DENGUE VIRUS INFECTION DURING POST-EPIDEMIC PERIOD IN DELHI, INDIA

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Abstract. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are major public health problems in India. During the period following an epidemic, a study was carried out using virological and serological tests for confirmation of suspected cases of dengue virus infection in fever cases presenting to the All India Institute of Medical Sciences. Serum samples of suspected DF/DHF cases were processed from January to December 1997. In 37 samples from patients with fever of less than 5-day duration, received on ice, virus isolation was attempted in C6/36 clone of *Aedes albopictus* cell line, followed by indirect fluorescent antibody staining with monoclonal antibodies to dengue viruses 1 to 4. One hundred and forty-three serum samples from patients with more than 5 days fever were tested for dengue specific IgM antibody by either MAC - ELISA or a rapid immunochromatographic assay. Dengue virus type 1 was demonstrated by culture in 8 (21.6%) of 37 serum samples and IgM antibody could be detected in 42 (29.4%) of the 143 serum samples by the serological methods. The peak of dengue virus infection was seen from September to November 1997.

INTRODUCTION

Dengue is currently the most important vector borne viral disease afflicting humanity, in terms of both morbidity and mortality (Halstead, 1984; Rosen, 1984). DF and DHF are emerging as major public health problems in tropical countries. The case fatality rates associated with early DHF epidemics were as high as 30-40% and in Southeast Asian countries where the disease has become endemic. It is also one of the leading causes of hospitalization and death among children (WHO, 1985). Effective prevention and control programs will depend on improved surveillance designed to provide early warning of dengue epidemics. Thus there is a need to study the occurrence of dengue virus infection during the period following an epidemic to provide information on the endemicity of dengue and to predict further epidemics. The study of dengue virus infection during the post - epidemic period can provide information about the serotypes usually circulating in the area. It can also give information regarding whether the serotype which was responsible for the preceding epidemic continues to circulate or is replaced by another serotype. There was an epidemic of DF/DHF in 1996 in Delhi which was due to dengue virus type 2 (Broor *et al*, 1997). We planned this study to look for the occurrence of dengue virus infection during the postepidemic period of 1997. Surveillance for dengue virus infection was carried out for one year during the post-epidemic period, in suspected cases of dengue in a hospital setting.

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MATERIAL AND METHODS

Clinical specimens

One hundred and eighty serum samples were received in virology laboratory from patients with clinical suspicion of dengue infection from January to December 1997, who presented to the All India Institute of Medical Sciences (AIIMS). Clinically suspected cases of dengue were defined as having symptoms of fever, arthritis, rash, and myalgia with or without hemorrhagic manifestations. Blood samples were collected from 180 patients from January to December 1997 in plain vial and transported to the virology laboratory on ice. Sera were separated and stored at -70°C for further processing.

Virus isolation

Virus isolation was carried out in the C6/36 clone of *Aedes albopictus* cell line as described by Boor *et al* (1997). The cell line was grown at 30°C in MEM containing 10% tryptose phosphate broth (2.9% stock) and 10% heat inactivated fetal calf serum (FCS). The cells were maintained in the above medium with 1% FCS, during virus isolation. One in ten dilution of each serum sample was inoculated on a confluent monolayer of C6/36 cell line and was incubated at 37°C for 1 hour for adsorption

followed by two washes with PBS (pH 7.2). Cells were then replenished with maintenance medium and incubated at 30°C for 7 - 10 days. Cells were harvested on both 7th days and 10th days and cell spots were made on a Teflon coated slide from each sample. Cells were fixed in chilled acetone at -20°C. Uninfected clone of *Aedes albopictus* cell line was used as negative control and cells line infected with dengue viruses 1 to 4 was used as positive control in each run. Indirect immunofluorescent antibody test (IFAT) was performed on these spots using monoclonal antibodies to dengue viruses 1-4 (Henchal *et al*, 1983) provided by kind courtesy of Dr Duane J Gubler, CDC Fort Collins, USA.

IgM-IgG capture ELISA

IgM-IgG capture ELISA was performed using dengue DUO ELISA test (Pan Bio Australia)as per the manufacturer's instructions. Results were expressed as the ratio of absorbance in test samples divided by the absorbance of the calibrator sera.

The recommended interpretation of the test was as follows:

a. Primary dengue was defined when IgM>1.0 and IgG: IgM<0.5.
b. Secondary dengue was when IgG>1.0 and IgG: IgM>1.0.
c. Suspected secondary dengue was defined when IgM<1.0 and IgG>1.0.

For confirmation of suspected secondary dengue, retest is recommended at 4 - 7 days which could not be done as only a single serum sample was collected.

Dengue IgM and IgG rapid immunochromatographic test

In the dengue rapid test (Pan Bio Australia), IgM and IgG antibodies to dengue virus are determined simultaneously by a rapid colloidal gold based immunochromatographic test for the separate determination of IgM and IgG antibodies in a capture assay format. The results were interpreted as per manufacturers instructions (Vaughn *et al*, 1998). Samples were classified into four categories, *ie,* negative, primary dengue; secondary dengues and suspected secondary dengue.

RESULTS

Of 37 serum samples which were processed for virus isolation, in 8 (21.62%) were positive for dengue-1 virus.(Table 1). IgM antibody could be detected in 42 (29.4%) of 143 serum samples by either DUO ELISA or rapid immunochromatographic test. (Table 1). Dengue was confirmed or suggested serologically in 55 (38.5%) cases. The distribution of serologically diagnosed cases into primary, secondary or suspected secondary cases is shown in Fig 1. A majority of the cases were either secondary dengue (confirmed) or suggestive of secondary dengue infection. The peak of dengue virus infection by virus isolation and serology was seen from September to December 1997 (Figs 2, 3).

DISCUSSION

Dengue is emerging as a major public health problem. Classical dengue fever is endemic in many parts of India including Delhi (Lall and Dhanda,

Table 1
Results of dengue virus isolation and IgM serology.

Test	No. received	No. positive (%)
Virus isolation	37	8 (21.6%)
IgM serology	143	42 (29.4%)

Table 2
Agewise distribution of serologically diagnosed cases of dengue.

Age group (year)	Primary dengue (8)	Secondary dengue (34)	Suggestive of secondary dengue (13)
0-1	25%	0%	0%
1-10	62.5%	20.6%	23.1%
11-20	0%	23.5%	30.8%
21-30	12.5%	44.1%	23.1%
31-40	0%	8.8%	7.7%
> 40	0%	2.9%	15.4%

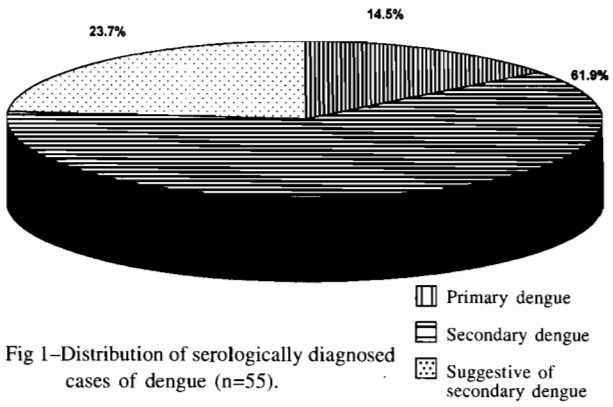

Fig 1–Distribution of serologically diagnosed cases of dengue (n=55).

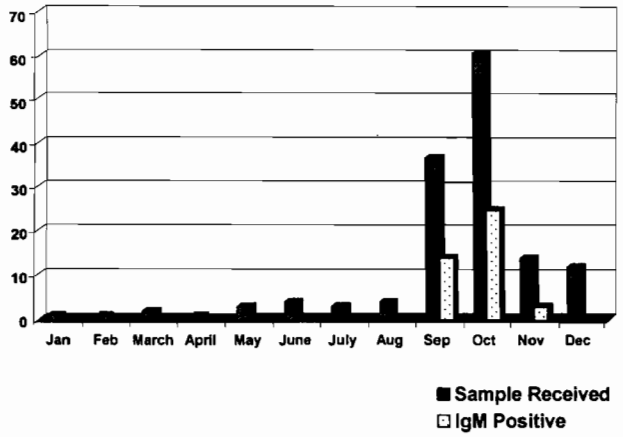

Fig 2–Monthwise distribution of cases from which dengue virus was isolated.

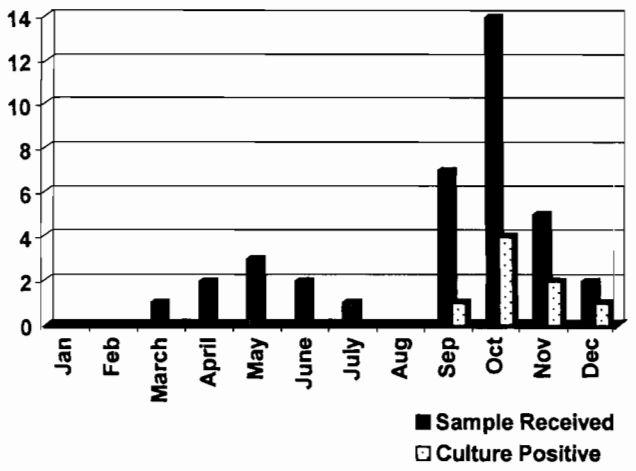

Fig 3–Monthwise distribution of cases positive for IgM antibodies to dengue virus.

1996). Virological and serological studies have been conducted during epidemics earlier in India (ICMR, 1980). However, serological and virological studies of dengue virus infection during period following epidemic are lacking from our country. The study of occurence of dengue virus infection during a postepidemic period will help in monitoring transmission in the community. It can also help in predicting the impending epidemic by detecting sudden increase in number of dengue fever cases occurring in an area or by detecting the change in serotype of dengue virus over the currently circulating strain. DEN-2 virus was responsible for the recent epidemic of DF/DHF in 1996 (Broor *et al,* 1997), while the prevalent serotype during postepidemic period was identified as DEN-1. Most of the epidemics of DF/DHF in India have been due to DEN-2 virus, while DEN-1 may be circulating endemically. The peak of dengue during the 1996 epidemic was in the months of September to November (Broor *et al,* 1997) and during the postepidemic period also the peak of infection was observed from September to December. This being the post monsoon season in Delhi there is an increase in mosquito breeding which may lead to increase in transmission of dengue virus (Lall and Dhanda, 1996). The age distribution of cases agreed with the preceding epidemiological background that suggested higher susceptibility of primary dengue in children (Chungue *et al,* 1989). The number of secondary dengue cases were maximum in adult population as most individuals in endemic area are already exposed to previous dengue infection.

This study demonstrates that DF is not an uncommon occurrence during period following an epidemic and culture and serology are useful methods for diagnosis. A large-scale proactive surveillance during non-epidemic period using virus isolation and serology is thus warranted for early indication of an epidemic.

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