Arch Virol (2014) 159:1567–1573
DOI 10.1007/s00705-014-2010-x

BRIEF REVIEW

Unusual and rare manifestations of dengue during a dengue outbreak in a tertiary care hospital in South India

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Received: 9 November 2013 / Accepted: 26 January 2014 / Published online: 9 February 2014

Abstract Dengue is the most rapidly spreading mosquito-borne viral disease in the world, and as a larger proportion of the population is being affected, more unusual manifestations are being reported. Very few studies have documented unusual manifestations of dengue in South India. This prospective study was undertaken from July 2011 to June 2013 to document rare manifestations of dengue fever in 175 hospitalized patients. The clinical diagnosis was confirmed by the detection of NS1Ag, dengue IgM, or IgG by ELISA and/or a RT-PCR and CDC real-time PCR for dengue virus (DENV) RNA. The daily profiles of the hematological and biochemical investigations were followed and recorded. Unusual and rare manifestations of dengue were documented for 115 patients (66 %). Hepatitis was observed in 70 % of the cases. Pleural effusion was seen in 11 %, acute renal failure in 10 %, neurological complications such as encephalitis in 7.4 %, myocarditis in 9 %, and bleeding gastric ulcers in 3.4 % of the cases. DENV serotype 2 was more prevalent in patients with unusual manifestations of dengue in our study. The WHO classification system does not include unusual and rare manifestations; hence, it is essential to be aware of these manifestations and closely monitor them for better clinical management and outcome of patients.

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Introduction

Dengue is the most common arboviral disease of humans and in recent years has become a major global public-health problem. Approximately 2.5 billion people, living mainly in urban areas of tropical and subtropical regions, are estimated to be at risk of acquiring dengue infection [1]. The resurgence of dengue has been observed in India, and dengue outbreaks have been frequently reported from different parts of the country in both urban and rural populations [2, 3].

Dengue infection presents classically as dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), which can be fatal. Rare manifestations are becoming more common.

Various manifestations of dengue may not have a distinct line of demarcation: apart from the classic features, reports of rare presentations have recently become more frequent [4, 5].

Based on the 1997 World Health Organization (WHO) classification, symptomatic dengue infection may be in the form of dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [6]. In 2009, in a revised WHO classification system, dengue fever was further divided into dengue with or without warning signs and severe dengue [7]. Since dengue is the most rapidly spreading mosquito-borne viral disease in the world [7] and as a larger proportion of population is being affected, more unusual manifestations are being reported. In 2011, the WHO classification guidelines for dengue were revised and dengue was divided into DF, DHF without shock or with shock (DSS), and expanded dengue syndrome. According to its severity, DHF is divided in to four grades: DHF grade I, DHF grade II – DHF, DHF grade III, and DHF grade IV – DSS [8].

Expanded dengue syndrome is a new entity added to the classification system to incorporate a wide spectrum of unusual manifestations of dengue infection affecting various organ systems, including the gastrointestinal, hepatic, neurological, pulmonary and renal systems. The endothelium is the target of the immunopathological mechanisms in dengue and DHF. The hallmark of these diseases is vascular permeability and coagulation disorders. These mechanisms can explain the varied systemic involvement in the majority of cases [4]. Dengue virus was isolated in India in 1944, but scientific studies addressing various problems of dengue disease have been carried out only at a limited number of centers, and very few studies have documented the unusual and rare manifestations of dengue, especially from South India.

This prospective study was undertaken between July 2011 and June 2013 to document unusual rare manifestations of dengue fever in hospitalized patients in Hyderabad, Andhra Pradesh State, South India, and compare them with the WHO dengue classification [8].

Materials and methods

The study included 175 hospitalized patients admitted with dengue-like illness from July 2011 to June 2013. The study was conducted following approval by the Institutional Ethics Committee of Nizam's Institute of Medical Sciences (EC/NIMS/1336/2012). Written informed consent was obtained from each of the 175 hospitalized patients. Acute-phase serum and plasma samples were obtained during days 1–15 after the onset of symptoms for serology and molecular assays, respectively. The dengue/DHF/DSS case proformas, prepared as per the WHO protocol [6], were filled in by the treating clinicians.

Hospitalized patients were diagnosed with DENV infection based on the WHO criteria (history of fever with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, maculopapular rash, hemorrhagic manifestation, and leucopenia), and laboratory diagnosis of dengue was established by demonstration of DENV NS1Ag, dengue IgG or IgM ELISA and/ or reverse transcription PCR [9] and CDC real-time PCR for DENV RNA.

The patients were prospectively followed from July 2011 to June 2013. A detailed history was obtained, and a general and systemic clinical examination was carried out. The daily profiles of the hematological and biochemical investigations were followed and recorded. Platelet counts were done sequentially for all of the patients from the day of admission until discharge. A platelet count of <100,000 cells/mm^3 was considered thrombocytopenia. Bleeding manifestations were recorded when petechiae, epistaxis, gum bleeding, hematemesis, melena, or a positive tourniquet test was observed. Signs of plasma leakage were assessed by chest radiography and abdominal ultrasonography. Hypotension or circulatory failure was recorded when the patient had cold, clammy skin and restlessness, tachycardia, and weak pulse with pulse pressure <20 mmHg. The clinical diagnosis of dengue encephalitis was based on symptoms including neck stiffness, altered sensorium, and behavioral disorders. Specific investigations were performed sequentially in patients who presented with neurological involvement (cerebrospinal fluid analysis, neuroimaging, electro-diagnostic studies). Hepatic failure was reported when screening for viral hepatitis markers and liver function tests with AST/ALT yielded values 10 times higher than the normal value (8-40 U). A peripheral smear and QBC for *Plasmodium falciparum*, Widal test for typhoid fever, and ELISA for leptospirosis were also performed. Cases of scrub typhus were identified based on a positive one-step scrub typhus antibody test (SD Bioloine Tsutsugamushi, a solid-phase immunochromatographic assay for rapid qualitative detection of IgG, IgM, IgA antibodies to *Orientia tsutsugamushi* in human serum or plasma), a positive Weil-Felix test (WFT), the presence of an eschar, or a combination of the three in a patient with an acute febrile illness [10]. Radiological examination included X-ray, electrocardiogram (ECG), ultrasonography, CT scan, echocardiography, and upper gastrointestinal endoscopy (UGIE), and these tests were performed sequentially from the day of admission until discharge. The management and therapy administered and the outcome were also noted.

Serological assays

NS1Ag detection

Panbio Dengue Early ELISA assay (Inverness Medical Innovations, Australia Pty Ltd) was performed to detect DENV NS1Ag in all 175 serum specimens, which were collected according to the manufacturer's instructions.

Dengue IgG and IgM antibody detection

Dengue IgG capture ELISA and Dengue IgM capture ELISA (Pan Bio pvt. Ltd, Queensland, Australia) was used to screen for anti IgG and anti IgM Dengue antibodies in all 175 samples according to the manufacturer's instructions.

A primary infection is indicated when the IgM-to-IgG index value ratio is 1.78, and secondary infection is indicated when the IgM-to-IgG ratio is less than 1.78 [11]. Taking these criteria into account, patients were categorized as suffering from primary or secondary infections [12].

Molecular assays

RNA extraction

Viral RNA for the nested RT-PCR assay was extracted from 140 µl of plasma specimens collected between days 1-7 after onset of fever using a QIAamp Viral RNA Mini Kit (QIAGEN, Germany). RNA was eluted in 50 µl of elution buffer and stored at −80 °C until testing.

RT-PCR

The RT-PCR protocol for DENV RNA detection and typing was carried out in 175 plasma samples as described previously [13]. Briefly, oligonucleotide consensus primers were designed to anneal to any of the four dengue virus types and to amplify a 511-bp product in a reverse transcription polymerase chain reaction (PCR). First, we produced a cDNA copy of a portion of the viral genome in a reverse transcription reaction using primer D2, and we then carried out a standard PCR (35 cycles of heat denaturation, annealing, and primer extension) with the addition of primer D1. The resulting double-stranded DNA product of the RT-PCR was typed by a second round of PCR amplification (nested PCR) with type-specific primers, which yielded DNA products, the unique sizes of which were diagnostic for each dengue virus serotype.

CDC DENV-1-4 real-time RT-PCR assay

A real-time RT-PCR assay was used for dengue virus serotype-specific identification in an ABI 7500 quantitative PCR system (ABI, USA). The assay is based on TaqMan chemistry, including a panel of oligonucleotide primers and dual-labeled hydrolysis probe sets [D1, D2,D3, D4] and employing an Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System. The amplification was carried out in a 25-µl reaction volume. The standard thermal profile for sample screening was as follows: reverse transcription 50 °C for 30 min, initial denaturation and enzyme inactivation at 95 °C for 2 min, 45 cycles of extension at 95 °C for 15 s and 60 °C for 1 min of denaturation and annealing extension, respectively [14]. Briefly, the reagents included 12.5 µl of 2× buffer (Invitrogen One-Step RT-PCR Kit, USA), 0.5 µl of enzyme mix, 0.5 µl of each D1/D3 both forward and reverse primer (5 nM), 0.25 µl of each D2/D4 forward and reverse primer (5 nM), 0.45 µl of each D1-D4 probe (1 nM), and DEPC-treated water in a total volume of 25 µl. Finally, 5 µl of viral RNA eluate extracted from different samples was added for the real-time RT-PCR assay.

Results

During the study period of two years, case records of 175 patients who were diagnosed with DENV infection and required hospitalization were studied. There were 107 males (61 %) and 68 females (39 %) (male: female ratio, 1.6:1). The ages ranged from 18 to 80 years, with the most affected age group being 21 to 35 years (37 %). The majority of the patients were admitted during the post-monsoon period, i.e. between August and November, in both of the years.

DENV-specific assays

Positive results in NS1Ag, IgG or IgM ELISA, RT-PCR and CDC real-time PCR for DENV RNA were obtained for all 175 patients, either singly or in combination, as shown in Table 1.

Clinical findings

Of 175 patients who fulfilled the WHO criteria for dengue, 159 (91%) had classic dengue fever (DF), 12 had dengue hemorrhagic fever (DHF), and four had dengue shock syndrome (DSS).

Comparison of clinical findings in patients with DF, DHF and DSS was done, and the most common clinical

Table 1 Laboratory findings of 175 hospitalized patients

RT-PCR and CDC real-time PCR P/T (%)	Serotype detected	NS1Ag only P/T (%) (no IgM/IgG)	NS1Ag +IgM P/T (%)	NS1Ag +IgM + IgG P/T (%)	IgM only P/T (%)	IgG only P/T (%)
80/175 (46 %)	DENV1-1 DENV2 –57 DENV3- 20 DENV 4-2	41/175 (23.4 %)	28/175 (16 %)	22/175 (12.5 %)	18/175 (10.3 %)	16/175 (9.1 %)

RT-PCR- and NS1-Ag-positive samples: 30

RT-PCR, reverse transcription PCR; DENV, dengue virus; IgG + IgM, immunoglobulins G and M; NS1 Ag, nonstructural protein 1 antigen; P, positive; T, tested

Table 2 Comparison of clinical findings in patients with DF, DHF, and DSS

Clinical findings	Number of cases N = 175 (%)	DF N = 159 (%)	DHF N = 12 (%)	DSS N = 4 (%)
Fever	175 (100)	159 (100)	12 (100)	4 (100)
Rash	68 (39)	56 (35)	9 (75)	3 (75)
Thrombocytopenia	175 (100)	159 (100)	12 (100)	4 (100)
Leucopenia	64 (36)	52 (33)	10 (83)	2 (50)
Elevated transaminases	122 (70)	107 (67)	11 (92)	4 (100)
Vomiting	81 (46)	72 (45)	7 (58)	2 (50)
Headache	79 (45)	70 (44)	5 (42)	4 (100)
Bleeding manifestations	70 (40)	57 (36)	9 (75)	4 (100)
Myalgia	66 (38)	58 (36)	6 (50)	2 (50)
Hypotension	20 (11)	14 (9)	2 (17)	4 (100)
Altered sensorium	18 (10)	14 (9)	2 (17)	2 (50)
Lymphadenitis	10 (5.7)	10 (6)	0	0

findings were rash, headache, myalgia, thrombocytopenia, leucopenia and bleeding manifestations, as shown in Table 2.

In addition to the above classical manifestations of dengue, unusual and rare manifestations of dengue were also documented in 115 patients (66 %). The remaining 60 patients had no unusual signs or symptoms. Adult patients with an age range of 21 to 35 years showed signs of unusual manifestations. The most frequent unusual presentation was hepatitis in 70 % of cases, in the form of elevated liver function tests, i.e., AST/ALT 10 times higher than normal values (8-40 U), icterus. Pleural effusion was seen in 11% of cases. Acute renal failure was observed in 10 % of the cases, neurological complications such as encephalitis were observed in 7.4 %, myocarditis in 9 %, and bleeding gastric ulcers in 3.4 % of the cases. A comparison of atypical manifestations observed in patients with DF, DHF, and DSS is shown in Table 3.

Unusual manifestations of DENV-2 infection were observed in 35 of 57 cases and 5 of 20 cases of DENV-3 infection ($P = 0.0084$, statistically significant by Fisher's test). A comparison of unusual manifestations of dengue in patients with the DENV-2 and DENV-3 serotypes is shown in Table 4.

More than two unusual manifestations were observed in two patients with DENV-2 infection.

Ten patients had co-infection with DENV and scrub typhus in our study, and eight of these patients had unusual manifestations of dengue. Three developed bilateral pleural effusion, three had acute renal failure, and two developed sepsis. All of the hospitalized patients were negative for Widal, leptospira, and malaria.

The mortality rate in our study was 9/175 (5.1 %). All nine of these patients, although admitted with dengue fever, developed unusual manifestations during the hospital stay. Four of them expired due to acute renal failure, three expired due to encephalopathy, and two expired due to cardiac failure associated with hypervolemia. Multiorgan involvement was also observed in these patients.

Discussion

Dengue (including DHF and DSS) is a growing major mosquito-borne disease of immense public-health importance in India. Classical DF is caused by infection with any one serotype of DENV, while DHF and DSS occur in individuals with secondary infections who had prior infection with one or more DENV serotypes. In the majority of infected people, dengue is an auto-limited disease that resolves in 5–7 days. However, approximately 500,000 people develop a severe form, leading to about 20,000 deaths annually [15]. Unusual and rare

Table 3 Comparison of unusual manifestations of dengue among 175 patients

Atypical clinical findings	Number of cases N = 175 (%)	DF N = 159 (%)	DHF N = 12 (%)	DSS N = 4 (%)
Acute renal failure	18 (10)	10 (6.3)	6 (50)	2 (50)
Pleural effusion	19 (11)	13 (8.2)	3 (25)	3 (75)
Shortness of breath	26 (15)	19 (12)	5 (42)	2 (50)
Gastric ulcers with UGI bleeding	6 (3.4)	1 (0.6)	4 (33)	1 (25)
Myocarditis	16 (9)	13 (8.2)	3 (25)	1 (25)
Encephalitis	13 (7.4)	10 (6.3)	2 (17)	1 (25)
Sepsis	13 (7.4)	8 (5)	3 (25)	2 (50)
Hepatitis	122 (70)	119 (75)	1 (8.3)	2 (50)
Oral candidiasis	6 (3.4)	5 (3.1)	0	1 (25)
Disseminated intravascular coagulation (DIC)	3 (1.7)	1 (0.6)	1 (8.3)	1 (25)

Table 4 Comparison of unusual manifestations of dengue in patients with the DENV-2 and DENV-3 serotypes

Atypical clinical findings	DENV-2 (N = 57)	DENV-3 (N = 20)
Acute renal failure	3	2
Pleural effusion	5	0
Shortness of breath	6	1
Gastric ulcers with UGI bleeding	1	0
Myocarditis	2	0
Encephalitis	3	0
Sepsis	1	1
Hepatitis	10	1
Oral candidiasis	1	0
Disseminated intravascular coagulation (DIC)	1	0

manifestations of dengue were documented in 115 patients (66 %) in our study.

Demonstration of DENV- 2 in 35 of 57 cases and DENV-3 in 5 of 20 cases by reverse transcription PCR and CDC real-time PCR indicated that unusual manifestations are probably more common with DENV-2 than with DENV-3. This is in concordance with studies done by Lum et al. [16], Leão et al. [17], Araujo et al. [18], and Thomas et al. [19]. None of the patients in our study showed clinical signs of infection with other arboviruses, including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV) and yellow fever virus (YFV). This was further confirmed by reviewing the patient's past medical history, recent travel history, and vaccination record (especially yellow fever vaccination) to determine the likelihood that the current acute febrile illness was due to an infection with dengue virus. All of the 175 cases included in our study fulfilled the WHO case definition criteria for dengue. None of the patients in our study had a history of travel to regions where WNV and YFW are endemic. JEV is the only other flavivirus reported in sporadic cases in this study area. We did not observe any cross-reactivity with JEV-positive samples, which may be due to their grouping in a different serogroup. Furthermore the results of RT-PCR and CDC real-time PCR demonstrated a high degree of specificity for the dengue virus serotypes, with no cross-reaction observed with in serotypes and with serologically related flaviviruses such as JEV, WNV, and HCV. The Platelia dengue NS1 Ag assay for laboratory confirmation of dengue used in this study is highly specific [20] and revealed no positive results for YFV, WNV and JEV in our study. However, the only limitation of our study is that the types of samples used to determine specificity were limited, and more samples, especially from other flaviviral pathogens, such as St. Louis encephalitis virus and other related viruses in this group, need to be included to ensure adequate coverage of possible cross-reactions. Obtaining such sera that are negative for DENV antibody is sometimes not possible due to the high endemicity in the tropical belt.

In the present study, 60 (34 %) out of 175 patients were without complications. Elevated liver enzymes, AST/ALT 10 times the normal value (8-40 U), were observed in 119 (75 %) cases of DF, which is suggestive of hepatitis. This is in concordance with a study done by Prakash et al. [21] in which 71 % had developed hepatitis. Elevated transaminases in DF are in concordance with a study done by Bowman et al. [22]. Pulmonary complications in dengue, although rarely seen, include pulmonary infiltration and small pleural effusion. Pleural effusion was observed in 11 % of our cases, and this is in concordance with studies done by Ejaz et al. [23].

Acute renal failure is rare in dengue fever and it mainly presents as shock-induced acute tubular necrosis. It has been observed as a complication of dengue fever in French Guiana by Hommel et al. in 1998 [24] and was found to occur in 0.3 % of cases in a series of 6154 patients with DHF [25]. Acute renal failure was observed in 50 % of DHF and DSS cases in our study. Haemodialysis and peritoneal dialysis were done for the management of these patients. Four of the nine expired patients in our study had acute renal failure. Dengue haemorrhagic fever can result in acute respiratory distress syndrome (ARDS). Dengue virus antigen is found in alveolar lining cells of the lung. Atypical respiratory manifestations such as pleural effusion were observed in 25 % of DHF and 75 % of DSS cases, and shortness of breath was observed in 42 % of DHF and 50 % of DSS cases in our study. Shortness of breath may be due to excessive accumulation of fluid in these patients. Heart involvement is infrequent in DENV infection, but atrial-ventricular blocks, atrial fibrillation, and other arrhythmias have been documented [26]. Cardiac involvement was observed in 25 % of DHF and 8.2 % of DF cases in our study.

Neurologic disorders have been increasingly reported in patients with dengue. These include disturbance of consciousness, seizures, coma, polyneuropathies, Guillain-Barré syndrome, and transverse myelitis [16, 17]. Solomon and others found dengue viruses in 4.2 % of patients with CNS infections [27]. These findings indicate that the virus can cross the hematoencephalic barrier and infect the brain by microvascular or frank hemorrhage and its intrinsic neurotropism. Alternatively, the virus may be transported to the CNS by infected macrophages, with consequent dengue-induced encephalitis. Some complications such as hypotension, cerebral edema, hyponatremia, and fulminant hepatic failure might also be implicated as possible causes of dengue encephalopathy. Dengue encephalitis was observed in 7.4 % of our cases and was caused by

serotypes 2 and 3. This is in concordance with studies done by Araujo et al. and Solomon et al. [18, 27]. Magnetic resonance imaging (MRI) of brain in our patients showed cerebral edema/hyperintense lesions in basal ganglion/ left temporal parietal bleed.

Bleeding manifestations including epistaxis (11 patients), bleeding gums (26 patients), hematemesis (6 patients) and melena (15 patients) were observed in 57 patients with dengue fever. Thirty-six out of 57 patients with dengue fever received blood/platelet-rich plasma transfusions. Some patients developed UGI bleeding and disseminated intravascular coagulation (DIC), for which supportive therapy with blood and blood products including platelets and fresh frozen plasma was given. A total of 50 out of 175 (28.57 %) patients needed transfusion of blood or blood products for their management.

Oral candidiasis was seen in six (3.4 %) of cases with DF. This is in contrast to a study done by Malavige et al. [28] in which no oral candidiasis was observed in an adult population. Dengue fever and scrub typhus are common infections in Asia that often present as acute febrile illness of unclear etiology. According to Watt et al. dengue virus infection is associated with hemorrhagic manifestations, particularly bleeding from the gums. A low platelet count (<140,000/mm^3) and low white blood cell count (<5,000/mm^3) are also strongly associated with dengue infections [29].

Of the 10 patients with DENV and scrub typhus confection, eight had unusual manifestations of dengue. Thrombocytopenia and elevated transaminases were observed in all eight patients. Bilateral pleural effusion in patients with DENV and scrub coinfection is in concordance with a study done by Iqbal et al. [30]. All eight of these patients were stable at discharge. Management of patients with scrub typhus was done with doxycycline. Management of patients with dengue of both groups (recovered and dead) was done by intravenous fluid therapy with 5 % dextrose in normal saline, transfusion of blood and blood products, antibiotics and proton pump inhibitors (PPI), peritoneal and hemodialysis in ARF patients and ventilator support for patients in shock.

Of the hospitalized patients, 5.1 % developed unusual manifestations and succumbed to illness due to multiorgan dysfunction. The remaining patients were managed by conservative treatment with intravenous fluid therapy and antipyretics, and all patients were stable at the time of discharge from the hospital. Primary infections were predominant in patients with unusual manifestations. Lack of awareness among primary physicians about the revised WHO criteria, which include expanded dengue syndrome, could probably be the reason for the mortality rate in our study.

This report of unusual manifestations may play an important role in better diagnosis, leading to improved treatment and prevention of fatal outcomes in patients with dengue infection.

Conclusion

Although the WHO classification system includes unusual and rare manifestations of dengue, lack of awareness among primary physicians may jeopardize clinical diagnosis. Since these manifestations are a result of microvascular leaks, they are considered to have an impact on the outcome of the patients. Hence, it is essential to be aware and closely monitor these unusual and rare manifestations for better clinical management and outcome of patients.

Acknowledgments The authors would like to thank the Director DRDE Gwalior and the Director of Nizam's Institute of Medical Sciences for giving permission to conduct the study.

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

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