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Original Article

Autopsy findings in fatal dengue haemorrhagic fever – 06 Cases

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ABSTRACT

Background: During recent outbreak of dengue fever in Delhi, there has been a significant increase in dengue-associated admission in hospitals. To better understand the pathology of dengue haemorrhagic fever, we conducted autopsies of dengue infections deaths within our hospital.

Method: This was an autopsy study of dengue-associated deaths at a large tertiary care hospital.

Results: From Sep 2009 to Dec 2010, a total of 1032 patients with serological evidence of dengue infection were admitted to our hospital. There were twelve deaths and autopsies were conducted in six. Adult respiratory distress syndrome, bleeding diathesis, hypotension, hepatic failure and acute renal failure were the common causes of death despite early hospitalization, intravenous fluid, and blood-product support.

Conclusion: Dengue is associated with severe disease, and deaths do occur despite current supportive management. Early predictors of disease severity and better clinical interventions are needed.

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Introduction

Dengue fever (DF) is an arthropod-borne viral infection, caused by 1 of 4 antigenically distinct but related single-stranded, positive-sense RNA viruses in the family Flaviviridae. Dengue viruses are transmitted through the bites of the mosquito *Aedes aegypti*. It causes a wide clinical spectrum of

disease that includes self-limited classic dengue fever, severe dengue haemorrhagic fever (DHF) with haemorrhagic manifestations, and dengue shock syndrome (DSS) with increased vascular permeability. Classic dengue fever is an acute, self-limiting illness characterized by fever, headache, bone pain, myalgia, rash, prostration, leukopenia and thrombocytopenia. Symptoms of DSS/DHF are cold and sweaty skin,

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gastrointestinal bleeding, and spontaneous bleeding from gums and nose. DHF/DSS has served as a useful classification of severe dengue to aid in disease identification for treatment, epidemiologic surveillance, and studies of dengue pathogenesis. However, as dengue spreads into new regions worldwide, atypical manifestations, geographic and age-related differences have necessitated a new classification. The revised classification by World Health Organization (WHO) has grouped dengue syndrome into two broad categories like dengue fever with or without signs and severe dengue.¹ This dengue classification scheme would allow appropriate triage of patients, guide clinical management, and facilitate the assessment of potential interventions.² Currently, there is no therapeutics available beyond supportive care. According to WHO, DSS/DHF is the leading cause of childhood mortality in some Asian countries. In India alone there have been major outbreaks in dengue from time to time suggesting the importance of thorough knowledge of the epidemiology, pathogenesis and prompt treatment of the disease.¹ The aim of the study was to better understand the pathology of dengue haemorrhagic fever in fatal cases.

Materials and methods

This was a prospective autopsy study of fatal cases due to seropositive dengue haemorrhagic fever in a large tertiary care hospital from 01 Sep 09 to 31 Dec 2010. Rapid test kits from Bio Standard Diagnostics Pvt Ltd were used to detect dengue IgM and IgG antibodies and dengue NS-1 Ag in the serum of the patients. Dengue IgM and IgG capture ELISA kits were also used. There were 12 fatal cases due to DHF during the period and complete autopsies using midline incision were performed in only 06 cases. All 06 cases included in this study were male, as the hospital being the garrison hospital for the soldiers. Broad criteria for admission were fever with rashes of 3 days duration or complications such as bleeding diathesis, hypotension, thrombocytopenia, hepatosplenomegaly, polyserositis and any serving soldiers with serological positive tests. All autopsies were conducted within 24 h of death. A meticulous external as well as internal gross examination was carried out. Multiple sections from brain, liver, spleen, lungs, heart, kidneys and adrenal glands were collected for light microscopy studies and stained by standard methods. In addition, clinical examination, biochemical, haematological, serological investigations and radiological findings were analyzed and compared with post-mortem findings.

Results

There were a total of 1032 serologically positive dengue patients admitted in this hospital from 01 Sep 09 to 31 Dec 2010; out of which 12 patients died. Complete autopsies were performed in 06 cases; all of these were male, between 29 and 47 years old and had rapidly progressive clinical deterioration. All the six were admitted with fever, chills and bodyache of 02–08 days duration. During hospital stay, they had haemorrhagic manifestations, thrombocytopenia, coagulopathy, hypotension and multi-organ failure in due course of

progression of disease. The condition of patients suddenly deteriorated after fever of 2–7 days duration and died within 24 h to 16 days of admission. Investigations revealed thrombocytopenia, high bilirubin level, deranged liver enzymes and progressive increase in blood urea level in all cases. A drop in the platelet count to below 1,00,000 per mm³ was found between third and eighth day of illness. All patients were treated with IV fluid, platelet infusion, antibiotics and other supportive therapies. Three patients had both dengue IgM and IgG antibodies and two patients had only IgG dengue antibodies elevated. IgG was detected elevated on fourth day onwards while IgM was found raised on seventh day onwards. One patient was found positive for NS-1 Ag on third day and subsequently IgM antibodies were also detected after 6 days in this case. Majority of the patients had evidence of overt bleeding. The clinical profile and investigations are summarized in Table 1.

Pleural and peritoneal effusions were noted universally on autopsy. In two cases pericardial effusion was also noted. Internal haemorrhage was also seen in the form of pulmonary alveolar haemorrhage, gastrointestinal tract (GIT) mucosal haemorrhage and in one case perirenal haemorrhage. Brain was oedematous in all cases and in one case petechial haemorrhagic spots were present in cerebrum. Tiny haemorrhagic spots on pericardium and heart in two cases (Fig. 1) and outer surface of both kidneys and mucosal surface of urinary bladder (Fig. 2) in one case were also noted. Hepatosplenomegaly was present universally. The gross autopsy findings have been shown in Table 1.

Microscopic examinations revealed pulmonary oedema with micro haemorrhages (Fig. 3), vascular congestion, features of acute tubular necrosis and cerebral oedema in majority of cases. Myocardial lymphocytic infiltration was noted in three cases (Fig. 4). Liver injury of varying degree was seen in all cases (Fig. 5). The microscopic findings are summarized in Table 1.

Discussion

Our data showed that all six patients were admitted with c/o fever, chills, rigors and bodyache and during hospitalization they had haemorrhagic manifestations leading to hypovolaemia, hypotension and shock. In DHF, the haemorrhagic manifestations usually appear by third day ranging from scattered petechiae over the trunk, limbs and axillae to frank bleeding from GIT or as haematuria.³ The cause of bleeding in DHF appears to be due to thrombocytopenia. However, in this study, one of the patients had upper GIT bleed without significant thrombocytopenia, suggesting some other mechanisms like platelet dysfunction or micro vascular injury as documented in literature could be cause of haemorrhage.⁴ Even, Lum et al reported that the platelet counts are not predictive of bleeding.⁵ One patient was admitted with altered sensorium and autopsy revealed only cerebral oedema. Encephalopathy in this case could be attributed to renal impairment or metabolic alteration such as liver failure, electrolyte imbalance and hypotension.

Dengue serology was positive in all cases. IgG was detected in five cases on fourth day onwards indicating severe

Table 1 – Antemortem, autopsy gross and histopathological findings in DHF fatalities.

Case no	Antemortem findings	Investigations	Autopsy gross findings	Histopathological findings
All cases	Fever	Positive dengue serology	Serous effusions, pul oedema, mucosal congestion of GIT, visceral congestion, hepatomegaly, cerebral oedema	Blood vessels dilated & congested, acute tubular necrosis
Case 1	- Jaundice, petechial spots, hepatomegaly, upper GI bleed and hypotension - Chronic alcoholic	Hb-5.5 g/dl, platelet-130,000/mm ³ , haematocrit-16, B. urea-175 mg/dl, S. creatinine-7.8 mg/dl, S. bilirubin-28 mg/dl, AST-121 IU/L, ALT-34 IU/L	Focal haemorrhage with consolidation of lungs, haemorrhagic spots over pericardium, epicardium, GIT mucosa and outer surface of kidneys. Liver: bile stained	Lungs: micro haemorrhages, vascular congestion, oedema & polymorphonuclear infiltrate. Liver: centrilobular necrosis, fatty changes, micro haemorrhages
Case 2	- Chills & rigors, periorbital puffiness myalgia, bloody diarrhoea, haematemesis & hypotension	Hb-8.2 g/dl TLC-1500/mm ³ , platelet-35,000/mm ³ haematocrit-25, B. urea-30 mg/dl, S. creatinine-1.7 mg/dl, S. bilirubin 3.3 mg/dl, AST-489 IU/L, ALT-111 IU/L	Rashes over extremities. Lungs: focal areas of consolidation	Lung: oedema & polymorphonuclear cells infiltrate. Heart: lymphocytic myocarditis. Liver: mononuclear portal tract infiltration & spotty necrosis
Case 3	- Breathlessness, altered sensorium pneumonia - Old case of chronic renal failure	Hb-7 g/dl, platelet-75,000/mm ³ , haematocrit-21, B. urea-323 mg/dl, S. creatinine-13.4 mg/dl, S. bilirubin-0.4 mg/dl, AST-77 IU/L, ALT-35 IU/L	Lungs: focal areas of consolidation	Lungs: micro haemorrhages and oedema. Liver: mononuclear portal tract infiltration
Case 4	- Chills, loose stools hypotension, GIT haemorrhage - Boil over nape of neck	Hb-17.1 g/dl, TLC-2000/mm ³ platelet-38,000/mm ³ , B. urea-88 mg/dl, S. creatinine-1.5 mg/dl, S. bilirubin-1.4 mg/dl, AST-118 IU/L, ALT-124 IU/L	Cellulitis nape of neck, Lungs: focal areas of consolidation. Hepatosplenomegaly	Lungs: micro haemorrhages and pul oedema. Liver: mononuclear portal tract infiltration
Case 5	- Bodyache, loose stools hypotension, oliguria, dyspnoea	Hb-13.2 gmdl, TLC-39400/mm ³ , platelet-72,000/mm ³ , B. urea-115 mg/dl, S. creatinine-3.4 mg/dl, S. bilirubin-2.8 mg/dl, AST-890 IU/L, ALT-917 IU/L	Stomach: mucosal haemorrhagic spots. Peritoneal blood clots, peri-renal haematoma hepatosplenomegaly	Lungs: patchy areas of bronchopneumonia, oedema and haemorrhage. Heart: lymphocytic myocarditis. Liver: spotty necrosis and fatty changes
Case 6	- Chills, bodyache, loose stools rashes, icterus, altered sensorium - Known case of diabetes mellitus and hypertension	Hb-9.4 mg/dl, platelet-30,000/mm ³ , haematocrit-23, B. urea-106 mg/dl, S. creatinine-1.6 mg/dl, S. bilirubin-1.6 mg/dl, AST-131 IU/L, ALT-171 IU/L	Skin: petechial haemorrhage. Mild ventricular hypertrophy. GIT and urinary bladder: mucosal haemorrhagic spots	Lungs: bronchopneumonia and oedema. Liver: central necrosis and fatty changes. Kidneys: micro haemorrhages, congestion and diabetic nephropathy

Fig. 1 – Gross photograph of heart showing petechial haemorrhagic spots over epicardium.

Fig. 3 – Microphotograph from a case of DHF showing pulmonary haemorrhage (100X, H&E).

secondary infection in most of these fatal cases. IgM was detected in four cases on seventh day onwards relatively in the late stage of disease process. In one case NS-1 Ag was tested on third day and it was found positive, an early marker for dengue. Subsequently, this case was also found positive for IgM. Other laboratory investigations reveal that thrombocytopaenia was a constant finding in DHF. Haemoconcentration is an important feature of DHF which is due to leakage of plasma, however, this could not be appreciated in this study, may be due to early start of management.

All six cases showed derangement of liver function tests indicating 100% liver involvement in DHF. It is noteworthy that one of our patients with DHF presented with features of liver failure like viral hepatitis with very high S. bilirubin (28 mg/dl). In other cases S. bilirubin was mild to moderately increased. Variable elevated levels of serum aminotransferases also suggested varying degree of injury to liver in all cases. In 4 cases, the histological findings in the liver were similar to those observed in infection due to known hepatotropic viruses and varied from hepatic necrosis to a pauci-

cellular acute hepatitis. The biochemical pattern of liver injury in patients with DHF is also similar to that observed among patients with acute viral hepatitis—marked elevation of serum aminotransferases. In view of this biochemical pattern, it is possible to confuse liver involvement in DHF with typical acute viral hepatitis. However, the presence of thrombocytopaenia and persistence of fever after the appearance of jaundice should help to make a diagnosis of DHF. The severity of liver injury is unlikely to be a pointer to the diagnosis since liver disease can be severe even in DHF.^{6,7} In this study, serological markers of hepatitis viruses had been tested and found negative in all cases. Blood urea nitrogen was elevated at the terminal stage of shock in all cases. Hypoalbuminaemia, and moderately elevated serum aminotransferases and blood urea nitrogen levels have been documented.^{8,9}

All the patients had effusions in the serous cavities, either haemorrhagic or straw coloured. This is due to increased vascular permeability causing selective plasma leakage. Based on high ascitic fluid protein concentration, this finding has

Fig. 2 – Gross photograph of urinary system showing petechial haemorrhagic spots over kidneys surface and mucosal surface of urinary bladder.

Fig. 4 – Microphotograph from a case of DHF showing mixed inflammatory cells predominantly lymphocytes infiltrating into the myocardium (200X, H&E).

Fig. 5 – Microphotograph from a case of DHF showing enlarged portal tract with mononuclear infiltration and focal necrotic changes in surrounding hepatocytes (200X, H&E).

been attributed to excessive leakage of plasma. Plasma leakage is caused by increased capillary permeability. Other common post-mortem gross findings are petechial haemorrhages in the skin, pericardial, pleural surfaces and external surface of kidneys, mucosal surface of urinary bladder and vascular congestion in almost all viscerae. Intracranial haemorrhage was seen in only one case, contrary to common findings, described by other authors.¹⁰

The histological changes were nonspecific and included haemorrhage, perivascular oedema and vascular congestion but no vasculitic or endothelial lesions. Acute tubular necrosis was seen in all cases. This could be due to shock, developed due to plasma leakage leading to hypovolaemia and hypotension. Three of our patients also showed features of lymphocytic myocarditis, indicating multi-organ involvement. This has also been described in literature.¹¹

Iatrogenic complications such as pneumonia and sepsis have been noted in this study, contributing to fatality. A very important observation in this series is that four out of six patients had some pre-morbid problems namely chronic alcoholism, diabetes mellitus, leprosy and chronic kidney disease, that might have contributed to final outcome of these patients.

Pathogenesis of DHF is not fully understood. It is hypothesized that there is enhancement of virus multiplication in macrophages by heterotypic antibodies which were formed during previous dengue infection. de Macedo et al in their series concluded that fatal dengue haemorrhagic fever is associated with acute, severe liver damage due primarily to massive direct infection of hepatocytes and Kupffer cells with minimal cytokine response.¹² Serotype cross-reactive antibodies and mediators from serotype cross-reactive dengue-specific T cells have been implicated in the pathogenesis. A complex interaction between virus, host immune response and endothelial cells likely impacts the barrier integrity and functions of endothelial cells leading to plasma leakage.¹³ Abnormal immune response of the host after dengue infection may also account for the progression of DHF.¹⁴ The major pathophysiological

abnormalities caused by DHF include the rapid onset of plasma leakage, altered haemostasis, and damage to the liver, resulting in severe fluid losses and bleeding.

We compared this series with a previous one from the same hospital pertaining to 1996–97 epidemic in which out of 160 cases of dengue, 7 had died and 4 underwent autopsy. Three out of the four had massive haemorrhages including intracranial and intraocular bleed in one. None of them had any significant effusion though frank pleural haemorrhage was present in one. Severe hepatitis was not there in any of the four though focal coagulative necrosis was present microscopically.¹⁵ Findings suggest that the natural history of dengue syndrome is community specific and is still evolving for Delhi region probably linked to the immune status of the community. In the previous study, the time from diagnosis to death ranged from 1 day to 5 days, whereas in this study the time from diagnosis to death ranged from 2 days to 15 days. This could be due to change in the treatment protocols. In the previous study, fresh whole blood transfusion was the important part of the management which was not easily available during the epidemic. In the recent outbreak, better platelet management was the key point of treatment protocol in DHF. In addition, majority of our patients had premorbid chronic diseases which had significant effect in progression of disease to fatal outcome.

Take home messages from this study is that all patients with fever/generalized weakness during outbreak of dengue should be investigated for dengue infection. Same way, a viral hepatitis case should also be investigated for dengue especially during epidemic. Secondly, a case of DHF with pre-morbid problem should be treated promptly.

Intellectual contribution

Study concept: Brig MM Arora, Col KR Rathi.

Drafting and manuscript revision: Col KR Rathi, Brig MM Arora, Col K Sahai, Col S Tripathi, Wg Cdr SP Singh, Lt Col DK Raman, Surg Cdr KB Anand.

Statistical analysis: Col KR Rathi.

Study supervision: Col KR Rathi, Brig MM Arora.

Conflicts of interest

All authors have none to declare.

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