A Comparative Study of Dengue Syndromes in a Tertiary Care Centre

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Abstract

Cyclic dengue epidemics have been reported from Kerala state since 2001. The DEN-1 and DEN-4 viruses had been detected since 1970s from the human sera in a few districts of Kerala. Circulation of multiple serotypes is associated with complications of dengue, namely Dengue Haemorrhagic Fever (DHF) / Dengue Shock Syndrome (DSS). A descriptive study was done from 16th August 2008 to 15th August 2009 in which clinical and laboratory parameters of dengue were documented. From the study group, blood samples were collected and IgG and IgM dengue antibodies were detected in the serum. Serotyping was done at Rajiv Gandhi Centre of Biotechnology by PCR. Dengue Fever (DF) without complications was seen in 94.03% primary and 68.42% secondary cases and DHF/DSS was seen in 5.9% of primary and 31.57% of secondary cases. Fever, headache, body-ache and myalgia were the common symptoms at presentation. Commonest bleeding manifestations were purpura and petechiae. Malena was more often associated with DHF/DSS. Facial puffiness was seen associated with DHF/DSS in the lower age groups. Retro-orbital pain was equally observed in DF and DHF/ DSS. DHF/DSS was observed in increased proportion in children; 36% of children had DHF/DSS, where as it was only 10% in the case of adults. Vomiting and abdominal pain were predominant in DHF/DSS group. Hepatomegaly was more in the DHF/DSS group. A positive tourniquet test showed greater association with DHF/DSS. Elevated liver enzymes and hypoproteinemia was observed in DHF/ DSS with greater significance. Serotype isolated in this outbreak was Dengue serotype 1.

Keywords: Dengue, Dengue Fever, Dengue Haemorrhagic Fever, Dengue Shock Syndrome

Background and Rationale

All dengue serotypes circulate in most countries of the Asian continent. The first extensive epidemic of DHF in India occurred during 1996 in the Northern states.1 The incidence of DHF from 1989 in Indian subcontinent was associated with the introduction of a new subtype, Den-3.2 Since 2001, occurrence of dengue increased in Kerala and outbreaks were reported repeatedly from most of the central and south districts. Aedes albopictus is the commonest species of mosquito incriminated in the transmission of dengue virus in Kerala.3

Repeated infections with different serotypes of the virus lead to complications (DHF/DSS). Secondary infections are commonly associated with complications. Based on the clinical and laboratory parameters WHO has classified dengue as dengue fever, dengue haemorrhagic fever and dengue shock syndrome.4 5 In this study we have attempted to point out the clinical and laboratory parameters that help us to categorise dengue as DF and DHF/DSS.

Objectives

1. To classify cases of dengue as Dengue Fever (DF) and DHF/ DSS among primary and secondary cases
2. To compare the clinical and laboratory features of Dengue Fever and DHF/DSS

Materials and Methods

This is a descriptive study done in the Departments of Paediatrics and Medicine, Government Medical College, Thiruvananthapuram over a one year from 16th August 2008 to 15th August 2009.

All cases of acute febrile illness admitted in the Departments of Medicine and Paediatrics which were clinically diagnosed as dengue fever as per the WHO criteria were included in the study.

Case definition by WHO:4 5

- Dengue Fever (DF)

Probable Dengue Fever: Acute febrile illness with 2 or more of the following manifestations:

- Headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leucopenia.

AND

A supportive serology (a reciprocal haemagglutination inhibition antibody titre<1280, a comparable IgG ELISA titre or a positive IgM antibody test on a late acute or convalescent phase serum)

OR


*See End Note for complete author details
Occurrence at the same location and time as other confirmed cases of dengue fever.

**Confirmed Dengue Fever:** A case confirmed by laboratory criteria.

**Reportable Dengue Fever:** Any probable or confirmed case should be reported.

**Laboratory criteria for confirmation of dengue fever are:**

- Isolation of the dengue virus from serum or autopsy samples
- Demonstration of a fourfold or greater increase in reciprocal IgG or IgM antibody titres to one or more dengue virus antigens in paired serum samples
- Demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by immunohistochemistry, immunofluorescence or ELISA
- Detection of dengue virus genomic sequences in autopsy tissue, serum or CSF samples by polymerase chain reaction.

**Dengue Hemorrhagic Fever (DHF):**

The following criteria must all be present:

- Fever/history of fever lasting 2 to 7 days, occasionally biphasic.
- Hemorrhagic tendencies evidenced by at least one of the following:
  - Positive Tourniquet Test
  - Bleeding from mucosa of GIT, injection site or other locations.
- Hematemesis or melena
- Petechiae, ecchymosis or purpura
- Thrombocytopenia (<100,000 cells/cmm)
- Evidence of plasma leakage due to vascular permeability as evidenced by one of the following:
  - A rise in hematocrit > 20% for age, sex and population or drop in hematocrit following volume replacement >20% of baseline.
  - Sign of plasma leakage such as pleural effusion, ascites, hypoproteinemia.

**Dengue Shock Syndrome (DSS)**

All of the above 4 criteria of DHF with any two of the following:

- Rapid weak pulse
- Narrow pulse pressure (< 20 mm of Hg) or Hypotension for age.
- Cold clammy skin and restlessness.

2126 blood samples were collected. IgM and IgG Dengue ELISA were done with the sera. Those tested positive were tested for IgG Dengue antibodies. From those who presented in the early days of fever (first 4 days), a sample was collected for PCR. IgG Dengue ELISA was done with these samples as well. Those tested positive for IgM with high titres of IgG were taken as secondary dengue infection. 685 cases were tested positive for dengue, 269 (39.27%) primary and 416 (60.73%) secondary.

150 primary and 150 secondary cases were selected randomly for analysing in detail the clinical and laboratory features. Patients presenting with fever less than 4 days were chosen for molecular diagnosis (Polymerase Chain Reaction).

IgM and IgG ELISA were done with the samples. IgM antibody was detected using Capture ELISA, NIV Pune and IgG using IVD Microwell ELISA (Indirect ELISA). Serotyping of the dengue virus was done at Rajiv Gandhi Centre for Biotechnology. The viral RNA was extracted using Viral RNA extraction kit (Qiagen, USA). The isolated RNA was subjected to reverse transcription and amplified by polymerase chain reaction, as per the protocols standardised in the laboratory. The Core-PrM region of the viral nucleic acid was amplified in the PCR using specific nucleic acid primers that can amplify any of the four types of dengue virus.

Those tested positive for IgM only were taken as primary and those with positive IgG with/ without IgM were considered secondary cases.

An informed written consent was obtained from the patient before collecting blood samples and taking history.

Data was entered in Microsoft Excel format and analysed using SPSS v13. The data was compiled to form proportions and compared using Chi-square tests.

**Results**

2126 samples were collected and 685 tested positive for dengue antibodies, 269 (39.27%) primary and 416 (60.73%) secondary. A total of 100 samples were taken for doing PCR, 33 tested positive for dengue. Serotype isolated in this outbreak was Dengue serotype 1.
The randomly selected 300 samples were studied in detail using the questionnaire prepared and the clinical and lab parameters were compared. This included 95 (31.4%) children and 205 (68.3%) adults.

All of the patients presented with fever (first week of fever). 43 (14.2%) had rash. Headache was present in 57 (37.7%) primary and 74 (48.7%) secondary cases of dengue fever. 65 (21.5%) had body-ache, 113 (37.3%) had associated myalgia and 33 (10.9%) had arthralgia; these symptoms were more among adults.

Purpuric spots were observed in 24 (8%) patients and 20 had petechiae, 2 had epistaxis and 2 had bleeding from gums. A total of 4 (1.3%) had ecchymosis and 5 (1.7%) of the females had menorrhagia. 1 (0.3%) with secondary dengue had retinal bleed. 1 (0.3%) patient had sub conjunctival haemorrhage. Haematuria was seen in 3 (1%) secondary cases. 1 (0.7%) primary and 2 (1.3%) secondary cases had hematemesis (2 in DHF). 14 patients with DF (5.7%) and 21 patients with DHF/DSS (24.6%) had malena.

Out of the total study group, 41 patients (15.5%) had conjunctival congestion and 18 (5.9%) had retro orbital pain. 11 patients with DHF/DSS (19.3%) had facial puffiness.

44 had DHF, 20 (9.62% adults) in adults and 24 (25.53% of children) in children. DSS was observed in 13 cases, 3 (1.44%) in adults and 10 (4.80%) in children. Among the DHF/DSS, 3 adults and 7 children had primary infection (Figure 1).

Elevated liver enzymes were noted in 147 (49%) patients. Elevated SGOT (AST) in 131/300 (43%) and elevated SGPT (ALT) in 97/300 (31.6%). Elevated SGOT was observed in 38 (66.7%) DHF/DSS and 93 (38.3%) DF cases (Figure 3). In the case of SGPT this was 25 (43.8%) and 72 (29.6%) respectively. Among those with DHF/DSS 29 (50.8%) had a hematocrit of >40. Haematocrit>40 was observed in 19 (55.8%) of paediatric population with DHF/DSS and it was only 10 (40.3%) in the adults.

Only 4 (1.6%) of DF and 7 (12.3%) of DHF/DSS had positive tourniquet test.

Among the DHF cases, 37 (41%) had a total protein of less than 7 and 34 (38%) had albumin levels less than 3.5. In the DSS group, 9 (53%) had a total protein of less than 7 and 8 (47%) had albumin level less than 3.5. Distribution of variables in the DHF/DSS and dengue fever group is given in Table 1.

Death rate in this study was low; two adults and a one year old child succumbed, resulting from uncontrolled bleeding or profound shock.
Table 1. Distribution of Various Parameters in the Study Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHF/DSS</th>
<th>Dengue Fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>33 (57.9%)</td>
<td>72 (29.3%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>17 (29.8%)</td>
<td>48 (19.5%)</td>
</tr>
<tr>
<td>Positive tourniquet test</td>
<td>7 (12.3%)</td>
<td>4 (1.6%)</td>
</tr>
<tr>
<td>Elevated SGOT</td>
<td>38 (66.7%)</td>
<td>93 (38.3%)</td>
</tr>
<tr>
<td>Elevated SGPT</td>
<td>25 (43.8%)</td>
<td>72 (29.6%)</td>
</tr>
</tbody>
</table>

Discussion

Each of the groups were divided as dengue fever and dengue fever with complications. This included 95 (25.83%) children and 205 (74.17%) adults. Dengue Fever (DF) without complications was seen in 142 (94.03%) of primary and 104 (68.42%) secondary and DHF/DSS was seen in 9 (5.9%) of primary and 48 (31.57%) of secondary cases, complications more often associated with secondary cases (p value 0.0001). Kabilan et al (Chennai 2001) reported DF in 65.5%, DHF in 11.2%, DSS in 23.8% of cases. Ratageri et al (Hubli 2003) reported DF in 18%, DHF in 60%, DSS in 22%. 6,7

43 (14.2%) had rash. It is difficult to interpret rash in Indian population. There was no difference in rash among the Dengue Fever and DHF/DSS group. 131 (43.7%) had headache, 65 (21.5%) had bodyache, 113 (37.3%) had associated myalgia and 33 (10.9%) had arthralgia with no significant association between the variables in DF and DHF/DSS group. Fever, fatigue, headache, retro orbital pain, myalgia, arthralgia/bone pain were the most common symptoms and occurred in almost equal frequency in DF and DHF/DSS groups a study at Chittagong, Bangladesh.8

24 out of 300 had purpuric spots (4.29%) and 20 had petechiae, 2 had epistaxis and 2 had bleeding from gums. These were associated with DF/DSS (28.1%), where as in DF it was only 3.3% (p value 0.0001).

Bleeding manifestations were seen in 42 patients (38.9%). Petechiae (15.7%) were the most frequent, followed by ecchymoses (7.4%), bleeding gums (6.5%) and haematemesis (5.6%). Vaginal bleeding was seen in 7 (15.9%) of 44 females, none of whom were menstruating at the time of infection. Haematuria and epistaxis were present in some. Mild bleeding manifestations (petechiae, ecchymoses) were also seen in 5 (15.2%) of 33 DF patients. Of the 42 patients (all DF) with bleeding manifestations, 13 (30.9%) needed platelet concentrates in one study.9 External bleeding (petechiae, positive tourniquet test, epistaxis, gingival bleeding) and chills were also present in more than 50% of children and adults with confirmed dengue. Fever, external hemorrhagic manifestations, and rash were present in more than 50% of infants in Nicaragua.10

Haematuria was seen in 3 (1%) secondary cases. 1 (0.7%) primary and 2 (1.3%) secondary cases had haematemesis (2 in DHF). Hematuria and menorrhagia were significantly more prevalent in adults than in children or infants and comprised the bulk of internal bleeding in adults in a study in Nicaragua.10

24.6% of DHF/DSS and 5.7% of dengue fever had malena, significant association (p value 0.0001) Malena (14.28%) was one of the common presentations in a study in India.11

Palmar erythema was noted in 17 cases (5.6%), itching in 8 (2.6%), flushed face in 11 (3.6%), sore throat in 6 (2%), facial puffiness in 34 (11.2%). Among these parameters there was no significant association between DF and DHF/DSS group, except for the few described below. Palmar erythema was observed more with lower age group, 10 (10.6%) in children and 7 (3.3%) in adults (p value 0.014). Facial puffiness was more associated with DHF/DSS 11 (19.3%) with a p value 0.033.

Facial puffiness was seen more in children, 20(21.3%) than in adults, 14 (6.7%), p value 0.0001. Flushed face was more associated with paediatric age group, 10(10.6%) when compared to adults which was only 1(0.5%), p value 0.0001. 41 (15.5%) had conjunctival congestion and 18 (5.9%) had retro orbital pain with no significant association with DHF/DSS. Bleeding in the loose connective tissue of the retro-orbital region leading to an increased intra-orbital pressure could be an initial, unnoticed bleeding manifestation in these patients and could contribute to the increased incidence of retro-orbital pain in the bleeders.12

Retro orbital pain, was present in more than 60% of children and adults with confirmed dengue and rash was present in more than 50% of infants in a study from Nicaragua.10 Retro orbital pain occurred in almost equal frequency in DF and DHF/DSS groups in one study group.13

44 had DHF, 20 (9.62% adults) in adults and 24 (25.53% of children) in children. 5 children had primary and 19 had secondary infection. 18 adults had secondary and 2 had primary dengue infection. DSS was observed in 13 cases, 3 (1.44%) in adults and 10 (4.80%) in children. 1 adult and 2 children had primary infection. DHF/DSS was observed in 23 (11%) adults and 34 (36.2%) children. The association of DHF/DSS with lower age group was significant (p value 0.0001). Antibody dependent enhancement leads to complications in infants following primary dengue. Inflammatory mediators contribute to DHF/DSS in primary dengue infections in adults. In the second theory, the dengue virus mutates as it replicates in the human and/or the mosquito. Some of these
mutations lead to more virulent viruses, these viruses causing DHF. Primary infections resulting in DHF has been described before.\(^7\)

In a study in Srilanka, 68 children with DHF had secondary and 18 had primary dengue infections.\(^14\) Another study from Singapore also reports DHF in primary dengue infections in adults.\(^15\) DSS following primary dengue infection was reported in a 10 year old boy from Kuala Lumpur.\(^16\) Of the 108 patients with mean age 26.6 years, included in a study in Srilanka, Dengue fever (DF) was seen in 33 (30.6%) and dengue haemorrhagic fever (DHF) in 75 (69.4%). Of the 37 (34.3%) with primary dengue infections, 19 (51.4%) developed DF and 18 (48.6%) developed DHF.\(^9\)

33 (57.9%) of DHF/DSS and 72 (29.3%) of dengue fever had vomiting, p value 0.0001. 17 (29.8%) of the DHF/DSS cases and 48 (19.5%) of DF had abdominal pain; though the association was not significant (p value 0.066). Abdominal pain is a prominent clinical manifestation of dengue infection which is not included in the WHO criteria. Almost all countries in South East Asia reported this symptom with high frequency.\(^17\) Other studies also show abdominal pain as a predominant symptom.\(^18,19\)

29 (9.6%) had diarrhoea, and there was no significant association DHF/DSS.

49 (19.9%) DF and 19 (33.3%) DHF/DSS had hepato-megaly, significant association, p value 0.025. Jaundice was noticed in 5 (1.7%) with no significant association with DF and DHF/DSS. Hepatomegaly (74%), epistaxis (26%), jaundice (25%), and petechial rashes (18%) were the common clinical manifestations of dengue infection in a study reported in 2000 from New Delhi.\(^20\) Hepatomegaly was observed in 15% of patients in New Delhi, more common in patients with secondary infection and some of these may be associated with an increase in liver transaminases. But in an outbreak in Chennai DF cases had significantly lesser hepatomegaly compared to DHF/DSS cases. The same study reported splenomegaly in 7 (11.8%) cases.\(^21\) Of the 32 patients in a study from Tamil Nadu on second to third day of fever, all showed gall bladder wall thickening and pericholecystic fluid, 21% had hepatomegaly, 6.25% had splenomegaly and right minimal pleural effusion.\(^22\) In our study myocarditis was seen in 8 (2.6%) cases with no significant association with DF and DHF/DSS. Though rare, myocarditis has been reported by some authors.\(^23,24\)

2 (0.7%) had pericardial effusion, 18 (31.6%) had pleural effusion, whereas it was 20 (35.1%) and 5 (2%) respectively in the case of ascites. A study from Massachusetts showed pleural effusion was the most common ultrasonographic sign of plasma leakage (62% of DHF cases one day after defervescence). Thickening of the gallbladder wall and ascites were detected less frequently (43% and 52% of DHF cases respectively) and resolved more rapidly than pleural effusions. Ultrasound detected plasma leakage in 12 of 17 DHF cases who did not meet the criteria for significant hemoconcentration.\(^25\) In Taiwan a study on patients with type 2 dengue fever had thickened gallbladder wall in 38 patients (59%), ascites in 24 patients (37%), splenomegaly in 22 patients (34%), and pleural effusion in 21 patients (32%); pleural effusion was either rightsided or bilateral sonologically.\(^26\)

No significant association was noted between leucopenia and DF and DHF/DSS. Leucopenia was observed in studies Malaysia.\(^16\) A retrospective observational study of dengue fever performed in Karachi from January 2001 to December 2006 observed 26.6% with leucopenia.\(^27\)

Among those with DHF/DSS 33 (76.7%) had a hematocrit of >40. Haematocrit >40 was observed in 19 (55.8%) of paediatric population with DHF/DSS and it was only 10 (43.4%) in the adults. The age-dependent differences in the outcome of dengue infection may be due to differences in vascular permeability; children have a greater propensity for vascular leakage, under normal physiologic conditions, than do adults. Many studies have showed that strictly adhering to WHO criteria for classifying cases, lead to difficulties especially with adults.\(^4\)

Elevated liver enzymes were noted in 147 (49%) patients. Elevated SGOT (AST) in 131/300 i.e 43% and elevated SGPT (ALT) in 97/300 i.e 31.6%. Elevated levels of SGOT (>100), were more associated with DHF/DSS, p value < 0.05. Liver enzymes were markedly elevated in more than 60% of the children who were dengue seropositive from Chennai. Aspartate aminotransferase (AST) was elevated in a larger proportion of the patients. There was no significant difference between the subgroups of dengue with respect to liver function tests. Kuo et al. in an evaluation of 270 dengue patients, observed abnormal levels of AST and ALT in 93.3% and 82.2%, respectively. They also reported that the elevation of AST levels was usually greater than for ALT, with a decrease to normal levels within three weeks. They noted significantly higher elevations of liver enzymes in patients with episodes of bleeding.\(^28\)

Among the DHF cases, 37 (41%) had a total protein of less than 7 and 34 (38%) had albumin levels less than 3.5. In the DSS group, 9 (53%) had a total protein of less than 7 and 8 (47%) had albumin level less than 3.5. The mean albumin level of DHF and DSS patients after plasma leakage was 4.1 and 3.6 gm% in a study conducted in Bangkok.\(^29\) Hypoalbuminemia was detected in ten patients (71%) in a study group with 14 patients in Brazil.\(^30\)

Only 4 (1.6%) DF and 7 (12.3%) of DHF/DSS had posi-
tive tourniquet test; associated with DHF/DSS (p value 0.001, which was significant). Previous reports showed low sensitivity of tourniquet test. A study from Chennai showed bleeding tendency in 39 patients. Tourniquet test was positive in 14 (23.7%) and this was the only bleeding tendency noted in 5 cases. Patients with DHF and DSS had a higher proportion of tourniquet test positivity (p = 0.02). The tourniquet test was positive in only 25.5% cases from Delhi. It was concluded that a negative tourniquet test may not be sufficient to exclude a diagnosis of DHF in a febrile patient.

Death rate in this study was low; two adults and a one year old child; resulting from uncontrolled bleeding or profound shock.

A total of 100 samples were taken for doing PCR, 33 tested positive for dengue. Serotype isolated in this outbreak was Dengue serotype 1.

Conclusions

Out of the 685 cases, 269 (39.27%) were primary and 416 (60.73%) were secondary dengue. Serotype of dengue virus isolated in this outbreak was Dengue serotype 1. Complications (DHF/DSS) were associated with secondary cases. Three adults with primary dengue infection had DHF/DSS. Headache, body-ache, myalgia, arthralgia were the common symptoms noted but there was no significant association between the variables in DF and DHF/DSS group. Though a prominent symptom, abdominal pain did not show a significant association. Retro-orbital pain showed no significant association. Petechiae, ecchymoses, bleeding gums and haematemesis were the bleeding manifestations noted in the DHF/DSS group. Malena, vomiting, hepatomegaly and elevated SGOT showed a statistically significant association in DHF/DSS. Leucopenia did not show a statistically significant association.

As our hospital is a referral centre, most of the cases (usually the more serious ones) reach here after 5 days and therefore the clinical spectrum cannot reliably be extrapolated to the actual population.

End Note

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List of Abbreviations

DF: Dengue Fever
DHF: Dengue Haemorrhagic Fever
DSS: Dengue Shock Syndrome

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