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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