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Episode of coexisting infections with multiple dengue virus serotypes in central Karnataka, India

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KEYWORDS
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Summary
Background: The co-circulation of multiple dengue virus serotypes has been reported in many parts of the world, including India; however, concurrent infection with more than one serotype of dengue virus in the same individual is rarely documented.
Method: An outbreak of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) occurred in and around Davangere, Karnataka, from June 2011—March 2012. This is the first report from India with a high percentage of concurrent infections with different dengue virus serotypes circulating during one outbreak. Acute phase sera from patients were tested for the presence of dengue virus RNA by RT-PCR.
Results: Of the 72 samples tested for dengue virus RNA, 42 (58.3%) were positive. All four dengue virus serotypes were found to be co-circulating in this outbreak, and DENV-2 was the predominant serotype. In addition, concurrent infection with more than one dengue virus serotype was identified in 18 (42.9%) dengue virus-positive samples.
Conclusion: Our study showed that serotype DEN-2 was dominant in the positive dengue virus-infected samples; the other serotype present was DEN-3. This is the first report of concurrent infections with different dengue virus serotypes in this part of the world.

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Introduction

Dengue infections are a significant cause of morbidity and mortality and lead to adverse economic effects in many developing tropical countries [1]. The incidence of dengue fever is on the rise worldwide, and in some areas of Asia, complications from the disease are a leading cause of serious illness and death in children [2,3].

Dengue illnesses are caused by four serologically related viruses, designated as DENV-1, DENV-2, DENV-3 and DENV-4 [4,5]. Infection with any one of these serotypes mostly causes a mild, self-limiting febrile illness (classical dengue fever (DF)); however, a few cases develop severe life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Clinical observation is important for the diagnosis of DF and DHF, and laboratory tests are essential for confirmation. An enzyme-linked immunosorbent assay has recently been used in many laboratories [6]. Viral isolation is a definitive test, but it is time consuming. Instead of virus isolation, reverse transcription polymerase chain reaction (RT-PCR) has been widely used for dengue diagnosis. It has been reported that RT-PCR is positive in serum samples collected in the febrile stage of primary dengue virus infection [7—9]. In recent years, the co-circulation of multiple dengue virus serotypes has been increasingly reported with concurrent infections. However, the association of concurrent infection with severe forms of the disease (DHF/DSS) requires further study [10].

In this study, we report the detection of dengue virus RNA by RT-PCR during a dengue outbreak that occurred in and around Davangere district from June 2011—March 2012.

Materials and methods

Two blood samples were collected from 72 patients with a <7-day history of fever who presented to outpatient departments from mid-June 2011—March 2012. One blood sample was sent on ice to the molecular laboratory for the detection of dengue viruses, the NS1 antigen and IgM and IgG antibodies. The second sample was used for a complete blood hemogram. The clinical basis for diagnosing patients with dengue virus infection was based on the WHO definitions. Because these diagnostic samples were received during an outbreak, no prior ethical clearance was required. However, patient information was de-linked from the sample information to protect patient privacy.

Viral RNA was extracted from serum samples using the QIAamp Viral RNA mini kit (Qiagen, Germany) according to the manufacturers' instructions. Extracted RNA was stored at −70 °C or immediately used for RT-PCR. RT and PCR were performed in one tube using a universal primer and a one-step RT kit (QIAGEN, GmbH, Hilden, Germany); the reaction was then placed in a thermal cycler (Eppendorf). The preliminary product was further used for nested PCR in another reaction tube [8]. Nested PCR was performed with a thermal cycler. The secondary PCR product was subjected to agarose gel electrophoresis using a 2% agarose gel (Bangalore Gene) in Tris—borate buffer, followed by staining with ethidium bromide and visualization on a UV transilluminator at 302 nm.

The NS1 antigen and IgM and IgG antibodies were detected with ICT. The test kit used was the dengue NS1 antigen and antibody Combi Card supplied by J. Mitra and Co. Pvt Ltd. (New Delhi, India) [6].

Statistical analysis

All statistical analyses were performed using SPSS version 16 software.

Results

Of 72 samples, 42 (58.3%) tested positive for dengue viral RNA by RT-PCR. Twenty-four cases were infected with a single DENV serotype, and 18 had a concurrent infection with two DENV serotypes. Of the 24 single-infection cases, 13 (54.2%) were typed as DENV-2, 7 (29.2%) as DENV-3, 2 (8.3%) as DENV-1 and 2 (8.3%) as DENV-4. DENV-2 dominated the outbreak, accounting for 54.2% of the positive samples, followed by DENV-3 (29.2%). The overall prevalence of concurrent infections was 42.9%. Co-infection with serotypes DENV-2 and DENV-3 was found to account for 66.7% of all concurrent infections. Other combinations included the following: DENV-1 and DENV-2 (3 of 18, 16.7%); DENV-1 and DENV-3 (2 of 18, 11.1%); and DENV-2 and DENV-4 (1 of 18, 5.6%). Thus, DENV-2 and DENV-3 were the most commonly combined serotypes observed during the outbreak. Of the 42 patients in whom dengue virus RNA was detected, 25 were male and 17 were female (Table 1). Forty-one samples were from the pediatric age group (<12 years of age), and one sample was from an adult (23 years of age). The mean age of patients with positive samples was 8.9 ± 7.8 years. The maximum number of dengue virus-positive cases occurred in patients 5—10 years of age (45.2%), followed by those >10 years of age

Table 1 Age and sex distribution of the RT-PCR-positive dengue cases.

Age (years)	Sex		Total
	Male	Female	
<5	5	6	11
6—10	11	8	19
>10	9	03	12
Total	25	17	42

Table 2 Clinical manifestations of patients with dengue infection.

Clinical features	Number (%)
Fever	42 (100)
Retro-orbital pain	25 (59.5)
Flushing	30 (71.4)
Rash	12 (28.6)
ARDS	13 (31.0)
Encephalopathy	05 (11.9)
Hepatomegaly	11 (26.2)
Splenomegaly	12 (28.6)
Ascites	05 (11.9)
Pleural effusion	03 (7.1)
Cyanosis	01 (2.4)
Convulsion	02 (4.8)
Oliguria	01 (2.4)
Hypoglycemia	01 (2.4)
Abscess	01 (2.4)
Pneumonia	03 (7.1)
Hematuria	02 (4.8)
Gum bleeding	01 (2.4)

(28.6%) and <5 years of age (26.2%). The clinical features of the patients in whom dengue virus RNA was detected are summarized in Table 2. Fever was the most common clinical presentation occurring in all patients on presentation. There was no specific pattern of fever, and the fever ranged from 38 °C to 40 °C. Other common clinical features included retro-orbital pain (59.5%), flushing (71.4%) and rashes (28.6%). ARDS was observed in 31.0% of patients, splenomegaly in 28.6%, ascites in 11.9% and encephalopathy in 11.9%. A platelet count <100,000 was observed in 37 (88.9%) patients, a platelet count of 50,000—100,000 was observed in 11 (26.2%) patients, a platelet count of 20,000—50,000 was observed in 20 (47.6%) patients, and a platelet count <20,000 was observed in 6 (14.3%) patients (Table 3). Per the classification based on the WHO definition (28), DF was observed in 18 cases with a single-serotype infection and 5 cases with concurrent infections, whereas DHF was present in 6 cases with a single-serotype infection and 11 cases with concurrent infections.

Table 4 Comparison of the efficacy of various dengue-specific parameters in RT-PCR-positive cases.

Parameters	Total	%
NS1	37	88.1%
NS1 + IgM	04	9.5%
IgM	01	2.4%
IgM + IgG	00	00
IgG	00	00
Total	42	100%

Of the 42 samples that were positive by RT-PCR, 37 (88.1%) were positive for NS1 antigen only, 4 (9.5%) were positive for NS1 and IgM, and 1 (2.4%) was positive for IgM only (Table 4).

Discussion

Dengue is a major public health problem in Davangere and surrounding districts in the state of Karnataka in south India. Over the last 5—6 years, we have observed varied clinical manifestations of dengue that differ from past reports from this region and other parts of the country. The first dengue epidemic in India occurred in Kolkata in 1963—64 [11], and since that time, the epidemiology of dengue virus has been evolving. This is the first study from this region to report concurrent infections with different dengue virus serotypes. In this study, we report a high percentage (42.9%) of concurrent infections.

The first case of concurrent infections with 2 dengue virus serotypes was reported in Puerto Rico

Table 3 Platelet count in dengue RT-PCR-positive cases.

Age (years)	Platelet count				Total
	<20,000	20,001—50,000	50,001—1,00,000	>1,00,000	
<5	1	6	4	0	11
6—10	5	9	3	2	19
>10	0	5	4	3	12
Total	6	20	11	5	42

in 1982 [10], and since then, other countries have also reported the occurrence of concurrent infections [12,13] in areas where multiple dengue virus serotypes co-circulate. In a study carried out in Delhi in 2006, 19% of the reported cases were concurrent infections. Previous studies with a lower percentage of concurrent infections have been reported in Taiwan (9.5%) and Indonesia (11%) and in Mexico, Puerto Rico and Indonesia together (5.5%) [12,13]. DENV-1 and DENV-3 were identified as the most frequent dengue virus serotype combination occurring during this outbreak. It has been postulated that concurrent infections with multiple dengue virus serotypes may influence the clinical course of the disease, and it is considered as the most important factor in the emergence of DHF.

During the dengue outbreaks of 1967, 1970 and 1982 in Delhi, no culture-confirmed cases of DHF/DSS were reported [12,13,15,16]. However, some cases of DHF were observed for the first time in 1988 [17]. In the present study, 17 cases of DHF were observed; 6 had single-serotype infections, and 11 had concurrent infections. Thus, a higher percentage of cases with concurrent infections had severe disease. However, the numbers are small, and larger studies are needed to prove this association.

The most unusual features observed in this series were encephalitis and a higher percentage of ARDS, whose diagnoses were based on clinical features and CSF examination. Dengue infection can cause neurological manifestations ranging from non-specific symptoms to encephalitis and, rarely, Guillain—Barré syndrome. The virus serotype may be involved, but DEN2 and DEN3 are most frequently reported as the cause of neurological sequelae. It is likely that we observed more encephalitis because of the prevalence of DEN2. The associations of other unusual features, such as URI, diarrhea, jaundice and lymphadenopathy, have been described in other studies.

The seasonal drift of dengue virus infection is revealed by the maximum positive cases observed from August to December, which is in agreement with previous outbreaks [19]. The highest number of cases positive for dengue virus by RT-PCR was observed in patients less than 10 years (71.4%) of age, as shown in Table 1.

The most challenging problem associated with patient management in dengue infection is rapid diagnosis. Although the commercially available MAC ELISAs or ICT offer improvements over other conventional assays for diagnosis, they do not offer serotype-specific diagnosis. Diagnosis based on the detection of the NS1 antigen can be achieved on the first day, but in the present study, NS1 was positive in only 88.1% of cases, and IgM antibodies were only detected after 5—7 days of illness. Concurrent infections can be detected by virus isolation in tissue culture followed by indirect immunofluorescence using serotype-specific monoclonal antibodies and/or RT-PCR [8]. However, RT-PCR offers accuracy and speed in addition to the serotype-specific diagnosis of various circulating dengue viruses and information about the co-circulation of different subtypes. It is now clear that epidemics caused by multiple dengue virus serotypes have become more frequent on a global basis in the past 18 years [6]. The general belief is that concurrent infections by different dengue serotypes occur during epidemics only, where multiple virus serotypes are being transmitted. The co-circulation of multiple dengue serotypes in the same region has been documented in several countries for decades [12—14].

In this outbreak, the detection of dengue virus RNA by RT-PCR showed that DENV-2 and DENV-3 were the most common etiologic agents, followed by DENV-1 and 4. All four dengue virus serotypes were found to co-circulate in the current outbreak. In Delhi and its surrounding areas, only two concurrent dengue cases have previously been identified [18], but the number has now significantly increased. The occurrence of recombination events may be of extreme importance in concurrent infections [19]. Viral recombination may lead to the emergence of more virulent strains.

Conclusion

This study revealed that DENV-2 was the dominant strain in this outbreak, even though all four serotypes were found to be co-circulating by RT-PCR. The increasing trend of the co-circulation of dengue virus serotypes suggests that Davangere district is becoming an endemic area. Although the sample size in this study was small, the study highlights the high percentage of concurrent infections with different dengue virus serotypes for the first time in India.

Conflict of interest

Funding: No funding sources.
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