

# A COMPARISON OF THE PATTERN OF LIVER INVOLVEMENT IN DENGUE HEMORRHAGIC FEVER WITH CLASSIC DENGUE FEVER

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**Abstract.** The impact of dengue on liver function was studied on fifty serologically confirmed dengue cases admitted to Hospital Universiti Kebangsaan Malaysia (HUKM). Twenty-five of these patients had classic dengue fever (DF) and 25 had grade 1 or 2 dengue hemorrhagic fever (DHF). There were more (60%) DHF patients with hepatomegaly compared to DF (40%) but the difference was not statistically significant. Analysis of the liver profile showed that liver dysfunction was commoner in DHF compared to DF, indicating that the degree of liver impairment may be related to the severity of DHF. Hyperbilirubinemia was noted in 3 (12%) DHF and 2 (8%) DF patients. The mean (range) serum bilirubin was higher in DHF [14.2(5-50)  $\mu\text{mol/l}$ ] compared to DF [10.9(5-30)  $\mu\text{mol/l}$ ] ( $p > 0.05$ ). Elevated levels of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were observed more frequently in DHF (20 and 12 patients respectively) compared to DF (16 and 8 patients respectively). Nine (36%) DHF and 6 (24%) DF patients had concomitant elevation of ALT and ALP levels. The mean (range) serum ALT levels were 109.3(23-325) U/l in DHF and 90.8(13-352) U/l in DF ( $p > 0.05$ ). The mean (range) serum ALP levels were 102.2(15-319) U/l in DHF and 93.3(34-258) U/l in DF ( $p > 0.05$ ). The ALT and ALP levels were significantly higher in DHF patients with spontaneous bleeding than those without bleeding ( $p < 0.05$ ). None of the patients developed fulminant hepatitis. The immunoregulatory cells, which include the T (CD3), B (CD 19), CD4, CD8, CD5 and natural killer (NK) cells were significantly lower in DHF compared to DF patients ( $p < 0.05$ ). However, the reduction in these cell counts did not correlate with the liver dysfunction seen in DHF patients. In conclusion, hepatomegaly and liver dysfunction were commoner in DHF compared to DF.

## INTRODUCTION

Dengue fever is caused by a mosquito-borne dengue virus types 1 to 4, and is endemic over large areas of the tropics and subtropics, especially in Southeast Asia (Halstead, 1980). Classic dengue fever (DF) is an acute, self-limiting illness characterized by fever, headache, bone pain, myalgia, rash, prostration, leukopenia and thrombocytopenia. Although DF can be quite debilitating, unlike dengue hemorrhagic fever (DHF) its outcome is seldom fatal. The major pathophysiologic hallmark that determines disease severity and distinguishes DHF from DF is plasma leakage due to an increase in vascular permeability. Hypovolemic shock occurs subsequent to critical plasma loss and if accompanied by massive bleeding confers a grave prognosis.

Unusual clinical manifestations of dengue fever

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have become more common in the last few years (George *et al*, 1988; Rosen and Khin, 1989). Although the liver is not a major target organ, pathologic findings including centrilobular necrosis, fatty change, Kupffer cell hyperplasia, acidophilic bodies, and monocyte infiltration of the portal tracts have been reported in patients with and/or dengue shock syndrome (DSS) (Bhamarapravati *et al*, 1967; Burke, 1968). With such involvement, it would be expected that liver function tests would be abnormal. However, liver function abnormalities and associated pathologic findings are seldom mentioned in dengue literature. Liver involvement in dengue infection has been reported to be mild and manifest by raised liver enzymes. Of late there have been reports of fulminant hepatitis with high mortality in-patients with dengue infection (George, 1987; Suvatte *et al*, 1990). Innis *et al* (1990) reviewed the clinical course and liver histopathology of 19 fatal cases of dengue infection. In this study acute liver failure had been identified as a cause of death in DHF by dengue virus types 1 to 3. The mode of liver injury whether a direct effect of the virus replication or a consequence of host response to infection could not be inferred.

Activation of immunoregulatory T lympho-

cyte subsets has been observed in dengue viral infection, being more evident in DHF than in DF (Kurane *et al*, 1991; Sarasombath *et al*, 1988). Activated lymphocyte subsets have been shown to be responsible for the severe thrombocytopenia and plasma leakage in DHF and thus these cells may also be involved in the mechanism of liver dysfunction seen in DHF.

In view of the emerging trend in dengue infection, we have undertaken a review on fifty dengue patients admitted to the medical wards of Hospital Universiti Kebangsaan Malaysia (HUKM). The purpose of this study was to examine the extent of the liver involvement in dengue infection in particular DHF, and to determine whether there is any association between the immunoregulatory cell counts that reflect the activated cellular immune status and the degree of liver dysfunction in DHF.

#### PATIENTS AND METHODS

Fifty patients (31 males and 19 females) with a mean  $\pm$  SD age of  $25 \pm 9.37$  years (range 16-48) with DF and  $28 \pm 8.91$  years (range 16-50) with DHF were included in this study. The age, sex and ethnic distribution were comparable between the two study groups. All patients showed typical clinical symptoms and signs of dengue fever and all were serologically positive for dengue IgM ELISA. They were admitted approximately five days after the onset of fever. Twenty-five of these patients had classical dengue fever (DF) and 25 were in grade 1 or 2 DHF, according to World Health Organization criteria (Moren, 1982). Patients were excluded from this study if they were pregnant, immunocompromised, if they had co-existing immunological or hematological disorders, had received hepatotoxic or myelotoxic drugs, or had just been transfused with blood or blood products. Fourteen (56%) DF and 21 (84%) DHF patients had episodes of spontaneous bleeding from the skin, gums, nasal cavity or respiratory tract. On admission blood was obtained for full blood count, blood urea and electrolytes, liver profile, coagulation profile and enumeration of lymphocyte subsets. The samples were processed within six hours after blood taking. The immunophenotypes of the lymphocytes were determined by flow cytometry. The blood samples were processed by "lysed whole blood" method and then lymphocyte subsets were analyzed on a FACScan (Becton Dickinson Immunocytometry Systems BHD) flow cytometer.

Hepatitis markers, including hepatitis surface antigen (HBs Ag), IgM antibodies to hepatitis A and C virus (IgM anti-HAV and IgM anti-HCV) were assayed in 12 cases using an enzyme immunoassay (EIA) in which acute hepatitis viral infection were suspected clinically.

#### Statistical analysis

Chi-square test and Mann Whitney U test were applied in comparing the clinical and laboratory characteristics between the two groups of patients. The relationship between the lymphocyte subsets and the liver biochemical tests was assessed by Spearman's correlation analysis. A p-value of less than 0.05 was taken as significant.

### RESULTS

#### Liver profile

Hepatomegaly (liver span ranged from 14 to 18 cm subcostally) was observed in 10 (20%) patients with DF and 15 (30%) patients with DHF. The enlarged liver was tender in one DF patient and 8 DHF patients. There were more DHF patients with hepatomegaly compared to DF but the difference was not statistically significant. The mean total protein and serum albumin in DHF were similar to DF patients. Hyperbilirubinemia was noted in 3 (12%) DHF and 2 (8%) DF patients. The mean serum bilirubin, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels was higher in DHF ( $p > 0.05$ ) (Table 1). Elevated levels of serum ALT and ALP were observed more frequently in DHF (20 and 12 patients respectively) compared to DF (16 and 8 patients respectively). Nine (36%) DHF and 6 (24%) DF patients had concomitant elevation of ALT and ALP levels. Four (16%) DHF and 3 (12%) DF patients had serum ALT levels of more than 200 U/l (Fig 1). The serum ALP levels were above 200 U/l in only 3 (12%) DHF and 2 (8%) DF patients (Fig 2). The patients with 5-fold or more elevations in serum transaminase levels were tested negative for HBs Ag, IgM anti-HAV and IgM anti-HCV. In patients with DHF and episodes of bleeding the mean serum ALT levels ( $118.2 \pm 18.12$ ) and ALP levels ( $116.12 \pm 18.42$ ) were significantly higher than those without hemorrhagic manifestations (ALT =  $62.25 \pm 12.96$ , ALP =  $28.25 \pm 7.4$ ;  $p < 0.05$ ).

#### The cellular immune status

The mean percentage of T (CD3) cells was

**Table 1**  
Comparison of the liver function test between DHF and DF patients.

Parameters	DHF	DF
Protein (g/l)		
Mean	70	71.6
Range	59-90	60-86
Albumin (g/l)		
Mean	42.4	41.8
Range	33-50	36-51
Bilirubin(μmol/l)		
Mean	14.2	10.9
Range	5-50	5-30
ALT (U/l)		
Mean	109.3	90.7
Range	23-325	13-352
ALP (U/l)		
Mean	102.2	93.2
Range	15-319	24-249

**Table 2**  
Distribution of the lymphocyte subsets in the peripheral blood of DHF and DF patients.

Lymphocyte subsets	DHF	DF	Normal adults
CD3 cell (%)			
Mean	22.28	46.1	67.5
Range	1-83	0.62-78	50.8-84.2
CD19 cell (%)			
Mean	9.0	17.96	12.4
Range	1-45	6-33	5-23.2
CD4 cell (%)			
Mean	14.52	26.96	35.5
Range	1-52	11-45	20.3-50.7
CD8 cell (%)			
Mean	16.12	34.96	36.8
Range	1-66	11-67	20.2-53.5
NK cell (%)			
Mean	15.0	12.6	17.9
Range	1-85	2-43	5.8-36.9
CD5 cell (%)			
Mean	24.4	53.2	Not available
Range	1-64	18-82	

The reference values for normal Malaysian adults are based on a previous study by Choong *et al* (1995).

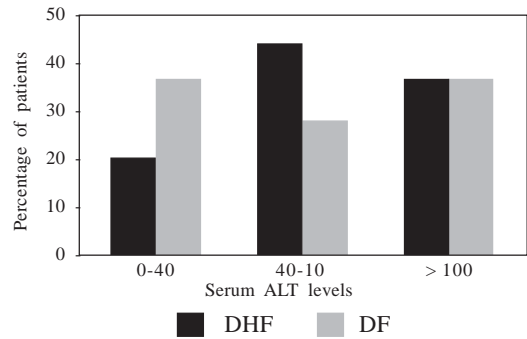


Fig 1 – The percentage of DHF and DF patients with normal and raised serum ALT (U/l) levels.

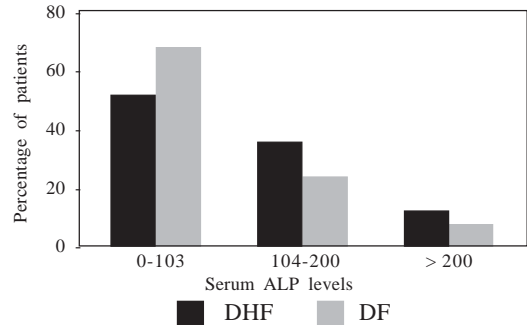


Fig 2 – The percentage of DHF and DF patients with normal and raised serum ALP (U/l) levels.

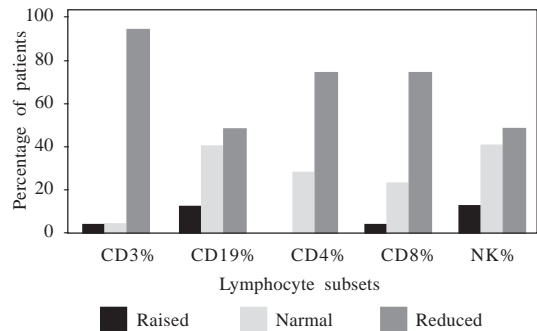


Fig 3–Comparison of the percentage of the lymphocyte subsets between DHF patients and normal adults.

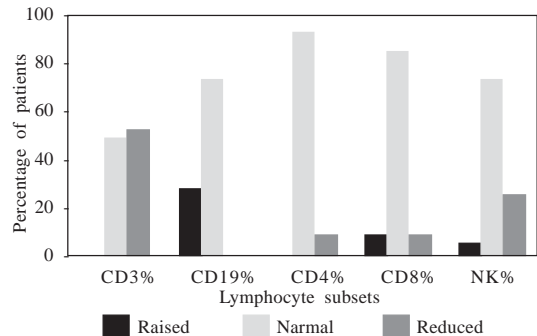


Fig 4–Comparison of the percentage of the lymphocyte subsets between DF patients and normal adults.

significantly lower in DHF compared to DF patients. Similarly, the mean percentage of B (CD19), CD4, CD8 and CD5 cells were also significantly lower in DHF patients compared to DF patients and controls ( $p < 0.05$ ) (Table 2). When compared with healthy Malaysian adults (Choong *et al.*, 1995), approximately 90% of the DHF patients had low CD3 cells, 70% had low CD4 and CD8 cells and 50% had low CD19 and NK cells (Fig 3). There was a significant correlation between the platelet counts and the CD3, CD4, CD5 and CD8 cells in DHF patients ( $r = 0.5, 0.47, 0.45$  and  $0.44$ ;  $p < 0.05$ ). However we failed to demonstrate a significant correlation between the lymphocyte subsets platelet count with the serum bilirubin, ALT or ALP levels in DHF patients ( $p > 0.05$ ).

## DISCUSSION

The results of the present study show that many patients with dengue viral infection had some degree of liver involvement as indicated by the abnormal liver function test results, especially in the serum transaminase levels. Histologic features characteristic of viral hepatitis has been observed in liver biopsies performed on DHF cases (Burke, 1968). These findings suggest that the liver may be involved in dengue viral infection particularly in DHF because of monocyte mobility and a broad immune response (Pang, 1983; Cornain *et al.*, 1987). The immunopathogenesis of DHF/dengue shock syndrome (DSS) differs from classic DF. It has been hypothesized that the pathophysiology of DHF is due to host immune response to dengue viral infection - the immune enhancement theory (Halstead and Rourke, 1977). The present study confirmed the previously reported significant decrease in T, CD4 and CD8 cells in DHF and demonstrated that these lymphocyte subsets were of some value in distinguishing DHF from DF during the acute febrile stage of the disease. Marked activation of immunoregulatory T lymphocyte subsets may contribute to the severe complications seen in DHF/DSS, including fulminant hepatitis. It should be noted that hepatomegaly and liver dysfunction were commoner and more severe in DHF compared to DF as has been demonstrated in the present study. The higher serum bilirubin, ALT and ALP levels in DHF compared to DF patients in this study suggest that activated cellular immune system may be involved in the mechanism of liver dysfunction in DHF. Although this study confirmed that T lymphocytes

are activated *in vivo* in response to dengue viral infection and the level of activation is higher in DHF than in DF patients, there was no significant correlation between the T lymphocyte subset count and the serum ALT or ALP levels among the DHF cases. This unexpected finding may be attributed to several factors including firstly, the changes in the T lymphocyte count and the levels of the liver enzymes do not occur simultaneously, *ie* the reduction of the T lymphocyte subsets begins as early as 3 days after the onset of fever whereas the transaminase levels usually increase to maximum levels 9 days after the onset of fever and gradually decreased to normal levels within 2 weeks, and secondly, the number of DHF patients in the study was too small.

Hepatomegaly was observed in up to 90% of Thai children and 60% of adults (Moren, 1982). Similar to this observation, hepatomegaly was seen in 15 (60%) DHF and 10 (40%) DF patients that we studied. Although hepatomegaly is common in DHF, it is not always associated with abnormal liver function tests. Among the 145 Thai children with DHF studied by Nimmannitya (1987), 98% had hepatomegaly, 74% had normal ALT levels, 18% had mildly elevated ALT levels (between 35-100 U/l) and 8% had ALT levels higher than 100 U/l. In the present study, 20% of the DHF patients had normal ALT, 44% had mildly elevated ALT levels and 36% had ALT levels higher than 100 U/l (16% had ALT levels above 200 U/l). Although the DHF cases in the present study were of comparable grades with those studied by Nimmannitya (1987), the greater liver function impairment in our study might be related to several factors such as the age of the patients, differences of dengue strains and virulence, and variation in the host immune system. Nimmannitya (1987) noted that the mean ALT levels were significantly different in grade 2 or 4 DHF cases, indicating that the liver function derangement is related to the severity of dengue viral infection. In agreement with the above study, the mean ALT levels in the DHF cases in the present study who were in grade 1 or 2 were only twice the normal level, and were not significantly different from those in DF patients and controls. However, the finding that DHF patients in the present study with spontaneous hemorrhage had significantly higher ALT and ALP levels than those without hemorrhage suggests that DHF patients who bled had more severe hepatocellular damage.

In conclusion, the liver is commonly involved in dengue viral infection, thus in endemic or epi-

demic areas; dengue fever should be included in the differential diagnosis of acute hepatitis. Hepatomegaly and liver impairment are commoner and more severe in DHF compared to DF suggesting that activated T lymphocytes subsets may cause the hepatocellular damage in DHF. The presence of spontaneous bleeding may be useful in predicting the extent of the hepatocellular damage observed in DHF.

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