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Sero diagnosis of dengue activity in an unknown febrile outbreak at the Siliguri Town, District Darjeeling, West Bengal

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ABSTRACT

Objective: To investigate the outbreak of unknown fever at Siliguri town, Darjeeling District on request from the State Health Department, Government of West Bengal. **Methods:** Investigations were made to the affected wards, Sub Divisional Hospital and the nursing homes of Siliguri Town. Duration of illness was 3-5 days. Interesting observations were made in some cases which had gastrointestinal disorders with high serum glutamate pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels. A total of 69 blood samples and 7 throat swabs (in Minimum Essential Media) were collected and brought to the ICMR Virus Unit, Kolkata for analysis. Mosquitoes from different affected areas were collected for the identification of the definite vector. **Results:** Amongst the 69 blood samples, 42 (60.86%) were positive to IgM antibody against dengue virus by Mac enzyme-linked immunosorbent assay (ELISA) test. No IgM antibody to Japanese encephalitis virus was detected among the collected blood samples. Based on the clinical symptoms, presence of IgM antibody to dengue virus and identification of Aedes mosquito, it amply proves that, the illness of those cases were due to dengue virus infection. **Conclusions:** Based on clinical-epidemiological observations of the investigations the possibility of a communicable disease of viral origin, the detection of IgM antibody and the identification of *Aedes egypti*, and the potential circulation of denge virus in Siliguri town for the first time were all suggested.

1. Introduction

Dengue virus with its four sero types is now classified within the flaviviridea family[1]. Among the four sero types, infection with any of them generally leads to a mild self limiting febrile illness [dengue fever(DF)]. A more severe form of the disease involving vascular and haemostatic abnormalities leads to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which is responsible for a high mortality rate, especially in children[2]. Dengue virus is responsible for a growing health problem in the tropical and sub tropical countries. It is one of the most important among human Arbovirus infections[3]. The global incidence of DF and DHF has increased dramatically in recent decades[4]. In India dengue was first isolated in 1946, and many epidemics have since been reported[5,6]. DHF was first reported in Calcutta, West Bengal, in 1963 again in 1964 and subsequently in Visakhapattanam in 1969 and in Jalore, Rajasthan in 1985. Delhi has experienced major epidemic of DHF in 1996[6]. The epidemic began in mid August and lasted up to November of that year . In addition to that, out breaks have also been reported at regular intervals from Maharashtra[7], Punjab[8,9], Tamil Nadu[10], and Madhya Pradesh[11].

Here, we report an outbreak of DF occurred in Siliguri town, Dist-Darjeeling from October-November 2005, which was intimated to us by the Director of Medical Education and Director of Health Services, Goverment of West Bengal, for necessary virological investigations. The purpose of this paper is to present a comprehensive report on the results of investigations of the disease from its first appearance up to the period of investigation which was from 18th to 22nd November 2005. Although the outbreak has tolled only one death, but no case of hemorrhagic manifestation was reported.

2. Materials and methods

According to the epidemiological information, this outbreak had two types of clinical cases at different periods. In the first type, mainly affecting children in whom mild dengue like features was predominant, started during

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the middle of July and had its peak in August and then gradually declined. There was no mortality in this group. In the second phase, (present investigation) showing an almost classical dengue like syndrome (without any shock and hemorrhage), started from 1st November, had its peak in the middle of November and then gradually declined. During this phase of illness, all the age groups (7 to 56) irrespective of sex differentiation, were affected and only one death was recorded. In the 2nd episode, all parts of the town were more or less affected.

Cases for investigation included those admitted to the Siliguri SD Hospital, as well as those who attended the Outpatient Department (OPD) of that hospital. During this investigation, the local nursing homes were also visited to evaluate and examine the fever cases, attending the OPD and admitted into those nursing homes. From 2nd November 2005 to 19th November 2005, daily distribution of unknown fever cases attending the OPD of Siliguri SD Hospital, could be collected. In addition to that, a good number of cases were referred to us by the local practitioners for proper investigations. In the matter of selection of cases the following criteria were taken into consideration: a) high fever, b) headache, c) joint pain, d) body ache, e) malaise, f) vomiting or with nausea g) generalized skin rash, h) spontaneous hemorrhage with or without circulating failure. In the present investigation, two or more of these criteria, apart from fever, were considered. The possibility of bacterial and prokaryotic etiology in the collected sample was excluded through investigations at the local hospitals/ nursing homes. Attempts were made to collect paired sera, after 15 days, from the positive cases and a total of 41 convalescent blood samples could be collected. All the cases, blood samples were collected by venous puncture.

Sera were separated from the collected blood samples and transported on wet ice to the virus unit, Calcutta, where they were stored at -80 ℃ until testing. All the sera were tested within 1-2 months from the date of collection. A total of 69 acute samples were collected from the cases of the affected area and screened for the presence of dengue/Japanese encephalitis (JE) IgM antibodies by IgM capture enzyme-linked immunosorbent assay (ELISA) (MAC-ELISA), using a kit (Prepared by National Institute of virology, Pune, India), following the prescribed protocol. optical density (OD) (in full name) was measured at 492 nm using an ELISA reader (Titertek Multiskan Plus, Lab systems Finland, Type- 314).

Attempts were made to isolate the virus in sucking infants (2-3 days old) Swiss mice, with the sera collected during the acute stage of the disease. The undiluted serum was inoculated intracerebrally in 1 L of 2-3 days old Swiss albino suckling mice and observed for 15 days. The brains of the sick mice were harvested and were inoculated into nutrient broth to exclude any bacterial contamination/ infection. If the mice did not show any illness, another blind passage was made. The acute phase sera were also inoculated into Vero cell cultures with two blind passages and observed for 15 days for cytopathic effects. On the other hand, hemagglutination inhibition (HAI) tests were done with the acute and convalescent sera for the rise of IgM antibody titre, if any, and also for the identification of the type of the virus, using the method described by Clark & Casals. In interpreting serological results, the standard criteria were followed. The samples which were positive to dengue virus by MAC ELISA method were subjected to HAI test against the four types of dengue antigen i.e. Den-1, Den-2, Den-3 and Den-4 for the identification of the sero type of the circulating strain. All the samples inhibited agglutination against Den-2 antigen. For confirmation of the etiologic agent, the convalescent sera along with the acute samples were subjected to HAI test against dengue and JE antigen. Samples were considered positive if they had a fourfold rise of titre in the convalescent paired sera.

Mosquitoes, resting inside the houses of the affected areas were collected and after identification, the pools of separate species of mosquitoes were ground in bovalbumin-phosphate saline containing antibodies. After centrifugation, the supernatant was inoculated aseptically into Vero cell line for isolation of virus.

A total of 69 acute samples could be collected from the cases of the affected area and screened for the presence of dengue/JE IgM antibodies by IgM capture ELISA (MAC-ELISA), using a kit (Prepared by National Institute of virology, Pune, India), following the prescribed protocol. OD was measured at 492 nm using an ELISA reader (Titertek Multiskan Plus, Lab systems Finland, Type- 314).

3. Results

Daily distribution of unknown fever cases at the OPD of Siliguri SD Hospital, were presented in Figure 1. Out of 69 acute samples, dengue virus specific IgM antibodies were observed only in 42 (60.86%) samples. IgM antibody to JE virus was not observed in any of the samples, collected from the acute cases. The maximum IgM positivity (72.41%) was observed in the age group of 11-20, followed by the age group of 0-10 (68.42%).In the age group of 21-30 and 31-40 the IgM antibody to dengue virus were 41.66% and 33.33%, respectively. In the upper age group no IgM antibody to dengue or JE virus was observed. Although attempts were made to isolate the virus from the acute samples and also from the mosquito pools, the inoculated mice and the cell culture did not produce any illness and cytopathic effect respectively and no virus could be isolated from any of them. The HAI test was used to detect rise of antibodies to dengue, if any, and also for the identification of the sero type of the circulating virus.

A total of 29 (70.73%) samples, out of 41 convalescent sera, revealed fourfold rise of antibody titre only against dengue antigen. Although the highest IgM antibody positivity (72.40%) to dengue was observed in the age group of 11-20 years, it was very close to the age group of 0-10, where the dengue positivity was 68.42%. An antibody to dengue was 41.66% and 33.33% in the age group of 21-30 and 31-40, respectively. No IgM antibody to dengue was observed in the age group of 41 and above. No samples were positive to JE antibodies alone, in this present investigation. Only 5 (17.24%) convalescent sera showed flavivirus Group reaction and had sharing with antibody titre to JE and dengue antigens. The rest of the 7 (24.13%) convalescent samples did not produce any rise of antibody titre either to dengue or to JE viruses.

For the isolation of virus, from the acute sera and from the mosquito pools, the inoculated mice and the cell culture did not produce any illness and cytopathic effect, respectively.

A total of 96 mosquitoes which were collected from the affected areas, only 11 were identified as *Aedes* sp. and these were from Gurung basti and Khal Para, the most affected area.

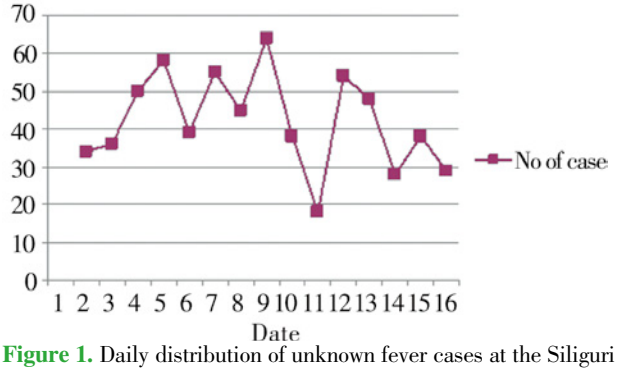

Figure 1. Daily distribution of unknown fever cases at the Siliguri SD Hospital.

4. Discussion

Outbreaks of dengue have been recorded in India on several occasions, although no such out break has yet been recorded from the district of Darjeeling or from its sadar town of Siliguri. In this study, all the cases were classical DF and there were no hemorrhagic manifestations except occasionally a few Petechae on the skin. This out break of dengue in Siliguri town occurred during the monsoon season (September-November), which is similar to most of the previous out breaks in India[9]. The highest no. of cases were reported during mid November is also similar with the previous reports of other different out breaks in India[9].

The detection of IgM antibody to dengue virus in the sera samples, collected from the affected area, by ELISA test amply proves that the febrile illness was due to the infection of dengue virus in the recent past. On the other hand, rise of antibody titre in the convalescent sera, as revealed by the HAI test against dengue antigen, again confirms the etiologic agent, of the febrile illness, was dengue virus. The serological result reveals that the acute samples inhibited agglutination against dengue-2 antigen and all the convalescent sera had the four fold rise of dengue antibody titre in them. In the older age group, group B flavivirus reaction indicates, their exposure to JE elsewhere in the remote past. So on the basis of laboratory investigation, epidemiological report and the identification of Aedes mosquitoes in the affected area confirms that the febrile illness was due to dengue-2 virus. In this outbreak, the young and young adult age groups were largely affected, which is similar to the observation of other dengue outbreak by other workers[12,13].

The identification of the Aedes mosquitoes collected from the affected area like Gurung basti and Khal para, the most affected area of the town Siliguri, amply proves the spread of the disease by the vector[14].

From the result, it is apparent that the possible etiological agent of the present illness was the Den-2 virus. In apparent infection of a large number of children during the period of July-August, possibly has played a great role in the current epidemic. Moreover, many of the ecological conditions in this town do not differ substantially from those where the disease has been prevalent. It was learnt during the investigation, that a major dengue outbreak took place in 2003 in the state of Sikkim, which is an adjacent state situated in the northern side of this town. Prompt control measures are taken including intensive household mosquitoes elimination programme, by the local administration. This might have sifted the affected mosquito population towards the Siliguri town and there by infecting the thickly populated area. Hence, it constitutes a new report from a rural district like Darjeeling in the Northern cold region of West Bengal and needs a continuous surveillance.

Conflict of interest statement

We declare that we have no conflict of interest.

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