>
<td>6.2</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>196</td>
<td>93.8</td>
</tr>
</tbody>
</table>

Discussion

Dengue is an important emerging disease of the tropical and sub-tropical regions today. It is clear that since last decade, dengue have been occurring regularly with periodic surges in a number of cases. In this study, 21.19% patients were serologically positive for dengue infection. Upon analyzing the year-wise distribution of dengue cases, an unsteady increase in the number of dengue patients over the past few years was noted. Of the total 209 cases, 111 (53.11%) were reported in the year 2019 whereas only few cases were reported during 2017 to 2018. However in 2017, of the 320 samples tested, 40 were positive and these findings are in accordance with other studies from India.19-21 Bihar is situated in North India on the bank of Ganges River. Thus there are lots of marshy places which provide excellent mosquito breeding places. Further, this may be partially attributed to the rapid unplanned urbanization with unchecked construction activities and poor sanitation facilities contributing fertile breeding grounds for mosquitoes; it is also true that an increase in the alertness among medical fraternity following the initial epidemic and the availability of diagnostic tools in the hospital have contributed to the increased detection of cases.

To identify the seasonal variation of the disease, analysis of the data on monthly basis were done. A gradual increase in cases was noticed from August with a peak in October, during all the three years of the study. The correlation between occurrence of dengue and monsoon season is clearly evident in this study and is further supported by similar findings from Delhi, Ludhiana, Chandigarh and Karachi.22-24 It may be because this season is very favorable for high breeding of the vector, that is, Aedes aegypti. This seasonal outbreak of disease transmission is very important at local level for effective control measures and that preventive measures against dengue infection should come into full swing during water stagnation periods after the initial bouts of rainfall and at the end of monsoon. The higher prevalence of dengue infection was noted among males than females. The male-to-female ratio was 2:1 which is congruent with other studies. 20,21 The age group of 11 to 15 years was highly affected with dengue (57.3%) and then 6-10 year (27.3 %) and followed by 0-5 year (15.4 %) these finding are not consistent with other Indian studies, as most of the other Indian studies have reported 15 to 45 years as the most affected age group. 20,21 However in several international studies, dengue has been reported to mainly a pediatric public health problem.22,23 It is a very significant finding because true endemicity of dengue is reached when the adult infection declines and only the new entrants into the population, that is, the children, are affected more by the disease. Another very important finding of this study is that among dengue infected patients, only 6.2% had primary infection whereas majority (93.8%) of patients had secondary dengue infection. Even secondary dengue infection was seen in infants as young as 6 months and it is a well established fact that complications like dengue haemorrhagic fever and dengue shock syndrome occur mainly in cases with secondary infections due to antibody mediated enhancement, Cross reactive T cell response with activation of TH-2 lineage cell and stimulation of soluble factors.24 The results of this study indicate that dengue infection is not going to wane away but is going to stay and will play havoc if immediate control measures are not taken. In absence of specific treatment for dengue fever, management is mainly supportive, further there are no vaccines currently available in market thus early diagnosis and vector control is the only method by which dengue can be controlled. Rapid immunochromatographic test to detect NS1 antigen and IgM antibodies should be available at primary and rural health centers, so that cases can be diagnosed early and thus properly managed. Secondly, the civic agencies have to wake up and rather than adopting a callous attitude and passing the buck, their workers have to ‘really work.’ Need for enhancing government-citizen partnership through well coordinated community participation efforts cannot be over emphasized. Involving resident welfare associations in urban areas and the Panchayats will help tremendously. The need of the hour is long-term vector control strategy; so that the outbreaks can be prevented and this will simultaneously solve the problem of other mosquito borne diseases like chikungunya, Japanese encephalitis, malaria and filaria.

Conclusion

The present study concluded that the disease afflicted almost all age groups but the incidence was more in 11-15 age group followed by 6 - 10 year and it was least effective in age group of 0-5 year. Males were more affected than females. The study also showed that the disease incidence increased in monsoon and post monsoon season. Therefore, preventive measures should be initiated each year with the advent of monsoon season. In addition,
vector and larval surveillance should be carried out at regular intervals to prevent the disease transmission.

References