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Dengue: An exaggeration or a nemesis? An hospital based study in the Northern part of West Bengal, India

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ABSTRACT

Dengue epidemics are becoming a regular scary event every few years leading to significant mortality and morbidity; involving the whole of West Bengal, India. A hospital record based cross-sectional study with complete enumeration was conducted to analyse the overall seropositivity among the clinically suspected dengue cases referred to the rural tertiary care hospital in the Northern part of West Bengal, India. Serum samples from 6855 clinically suspected dengue cases were included in the study over a period of three years. Samples were tested for non-structural protein-1 (NS1) Antigen and IgM MAC enzyme-linked immunosorbent assay (ELISA) depending on the duration of fever. Of the 6855 samples screened, 62.91% (4313) were positive either for dengue NS1Ag or IgM antibody ELISA. Out of 4313 positive samples, 72.40% (3123) were positive for NS1Ag exclusively and 27.59% (1190) for IgM. More females were affected than males. The most affected was the primary school age group (64.75%). There was an increased occurrence during the post-monsoon season. The findings of this study revealed that containing dengue has been partially successful in the Northern part of West Bengal. However, dengue may again resurge and cause unprecedented panic among the people of the region if appropriate vector control measures are not continued.

INRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral diseases in the world. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries (WHO, 2009). Dengue fever was first referred as “water poison” associated with flying insects in a Chinese medical encyclopaedia from the Jin dynasty (265-420 AD). The word ‘dengue’ is derived from Swahili phrase Ka-dinga pepo, meaning “cramp like seizure”. 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 (Gupta et al., 2012). DV 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* (Gupta et al., 2012).

Dengue virus infection produces a spectrum of clinical illnesses which range from asymptomatic or mild febrile illnesses, to classic dengue fever (DF), to the most severe form of illness, dengue haemorrhagic fever (DHF)

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(Garg et al., 2011).

Viral isolation by cell culture and subsequent detection by immunofluorescence, though the gold standard tests for identification of dengue infection are not within the reach of peripheral and even most tertiary care laboratories (Chakravarti et al., 2006). Differential diagnosis based on symptoms is challenging due to dengue's non-specific symptoms such as fever, aches and fatigue that overlap with other endemic infections. A positive laboratory test often alerts physicians to closely monitor platelet levels and other disease specific warning symptoms. The most widely used method for diagnosing dengue is an enzyme-linked immunosorbent assay (ELISA) which measures anti-Dengue IgM or IgG antibodies in patient's serum. These antibodies are not reliably detectable until 3-4 days post onset of symptom. In 2000, the first ELISA capable of detecting DV non-structural protein-1(NS1) was developed. Importantly NS1 is detectable early during the acute phase (Day 0 to 6) of both primary and secondary dengue (Pal et al., 2014). NS1 is shown to be highly specific viral marker making it an extremely reliable parameter for diagnosis of dengue infection from day 1 of fever (Datta et al., 2010).

The first epidemic of clinical dengue-like illness was recorded in Chennai in 1780 and the first virologically proved epidemic of DF in India occurred in Kolkata and Eastern Coast of India in 1963-1964 (Sarkar et al., 1964; Chatterjee et al., 1965; Carey et al., 1966). Thereafter several outbreaks occurred in West Bengal with the last one being reported in 2012 (Bandopadhyay et al., 2013). It has generated unprecedented panic amongst the general population. We, therefore, analysed the data available in our hospital for NS1Ag and IgM seropositivity over a period of 3 years (2013-2015) from patients with dengue fever like illness with an objective to determine the magnitude of problem of dengue as well as to assess the pattern of dengue fever among the patients attending the rural tertiary care hospital in India.

MATERIALS AND METHODS

Study setting

The study was conducted in the Department of Microbiology, North Bengal Medical College, West Bengal, India. North Bengal Medical College is a rural tertiary care hospital in the northern part of West Bengal which caters to the needs of patients from all the six districts of the region, from neighbouring Indian States of Bihar, Assam and Sikkim as well as from allied border regions of other three countries like Bangladesh, Bhutan and Nepal. The climate of North Bengal varies between the tropical heat of the valleys and alpine cold of the snowy regions. With rainfall averaging 348 cm, it is the most humid region of the Himalayas.

Inclusion criteria

Patients with clinical suspicion of dengue fever like illness as per WHO criteria of all ages and both sexes who presented to the outpatient department or were admitted in the wards of the hospital in between January 2013-December 2015.

Serum samples received from the various district, block level hospitals and samples sent by physicians in private practice of the region for confirmation by ELISA were also included.

Exclusion criteria

Patients with history of prolonged fever of more than one month duration and patients with any other proven febrile illness like malaria, typhoid etc.

Study population

A total of 6855 consecutive, non-repetitive serum samples of all age and both sexes received during this period were included in the study.

Sample collection

Under aseptic measures, about 5 ml of blood was collected in a sterile vial from clinically suspected dengue cases. The vial was left at room temperature and the blood was allowed to clot for separation of serum. The serum was then separated by centrifuging the blood in a centrifuge machine at 3000 rpm for 5 min. The separated serum was then transferred to a sterile vial, labelled, and stored at -20°C till the assay was done (as per ELISA kit). Samples received from the different laboratories of the region were transported maintaining the cold chain.

Laboratory investigation

Serum samples obtained within 5 days of onset of fever (n=4272) were tested for dengue NS1Ag using Pan Bio (Australia) NS1Ag ELISA kit. Serum samples with fever history of ≥5 days (n=2583) were screened for dengue specific IgM antibody by IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA) kit prepared by National Institute of Virology, Pune, India (as an integral part of National Vector borne Disease Control Programme). The tests were performed strictly following manufacturer's instructions.

Data collection and analysis

Data based on hospital records were compiled in MS

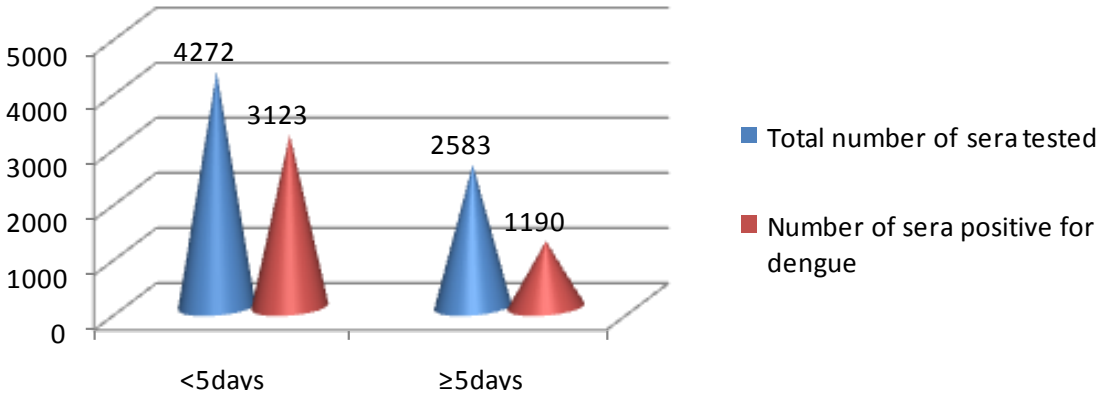

Figure 1. Shows dengue NS1Ag and IgM positive cases (N= 6855). Proportion of case detection by NS1Ag ELISA was more than IgM MAC ELISA in this study (Chi square=504.2, p<0.000, highly significant).

Table 1. Trend of dengue positivity during the three year study period (2013-2015) (N=6855).

Year	Total cases examined	No. of dengue positive cases	Percentage
2013	5811	3937	67.75%
2014	456	213	46.71%
2015	588	163	27.72%

The rate of decrease in dengue positivity in the period 2014-2015 was higher (1.68 fold) than that in 2013-2014 (1.45 fold).

excel sheet and analysis was done using IBM SPSS 22.0 version.

RESULTS

During the study period, a total of 6855 serum samples were screened of which 62.91% (4313) were serologically positive either for dengue NS1Ag or IgM antibody. Year wise distribution of dengue positive cases over the three year study period is shown in Table 1. The rate of decrease in dengue positivity in the period 2014-2015 was higher (1.68 fold) than that in 2013-2014 (1.45 fold). Of the 4313 positive samples, 72.40% (3123) were positive exclusively for NS1Ag and 27.59% (1190) were positive for IgM. Out of 6855 samples, 4272 were received in acute stage of fever (within 5 days of onset of fever), of which 73.10% (3123) were found to be positive for dengue NS1Ag ELISA. The remaining 2583 samples which were received in early convalescent stage (post 5 days of onset of fever) were tested for IgM MAC ELISA and 46.07% (1190) were found to be positive for IgM antibody (Figure 1).

Of the total samples, 4085 were males and 2770 were females. The overall positivity seen in females (64.94%) were more than the males (61.54%) (Figure 2). The pattern was same each year during the three-year study period. The most affected (64.75%) was the primary school age group (5-9 years) (Table 2). The affected patients were mostly from urban areas of Darjeeling district.

Seasonal trend showed that there were very few positive cases from January to June, the cases started increasing by end of July with peak from September to November, then slowly tapered by December each year. Maximum positivity occurred during the month of September in 2013 and 2015 (74.65% and 46.42%) but in the year 2014 maximum positive cases (68.07%) were recorded in November (Figure 3).

DISCUSSION

Dengue is an important emerging disease of the tropical and subtropical regions today (Jain et al., 2015). Dengue infection presents with nonspecific fever that mimics other viral illnesses. To prevent the outbreaks it is necessary to diagnose the dengue virus infection as early as possible. In 2012, India experienced a massive outbreak of dengue fever. Tamil Nadu reported the highest number of cases of dengue (9,249) in the country, followed by West Bengal that reported 6,067

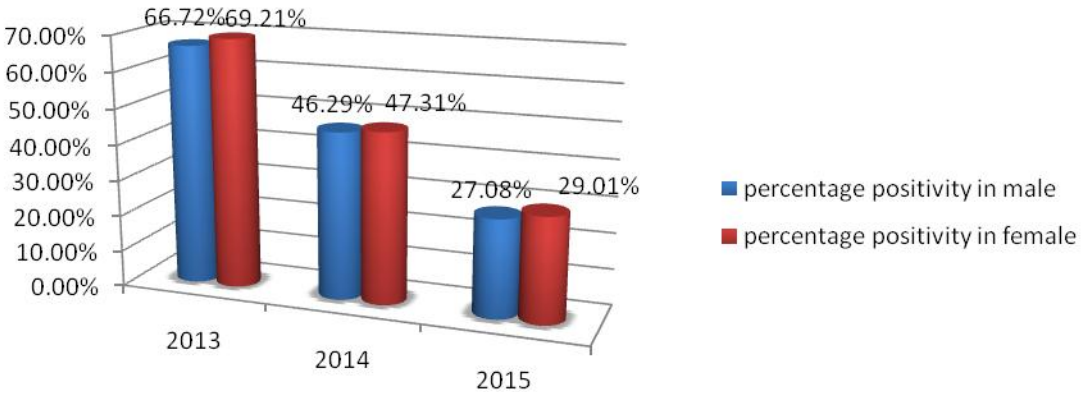

Figure 2. Sex-wise distribution of dengue positive cases during the three year study period (n= 4313). Females were significantly more affected than males (Chi-square 8.196, p<0.005); n indicates total number of positive cases.

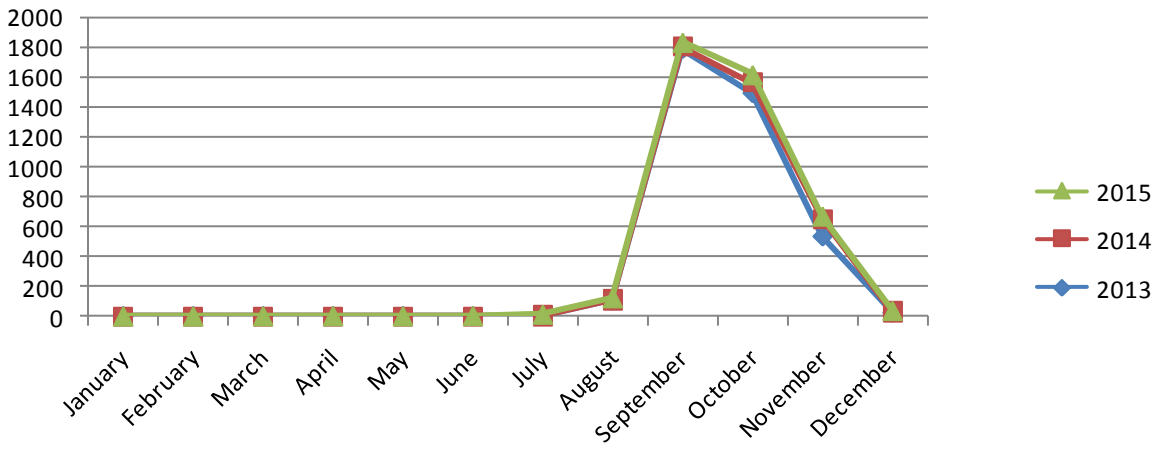

Figure 3. Month wise distribution of serologically positive dengue cases (n=4313).

Table 2. Age group wise distribution of study population and their dengue positive status (N=6855).

Age	2013	2014	2015	Total number of sera positive	Percentage positivity
<5 years	53(76)	3(13)	7(34)	63 (123)	51.2
5-9 years	156(220)	7(12)	6(29)	169 (261)	64.7
10-20 years	1042(1535)	60(115)	38(132)	1140 (1782)	63.9
21-40 years	1889(2781)	105(209)	80(283)	2074 (3273)	63.3
41-60 years	672(986)	25(78)	29(84)	726 (1148)	63.2
>60 years	125(213)	13(29)	3(26)	141 (268)	52.6

People in extremes of age (<5 years and >60 years) were least affected than others (Chi-square 17.68, p< 0.000). Numbers in parenthesis denote number of sera tested.

cases (Accharya et al., 2016). Though dengue was first documented in Kolkata (Calcutta) in 1824 and several epidemics took place in the city during the years 1836, 1906, 1911 and 1972 (affecting 40% of the city people) (Bandopadhyay et al., 2013), no large-scale epidemiological study for laboratory confirmation of dengue cases

was attempted in Northern part of West Bengal. Keeping this in mind this study was attempted to bring out the dengue scenario in this part of Bengal.

NS1 Ag circulates uniformly in all serotypes of dengue virus at a high level during the initial days of illness, therefore has a higher detection rate in acute phase sera (Bessof et al., 2008). In the present study 72.40% of the samples were positive for NS1Ag ELISA exclusively. Similar finding of 71.42% was reported by Datta et al. (2010) from Delhi. On the contrary lesser values of 31.20% (Ranganath and Peerapur, 2016) and 35.98% (Bhattacharya et al., 2014) as well as higher values of 83.1% (Pothapregeda et al., 2016) were reported from various studies for NS1Ag ELISA. Dengue specific IgM is detectable within 3-5 days of clinical illness. The titer of IgM rises quickly within 2 weeks and wanes to undetectable levels after 2-3 months. In our study, 27.59% were positive for IgM ELISA only. Our finding was similar to Vijaykumar et al. (2005) who reported dengue positivity of 29.7% by MAC ELISA from Southern India. During the three year study period, proportion of positive case detection by NS1Ag ELISA was found to be more than MAC ELISA (Chi square=504.2, $p<0.000$, highly significant). Dengue infection is common in the tropical countries where several other infections resembling dengue like malaria, enteric fever are endemic and diagnosis based merely on clinical symptoms is unreliable. Therefore dengue can be easily under diagnosed in the absence of adequate and quality laboratory diagnostic method.

From the study, seropositivity was seen maximum in the primary school age group (5-9 years). People in extremes of age group (<5 years and >60 years) were least affected than others and the association was found to be highly significant (Chi square 17.68, $p<0.000$). Previously a study from Darjeeling district, found the most affected age group to be 11-20 years (Taraphdar et al., 2010).

Studies have shown that in areas where dengue infection is either endemic or epidemics are more frequent, a shift occurs in the predominant age group involved. This shift in the affected age group may be attributable to changes in locations where disease transmission takes place (Gupta et al., 2005). Our findings were similar to studies from other parts of India (Biradar et al., 2016; Sood et al., 2013; Gunasekaran et al., 2011). Several international studies also reported dengue to be mainly a paediatric public health problem (Meng Chour et al., 2002; Capeding et al., 2015). It is suggested that over a period of time as the duration of co-circulation of multiple serotypes of dengue in a particular geographic area increases, adults have a lower probability of remaining susceptible to infection. This results in the young population to become completely susceptible individuals. Therefore, monotypically immune individuals are more likely to be from younger age groups.

The present study shows a decrease in dengue cases in these 3 years from 2013 to 2015 with highest number of cases being reported in 2013 (67.75%) and lowest in 2015 (27.72%). A study from Thailand reported that greater the incidence of dengue infection of the previous year's epidemic, the milder the subsequent year's disease severity (Sharma et al., 2012). This raises the interesting possibility that herd immunity is an important contributor to disease severity. Also the decrease in cases in consecutive years may be due to upgraded laboratory set up in various district level hospitals which curbed down the need to be referred to tertiary care centre for diagnosis to some extent. Lessons learnt from 2012 outbreak might have prompted government to take intensified control measures like household mosquito elimination programmes, public awareness etc. which in turn also helped in curbing down dengue cases in the subsequent years.

The study clearly depicts seasonal trend of dengue in North Bengal with its peak in the post monsoon seasons. The correlation between the occurrence of dengue and the monsoon season is evident and was further supported by similar findings from Chennai, Delhi and Karachi (Pakistan) (Gunasekaran et al., 2011; Jain et al., 2015; Khan et al., 2007). This shows that the presence of stagnating water after rainfall favours breeding of the mosquito vector resulting in an increased incidence of dengue. These findings also indicate that preventive measures against dengue should probably come into full-swing during the monsoon and post monsoon months. Also distribution of stray cases throughout the year pointed out that perennial transmission is going on which requires attention, because this can be effectively reduced through proper vector control measures. A higher prevalence of dengue infection was noted in females than males (Chi-square 8.196, $p<0.005$). Similar finding was reported by Gunasekaran et al. (2011). This is in contrast to the findings reported by other study (Biradar et al., 2016).

There were several limitations to the present study. Firstly, the study was cross-sectional and hospital record based, so clinical findings of the dengue cases could not be elaborated. Secondly, dengue specific NS1Ag and IgM ELISA if done simultaneously on a single sample could have increased the detection rate of dengue fever. Thirdly, virus isolation and specific serotype detection facility for dengue was not available at the institutional level.

Conclusion

An increase in public awareness, early diagnosis, long term vector control, constant vigilance by health care officials and upgraded laboratory set up has helped in

curbing down the dengue fever cases in Northern part of West Bengal in the last few years. So long we understand the disease process, we can undertake steps to control the menace proving it to be an exaggeration, but whenever we falter or fail to adhere to strategical regulation we may see dengue raising its ugly head in society and proving itself to be nemesis as it has been termed time and again.

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