Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India


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Dengue is one of the most serious mosquitos 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. As effective control and preventive programmes depend upon improved surveillance data, this study was done to report the seroprevalence of Dengue virus infection at Kanpur, North India. The laboratory records of clinically suspected Dengue patients from January 2006 to December 2010 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). A total of 1227 serum samples were analyzed. Out of which 242 samples (19.7%) were found positive for dengue virus infection. Maximum positive cases were seen in 2010 (46.5%). Seasonal trend showed that infection started appearing in August, peaked in October and slowly tapered by December. The most affected age group was 0 to 15 years of age (Pediatric population), followed by 16 to 30 years group and majority of cases were found to be of secondary dengue virus infection (92%). The present outbreak thus emphasizes the need for continuous sero epidemiological surveillance for the timely formulation and implementation of effective dengue control programme.

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

INTRODUCTION

Dengue virus is a positive-strand RNA virus of the Flaviviridae family with 4 distinct serotypes (DV1-4), and is transmitted to humans by several species of the Aedes mosquito. It is a major cause of morbidity throughout the tropical and subtropical regions of the world (Gubler, 1998). Dengue virus infection produces a spectrum of clinical illness, ranging from an asymptomatic or mild febrile illness to classic dengue fever (DF) to the most severe form of illness, dengue hemorrhagic fever (DHF). Population-based studies suggest that asymptomatic infections are the main outcome of dengue virus exposure. However whenever DHF occurs, it is associated with high morbidity and mortality (Martina et al., 2009).

The global prevalence of dengue has grown dramatically in recent decade. The disease is now endemic in more than 100 countries in Africa, the Americas, the eastern Mediterranean, Southeast Asia, and the Western Pacific, threatening more than 2.5 billion people (Dengue, 2010). Dengue is believed to infect 50 to 100 million people worldwide a year with half a million life-threatening infections requiring hospitalization, resulting in approximately 12,500 to 25,000 deaths (Dengue, 2010). Although dengue has a global distribution, the world health organization (WHO) South-East Asia region together with Western Pacific region bears nearly 75% of the current global disease burden. The South East Asia region is currently experiencing an upsurge in reported cases of dengue in a number of countries, including India, Sri Lanka, and Thailand (Dengue, 2010). The epidemics from India include those from Calcutta (1963),

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Early laboratory diagnosis of dengue virus infection is important and is routinely done by serological test. Anti dengue IgM antibodies appears as early as 3 days after infection and remain in circulation for 1 to 2 month. Anti dengue IgG antibodies appears after 1 week, peaks after 2 to 3 weeks and remain lifelong in circulation. Recently, many laboratories are also performing NS1 dengue antigen detection test, and it is showing promising results (World Health Organization, 1997). As effective control and preventive programmes for dengue infection are based upon improved surveillance data, this study was done to report the seroprevalence of dengue virus infection at Kanpur, a major industrial town of North India.

MATERIALS AND METHODS

In this retrospective study, blood samples from the clinically suspected cases of Dengue were reviewed for a period of 5 years from January 2006 to December 2010. Samples were collected from out-patient department as well as patients admitted in different clinical wards of L. L. R. M. Hospital, Kanpur. 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, G. S. V. M. Medical College, Kanpur, 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. 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, Australia). Briefly the procedure was done as follows: 125 µl of peroxidase-labelled antidiengue monoclonal antibody conjugate was added in the microwell containing dengue 1 to 4 antigens (antigen plate), resulting in the formation of antigenantibody 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 microwell 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 microwells were washed and 100 µl of tetramethylbenzidine/ hydrogen peroxide (TMB/H₂O₂) 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 (Shu et al., 2003). All collected data was later on statistically analyzed and presented.

RESULTS

During the five year study period, 1227 serum samples were analyzed. Out of these, 242 samples (19.7%) were positive for dengue virus infection. From 2006 to 2009, dengue fever was moderately low in prevalence, but its prevalence suddenly jumped in 2010 (46.5%) 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 0 to 15 years of age (Pediatric population), followed by 16 to 30 years group. Interestingly, dengue cases in pediatric patients rose from 25% in 2006 to 43% in 2010. The youngest age showing positive result was 5 months and oldest case was 78 years old. Male to female ratio was 2:1 and majority of cases were of secondary dengue virus infection (92%) (Table 1 and Figure 2).

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 (Singh, 2007). In this study, 19.7% 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 390 cases, 181 (46.5%) were reported in the year 2010 whereas only few cases were reported during 2007 to 2009.

However in 2006, of the 400 samples tested, 34 were positive and these findings are in accordance with other studies from India (Padbidri et al., 1973; Balaya et al., 1967; Dar et al., 1999; Gupta et al., 2005). Kanpur 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 five 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 (Lal et al., 2007; Ratho et al., 2005; Ahmed et al., 2008; Ukey et al.,
Figure 1. Showing year-wise distribution of dengue cases. Blue bar is showing total samples received whereas red bar is showing total positive samples for dengue.

Table 1. Showing demographic and immunological characteristics of dengue cases.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Age in years</td>
<td></td>
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<tr>
<td>0-15</td>
<td>103</td>
<td>42.5</td>
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<tr>
<td>16-30</td>
<td>93</td>
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<td>5.7</td>
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<td>≥ 61</td>
<td>8</td>
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<table>
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<tr>
<td>Male</td>
<td>162</td>
<td>67</td>
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<tr>
<td>Female</td>
<td>80</td>
<td>33</td>
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<table>
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<th>Immunological type</th>
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<tr>
<td>Primary infection</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>224</td>
<td>92</td>
</tr>
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2010 Lal et al., 2007; Ratho et al., 2005; Ahmed et al., 2008; Ukey et al., 2010. 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 (Gupta et al., 2005; Ukey et al., 2010; Kumar et al., 2010). The age group of 0 to 15 years was highly affected with dengue and 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 (Gupta et al., 2005; Ukey et al., 2010; Kumar et al., 2010). However in several international studies, dengue has been reported to mainly a pediatric public health problem (Shah et al., 2006; Anderson et al., 2007). 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 8% had primary infection whereas majority (92%) 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 (Martina et al., 2009). 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 can not be overemphasized. 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.

REFERENCES


