LIVER FUNCTION TEST RESULTS AND OUTCOMES IN CHILDREN WITH ACUTE LIVER FAILURE DUE TO DENGUE INFECTION

Voranush Chongsrisawat[1], Yanee Hutagalung[2] and Yong Poovorawan[1]

[1]Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok; [2]Glaxo SmithKline, Thailand

Abstract. This retrospective study compared the liver function test results and outcomes between children with acute liver failure (ALF) due to dengue hemorrhagic fever (DHF) and due to other causes. We retrospectively reviewed patients less than 15 years old with a diagnosis of ALF admitted to 13 participating centers from different parts of Thailand for the years 2000 and 2001, and those admitted to King Chulalongkorn Memorial Hospital for the year 1997 to 2004. The diagnosis of ALF was based on prothrombin time (PT) prolongation to greater than 2 times the normal control value and the presence of encephalopathy without pre-existing liver disease. The patients were divided into 2 groups: group I (*n*=16) had DHF with ALF and group II (*n*=37) had ALF due to other causes. DHF patients had AST levels significantly higher than ALT levels. The mortality rate in group I (50%) was lower than in group II (72.9%), although the difference was not statistically significant. The non-DHF patients who died had a significantly longer duration of jaundice before the onset of encephalopathy and a significantly higher PT ratio compared to survivors. There were no significant differences in the duration of jaundice before the onset of encephalopathy and liver function between dengue patients who died and those who survived.

INTRODUCTION

Dengue infection is prevalent in tropical countries. It has been estimated that at least 2.5 billion people worldwide live in areas where there is a significant risk of infection from the dengue virus (WHO, 1999). Estimates suggest that annually over 50 million cases of dengue infection and about 400,000 cases of dengue hemorrhagic fever (DHF) occur in Asian countries with a case fatality rate of less than 5% (WHO, 1999; Deen *et al*, 2006). Of those with DHF, at least 90% are children younger than 15 years old (WHO, 1999).

Infection with dengue virus can cause a spectrum of illnesses including asymptomatic, fever and relatively mild disease, known as classic dengue fever (DF), a more severe form known as DHF and less frequently acute hepatitis with liver failure and encephalopathy. Acute liver failure (ALF) caused by dengue virus has been reported, mostly in case reports and small case series (Alvarez and Ramirez-Ronda *et al*, 1985; George *et al*, 1988; Lum *et al*, 1993). The unusual clinical forms of this disease are frequently associated with more serious states, and they often result from multifactorial con-

Correspondence: Professor Yong Poovorawan, Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Rama IV Road, Patumwan, Bangkok 10330, Thailand.
Tel: 66 (0) 2256 4909; Fax: 66 (0) 2256 4929
E-mail: yong.p@chula.ac.th

ditions, such as the use of hepatotoxic drugs, as well as the direct effect of the dengue virus (Nimmannitya *et al*, 1987; George *et al*, 1988; Lum *et al*, 1993).

The management of ALF includes treating the specific cause and supporting multiple organ system failure. Frequently associated disorders that necessitate prompt recognition and treatment include cerebral edema, coagulation abnormalities, hypoglycemia, and infection. Use of novel treatment alternatives, such as liver assist devices and hepatocyte transplantation, are still in the experimental stage. Although liver transplantation is an established mode of treatment in irreversible ALF, thus far there have been no reports regarding this treatment in ALF caused by dengue virus.

This study compared liver function test results and clinical outcomes in children with ALF due to dengue infection and ALF due to other etiologies.

MATERIALS AND METHODS

We conducted a retrospective review of patients less than 15 years old diagnosed with ALF admitted to 13 participating centers from different parts of Thailand during 2000 and 2001 and those admitted to King Chulalongkorn Memorial Hospital from 1997 to 2004. A number of these subjects were included in a study of the etiology of ALF (Poovorawan *et al*, 2006). This study was approved by the ethics committee of the Faculty of Medicine, Chulalongkorn University. Written informed consent was received from the parents or custodians of each of the subjects.

ALF was characterized as having clinical or laboratory evidence of liver failure (patient prothrombin time/laboratory prothrombin time for the day, so called prothrombin time ratio; the cut-off level was 2) complicated by encephalopathy in patients without a previous history of liver disease, with onset of encephalopathy ≤ 4 weeks after the onset of symptoms. Encephalopathy was defined following the standard classification of severity adapted for children (Devictor *et al*, 1992): grade 1, child is confused and has mood changes; grade 2, child is drowsy and displays inappropriate behavior; grade 3, child is stuporous but obeys simple commands; grade 4a, child is comatose but arousable by simple commands; grade 4b child is in a deep coma and does not respond to any stimuli. During the 6 month period prior to the study, none of the subjects had: exposure to anesthetic drugs, blood transfusion, intravenous drug use or surgery.

The patients were divided into 2 groups: group I (n=16) those with ALF due to DHF (n=4 and n=12 for those with DHF grades I/II and III/IV, respectively) and group II (n=37), those with ALF due to other causes (5 had ischemic hepatitis, 3 had Wilson's disease, 2 had acetaminophen overdose, 2 had lymphoma, 2 had acute hepatitis B infection, 1 had varicella infection, 1 had hemophagocytic syndrome, 1 had mushroom poisoning, 1 had Reye syndrome, and 19 had an unknown etiology). Patients with an unknown etiology for their ALF had negative serologic results for hepatitis A, B, C, cytomegalovirus, and Epstein-Barr virus. Blood from dengue patients was collected during the febrile period. The diagnosis of dengue infection was made by clinical examination and serological testing using the enzyme-linked immunosorbent assay (ELISA) method. Dengue patients were classified into different groups based on clinical severity: DHF grades I/ II (DHF without shock) and grades III/ IV (DHF with shock) according to WHO criteria (WHO, 1999). Liver function testing evaluated peak total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and prothrombin time

(PT) ratio; these were compared between the two groups. Data is expressed as mean± standard error of mean. Differences between the groups were analyzed by chi-square or *t*-test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

The children in group I (M:F=6:10) tended to be older than those in group II (M:F=16:21) (age 8.18±1.21 years and 5.94±0.74 years, p=0.11, respectively). The presence of jaundice did not significantly differ between the two groups (8 of 16 in group I and 26 of 37 in group II; p=0.16). Non-dengue patients had significantly higher TB levels and PT ratios (Table 1). Dengue patients had AST levels significantly higher than ALT levels (7,524.19±1,328.95 and 3,049.69±586.37 U/l, respectively; p=0.01). There were no significant differences in liver function among dengue patients with varying severities (Table 2).

The mortality rate was higher in non-dengue patients (72.9%) than in dengue patients (50%), but the difference was not statistically significant (p=0.1). Liver transplantation was performed on only one patient in group II without success due to irreversible brain damage. The mortality rate in patients with DHF without shock was not significantly different from those with shock (25% *vs* 58.3%, p=0.57). The age differences between the dengue patients who died and those who survived was not statistically significant (8.74±1.05 years and 7.62±2.26 years; p=0.66, respectively).

Non-dengue patients who died had sig-

Table 1
Comparison of liver function tests between patients with and without dengue infection.

Liver function tests	Group		*p*-value
	Dengue (*n*=16)	Non-dengue (*n*=37)	
Total bilirubin (mg/dl)	8.95 ± 2.84	20.30 ± 3.05	0.01
Direct bilirubin (mg/dl)	5.79 ± 2.01	11.64 ± 1.95	0.08
ALT (U/l)	3,049.69 ± 586.37	4,082.62 ± 909.97	0.34
AST (U/l)	7,524.19 ± 1,328.95	5,711.78 ± 1,298.67	0.41
Prothrombin time ratio	4.51 ± 0.67	7.48 ± 0.96	0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Data expressed as mean±standard error of mean.

Table 2
Comparison of liver function tests among patients with dengue infection.

Liver function tests	Grading		*p*-value
	DHF I/II (*n*=4)	DHF III/IV (*n*=12)	
Total bilirubin (mg/dl)	17.30 ± 9.42	6.17 ± 1.95	0.33
Direct bilirubin (mg/dl)	11.55 ± 6.92	3.87 ± 1.29	0.35
ALT (U/l)	2,135.75 ± 657.85	3,354.33 ± 743.00	0.39
AST (U/l)	8,409.00 ± 2,777.35	7,229.25 ± 1,574.25	0.72
Prothrombin time ratio	5.23 ± 1.45	4.27 ± 0.78	0.56

Table 3
Comparison of liver function tests and outcomes among patients with dengue infection.

Liver function tests	Outcome		*p*-value
	Died (*n*=8)	Survived (*n*=8)	
Total bilirubin (mg/dl)	10.79±5.36	7.12±2.22	0.54
Direct bilirubin (mg/dl)	7.63±3.87	3.94±1.18	0.39
ALT (U/l)	2,962.25±574.55	3,137.13±1,068.31	0.89
AST (U/l)	7,495.75±1,767.76	7,552.63±2,108.05	0.98
Prothrombin time ratio	5.54±1.07	3.49±0.71	0.14

nificantly longer durations of jaundice before the onset of encephalopathy (5.72±1.14 *vs* 1.63±0.42 days, respectively, p=0.003) and had significantly higher PT ratios (8.66±1.21 *vs* 4.28±0.67, respectively, p=0.003) when compared with survivors. There were no significant differences in duration of jaundice before the onset of encephalopathy between dengue patients who died and survived (Table 3).

DISCUSSION

Acute liver failure is a grave complication of acute hepatic illness resulting from various causes. Worldwide, the most frequent causes of ALF are acute viral hepatitis and drug-induced hepatocellular injury. In this study, hepatitis B virus (HBV) was the cause of ALF in only 2 of 37 cases. The lower prevalence of HBV infection is a result of the program for universal HBV vaccination (Chongsrisawat *et al*, 2006). Our findings are in agreement with a study from Taiwan where HBV is rarely the cause of fulminant hepatitis in children above 1 year old after the universal vaccination program (Chen *et al*, 2004). None of the patients in this study were seropositive for hepatitis A virus (HAV). Improved hygiene standards and socioeconomic conditions have led to a reduction in exposure to HAV in childhood.

Severe dengue infections may give rise to many complications, such as liver failure, disseminated intravascular coagulation, encephalopathy, myocarditis, acute renal failure, and hemolytic uremic syndrome (WHO, 1999). ALF following dengue infection has been reported in many countries in both children and adults (Alvarez and Ramirez-Ronda *et al*, 1985; Kuo *et al*, 1992; Lum *et al*, 1993; Miagostovich *et al*, 1997; Nguyen *et al*, 1997; Couvelard *et al*, 1999; Sirivichayakul *et al*, 2000; Lawn *et al*, 2003). Nonfatal Reye's syndrome confirmed by liver biopsy has also been described as a complication of DHF (Terry *et al*, 1980; Kho *et al*, 1981). Liver involvement is common in all forms of dengue infection (Kho *et al*, 1981). Its severity varies with the overall severity of the dengue infection. In agreement with previous studies (Kuo *et al*, 1992), the dengue patients in this study had higher AST levels (7,524.19±1,328.95 U/l) than ALT levels (3,049.69±586.37 U/l). This may be due to the release of AST from myocyte damage in dengue infection. For most cases, hepatic involvement prolongs the clinical course of dengue infection, but does not constitute a sign of worse prognosis (Kuo *et al*, 1992; Nguyen *et al*, 1997; Mohan *et al*, 2000). Previous studies have found AST and ALT levels are significantly higher, and globulins significantly lower in patients with the more severe grades of DHF (Mohan *et al*, 2000). Our study found that liver function was not

significantly different between dengue patients with and without shock. This finding supports that liver failure attributable to dengue virus does not result from ischemia with grade III/IV DHF [dengue shock syndrome (DSS)]. It has been suggested that infection with dengue serotypes 3 and 4 produces higher liver enzyme levels compared with infections due to the other two serotypes (Kalayanarooj and Nimmannitya, 2000). We did not perform serotyping, therefore we cannot evaluate this finding. Another limitation of this study was the lack of data regarding acetaminophen usage, which may influence the course of ALF in addition to the dengue virus infection.

Attention has been drawn to the involvement of the liver in DHF by the detection of viral antigens in hepatocytes and Kupffer cells and by virus recovery from liver biopsies (Rosen *et al*, 1989; Hall *et al*, 1991; Guzman and Kouri *et al*, 2002). Cell degeneration in centrilobular and midzonal areas of the liver with Councilman bodies resembling those found in yellow fever cases has been described (Hall *et al*, 1991; Huerre *et al*, 2001). Such bodies are believed to be hepatocytes that have undergone apoptosis in response to dengue infection (Lacronique *et al*, 1996; Marianneau *et al*, 1998; Couvelard *et al*, 1999). Infected hepatocytes appear to be subjected to apoptosis *in vitro*, and this may be a key element in the pathophysiology of liver failure associated with DHF (Despres *et al*, 1996; Marianneau *et al*, 1997).

Despite aggressive therapy, the prognosis for ALF due to dengue infection was as poor as those attributable to other etiologies. The case fatality rate for dengue patients with ALF in this study was 50%, which is much higher than the overall fatality rate of less than 0.3% from dengue infection in Thailand (WHO, 1999). From our study, the mortality rate for ALF following DHF does not depend on the severity of dengue infection since it is comparable between patients without shock and DSS patients. Current prognostic criteria to predict the outcome of ALF, based on analysis of a case series of patients of all ages referred to King's College Hospital (O'Grady *et al*, 1989) are age, etiology, duration of jaundice before encephalopathy, serum bilirubin and prothrombin time. From our study, none of those factors predicted the outcome in liver failure due to dengue infection. Despite the importance of the liver as a target organ in the pathogenesis of DHF, the nature of the interaction between dengue viruses and hepatocytes remains largely unexplored.

In conclusion, liver failure is a rare but important complication of DHF. DHF should be included in the differential diagnosis of infective hepatitis in individuals who have been in an endemic area. One characteristic of hepatic involvement with dengue infection is a greater elevation in AST than ALT levels. This information is useful to distinguish between liver failure caused by dengue infection and that caused by other etiologies, in which this phenomenon is infrequently observed. Duration of jaundice before the onset of hepatic encephalopathy and PT ratio were prognostic indicators in ALF from other causes but not with dengue infection.

ACKNOWLEDGEMENTS

We would like to thank The Thailand Research Fund, (the Commission on Higher Education) and Center of Excellence in Clinical Virology, Chulalongkorn University, for their generous support. We are grateful to the University Hospital and Regional Hospital Center staff who have contributed to this most interesting data and for their patient care in this study. This study was partially supported by a grant from GlaxoSmithKline Biologicals, Rixensart, Belgium.

REFERENCES

Alvarez ME, Ramirez-Ronda CH. Dengue and hepatic failure. *Am J Med* 1985; 79:670-4.

Chen HL, Chang CJ, Kong MS, *et al*. Fulminant hepatic failure in children in endemic area of hepatitis B virus infection: 15 years after universal hepatitis B vaccination. *Hepatology* 2004; 39: 58-63.

Chongsrisawat V, Yoocharoen P, Theamboonlers A, *et al*. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health* 2006; 11: 1496-502.

Couvelard A, Marianneau P, Bedel C, *et al*. Report of a fatal case of dengue infection with hepatitis: demonstration of dengue antigens in hepatocytes and liver apoptosis. *Hum Pathol* 1999; 30: 1106-10.

Deen JL, Harris E, Wills B, *et al*. The WHO dengue classification and case definitions: time for a reassessment. *Lancet* 2006; 368: 170-3.

Despres P, Flamand M, Ceccaldi PE, Deubel V. Human isolates of dengue type 1 virus induce apoptosis in mouse neuroblastoma cells. *J Virol* 1996; 70: 4090-6.

Devictor D, Desplanques L, Debray D, *et al*. Emergency liver transplantation for fulminant liver failure in infants and children. *Hepatology* 1992; 16: 1156-62.

George R, Liam CK, Chua CT, *et al*. Unusual clinical manifestations of dengue virus infection. *Southeast Asian J Trop Med Public Health* 1988; 19: 585-90.

Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis* 2002; 2: 33-42.

Hall WC, Crowell TP, Watts DM, *et al*. Demonstration of yellow fever and dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistological analysis. *Am J Trop Med Hyg* 1991; 45: 408-17.

Huerre MR, Lan NT, Marianneau P, *et al*. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Arch* 2001; 438: 107-15.

Kalayanarooj S, Nimmannitya S. Clinical and labarotory presentations of dengue patients with different serotypes. WHO SEARO Regional Publication, 2000; 24. [Cited 2008 Aug 30]. Available from: URL: http://www.searo.who.int/en/section10/section332/section522_2514.htm

Kho LK, Sumarmo, Wulur H, Jahja EC, Gubler DJ. Dengue hemorrhagic fever accompanied by encephalopathy in Jakarta. *Southeast Asian J Trop Med Public Health* 1981; 12: 83-6.

Kuo CH, Tai DI, Chang-Chien CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. *Am J Trop Med Hyg* 1992; 47: 265-70.

Lacronique V, Mignon A, Fabre M, *et al*. Bcl-2 protects from lethal hepatic apoptosis induced by an anti-Fas antibody in mice. *Nat Med* 1996; 2: 80-6.

Lawn SD, Tilley R, Lloyd G, *et al*. Dengue hemorrhagic fever with fulminant hepatic failure in an immigrant returning to Bangladesh. *Clin Infect Dis* 2003; 37: 1-4.

Lum LC, Lam SK, George R, Devi S. Fulminant hepatitis in dengue infection. *Southeast Asian J Trop Med Public Health* 1993; 24: 467-71.

Marianneau P, Cardona A, Edelman L, Deubel V, Despres P. Dengue virus replication in human hepatoma cells activates NF-kB which in turn induces apoptotic cell death. *J Virol* 1997; 71: 3244-9.

Marianneau P, Flamand M, Deubel V, Despres P. Apoptotic cell death in response to dengue virus infection: the pathogenesis of dengue haemorrhagic fever revisited. *Clin Diagn Virol* 1998; 10: 113-9.

Miagostovich MP, Ramos RG, Nicol AF, *et al*. Retrospective study on dengue fatal cases. *Clin Neuropathol* 1997; 16: 204-8.

Mohan B, Patwari AK, Anand VK. Hepatic dysfunction in childhood dengue infection. *J Trop Pediatr* 2000; 46: 40-3.

Nguyen TL, Nguyen TH, Tieu NT. The impact of dengue haemorrhagic fever on liver function. *Res Virol* 1997; 148: 273-7.

Nimmannitya S, Thisyakorn U, Hemsrichart V.

Dengue hemorrhagic fever with unusual manifestations. *Southeast Asian J Trop Med Public Health* 1987; 18: 398-406.

O'Grady JG, Alexander GLM, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* 1989; 97: 439-45.

Poovorawan Y, Hutagalung Y, Chongsrisawat V, Boudville I, Bock HL. Dengue virus infection: a major cause of acute hepatic failure in Thai children. *Ann Trop Paediatr* 2006; 26: 17-23.

Rosen L, Khin MM, UT. Recovery of virus from the liver of children with fatal dengue: reflections on the pathogenesis of the disease and its possible analogy with that of yellow fever. *Res Virol* 1989; 140: 351-60.

Sirivichayakul C, Sabcharoen A, Chanthavanich P, Pengsaa K, Chokejindachai W, Prarinyanupharb V. Dengue infection with unusual manifestations: a case report. *J Med Assoc Thai* 2000; 83: 325-9.

Terry SI, Golden MH, Hanchard B, Bain B. Adult Reye's syndrome after dengue. *Gut* 1980; 21: 436-8.

World Health Organisation. Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. WHO SEARO Regional Publication, 1999; 29. [Cited 2008 Aug 30]. Available from: URL: http://www.searo.who.int/en/Section10/Section332/Section554_2585.htm