

AN EVIDENCE OF INTESTINAL MUCOSAL INJURY IN DENGUE INFECTION

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Abstract. The aim of this study was to determine whether there is an association between dengue infection and intestinal mucosal injury. Serum levels of intestinal fatty acid binding protein (I-FABP) were used as a specific marker for mucosal injury. The diagnosis of all patients was serologically confirmed by anti-dengue IgG or IgM. Serum I-FABP levels of 120 patients were determined and compared to those of 25 controls using ELISA. In order to investigate serum I-FABP among dengue patients, they were categorized into 5 groups according to disease severity: dengue fever (DF), dengue hemorrhagic fever (DHF) grade I through DHF grade IV. The dengue patients had higher levels of serum I-FABP compared to 25 controls (408.0 ± 499.3 vs 124.72 ± 147.81 pg/ml, $p=0.006$). The patients with DHF grade IV had the highest levels of serum I-FABP, ALT, and AST compared to the other groups. However, there were no differences in serum I-FABP, ALT, and AST levels among patients with DF, DHF grade I, grade II, and grade III. Evidence of intestinal mucosal injury in patients with dengue infection was demonstrated. Patients with DHF grade IV had high serum I-FABP levels and had associated liver injury.

INTRODUCTION

Dengue infection is one of the most important mosquito-borne viral diseases in humans. It is characterized by fever, bleeding diathesis, and a tendency to develop a potentially fatal shock syndrome. The pathological process of dengue hemorrhagic fever is an acute increase in vascular permeability, resulting in hypovolemic shock (Pancharoen *et al*, 2002). Theoretically, during the course of the disease, intestinal mucosal perfusion is probably compromised due to increased capillary permeability (Malavige *et al*, 2004). However, the proof of intestinal mucosal injury in dengue infection has not been investigated. Since acute liver failure and encephalopathy are complications in severe dengue infection (Thisyakorn *et al*, 1999), together with the fact that organ failure can be induced by intestinal mucosal injury (Lieberman *et al*, 1998),

it is probable that intestinal ischemia or injury plays a pathophysiological role in dengue infection.

Intestinal fatty acid binding protein (I-FABP) is a 15 kDa protein that is uniquely located at the tips of intestinal mucosal villi. It constitutes approximately 2% to 3% of the protein of the enterocyte and is normally undetectable in the circulation (Kanda *et al*, 1996). Several studies have shown that serum I-FABP is a useful diagnostic marker for intestinal damage both *in vivo* and *in vitro* (Lieberman *et al*, 1997; Pelters *et al*, 2003; Niewold *et al*, 2004). The objectives of this study were to investigate whether there is an evidence of intestinal mucosal injury in dengue patients by determining a diagnostic serum marker, namely I-FABP, and to evaluate whether there is an association between the severity of dengue infection and serum I-FABP levels.

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MATERIALS AND METHODS

The protocol of the study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. All patients were in-

formed of the purpose of the study. Patients were recruited from King Chulalongkorn Hospital and three other provincial hospitals, Ayutthaya, Sakon Nakhon, and Uttaradit Hospitals, between January 2003 and January 2004. The diagnosis of dengue infection in each patient was serologically confirmed by anti-dengue IgM or IgG ELISA (NovaTec Immundiagnostica GmbH[†], Technologie and Waldpark, Dietzenbach, Germany). All of the patients were diagnosed and treated by responsible medical experts in infectious diseases.

The patients' sera were collected on the day of admission during the febrile stage of the disease. The control group was comprised of 25 healthy adults from the blood bank. Peripheral venous whole blood was drawn with a sterile syringe, transferred to a centrifuge tube, allowed to clot and then centrifuged at 4°C. The serum was stored at -70°C until assayed. All demographic and clinical data were extracted from the patients' files. The patients were categorized into 5 groups according to their disease severity (Pancharoen *et al*, 2002) (WHO classification): dengue fever or DF (fever without capillary leakage), dengue hemorrhagic fever or DHF grade I (a positive tourniquet test), DHF grade II (spontaneous bleeding), DHF grade III (circulatory failure with narrowing of the pulse pressure and a rapid and weak pulse), and DHF grade IV (profound shock with no detectable blood pressure or pulse). Serum I-FABP was determined using a commercially available ELISA kit (HK406, HBT, the Netherlands), based on the sandwich principle. The serum I-FABP levels are expressed as mean and SD in terms of pg/ml.

The analyses include a description of clinical data, a comparison of serum I-FABP levels between the patients and normal controls. The association between serum I-FABP and the disease's severity was analyzed using one-way ANOVA with post-hoc Tukey comparisons. Significant differences were established as $p < 0.05$. For all statistical analyses, either GraphPad Prism version 3.02 (GraphPad Software, California, USA) or SPSS software version 10.0 (SPSS, Chicago, IL) was used.

RESULTS

The diagnosis of dengue infection in 120 patients (70 boys and 50 girls) was serologically confirmed during the studied period. There was no difference in gender between the patients and the controls (12 males and 13 females). Patients with dengue infection had higher levels of serum I-FABP compared to normal controls (408.0 ± 499.3 pg/ml vs 124.72 ± 147.81 pg/ml, $p = 0.006$ using unpaired *t*-test). The demographic and clinical data of the dengue patients are shown in Table 1. The patients with DHF grade IV had the highest levels of serum I-FABP, ALT, and AST compared to the other groups (Fig 1). However, there were no differences in serum I-FABP, ALT, or AST levels in patients with DF, DHF grade I, grade II, or grade III.

DISCUSSION

Dengue infection is the most common mosquito-borne viral illness in humans. It is known to be caused by a single-stranded RNA virus in the family Flaviviridae. An estimated 2.5-3 bil-

Table 1
Demographic data, serum I-FABP, ALT, and AST levels in patients according to WHO classification.

	Control(N=25)	DF(N=30)	DHF I(N=27)	DHF II(N=23)	DHF III(N=26)	DHF IV(N=14)
Age (years)	-	10.43±2.94	10.68±2.32	11.19±2.44	9.32±3.79	9.05±4.35
Gender (M/F)	12/13	18/12	13/14	15/8	15/11	9/5
Serum I-FABP (pg/ml)	124.72±147.81	286.79±220.39	385.44±319.35	327.06±273.21	304.96±297.45	1,035.54±1,086.76*
ALT (IU/l)	-	49.53±32.55	66.70±47.89	97.00±85.14	158.50±325.00	4,230.75±6,399.92*
AST (IU/l)	-	93.73±65.55	136.15±84.89	225.91±171.62	437.15±993.91	3,520.92±6,268.49*

* $p < 0.05$ compared to the other groups

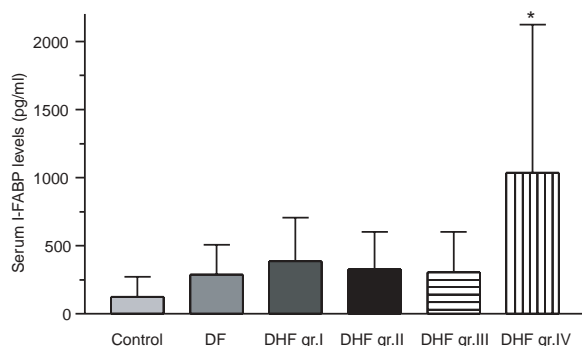


Fig 1–Serum I-FABP levels according to the severity of dengue infection. * $p < 0.05$ compared to the other groups.

lion people live in areas at risk for dengue infection. Each year, 20-100 million people are infected with DF, and DHF develops in 250,000-500,000 of these individuals. Approximately 24,000 deaths around the world are annually attributed to this disease (Malavige *et al*, 2004). The dengue virus has 4 closely related but distinct serotypes, DEN1-DEN4. It maintains an infection cycle that uses mosquitoes, mostly the *Aedes aegypti* mosquito, as vectors to human hosts and as sources of viral amplification (Pancharoen *et al*, 2002). The clinical presentation of dengue infection involves a wide spectrum of findings, from asymptomatic or mild self-limiting infection of dengue fever to potentially fatal hemorrhage and shock.

The main clinical picture of DHF is characterized by plasma leakage. This results from endothelial gaps in the peripheral vascular bed without necrotic or inflammatory changes in the endothelium leading to reduction in intravascular volume and tissue hypoperfusion. However, gastrointestinal manifestation in patients with dengue infection is uncommon. An acute abdomen may occur and its symptoms are thought to be caused by intraperitoneal bleeding or a mesenteric hematoma, but not by ischemic pain from the gastrointestinal tract (Wang *et al*, 1990; Pancharoen *et al*, 2001; Khanna *et al*, 2004). Theoretically, intestinal ischemia can happen in dengue patients. Evidence for intestinal mucosal injury or ischemia with dengue infection has not been characterized.

Our study clearly demonstrates that some extent of intestinal injury occurs in patients with dengue infection, as confirmed by elevated serum I-FABP levels compared to normal controls. Patients with DHF grade IV and acute liver injury had the highest levels of I-FABP reflecting intestinal injury. It has been shown that intestinal injury or ischemia induces systemic inflammatory response syndrome resulting in multi-system organ dysfunction, including the lungs, liver, and heart. It has been demonstrated that liver failure is induced by intestinal ischemia-reperfusion (Vejchapipat *et al*, 2001). Interactions between leukocytes and endothelial cells have been thought to be due to liver dysfunction during intestinal reperfusion (Horie *et al*, 1997). It is possible that acute liver injury in patients with DHF grade IV was secondary to intestinal injury. The significant increase in liver enzymes in DHF grade IV might have been due to either liver ischemia from circulatory collapse or dengue viral infection of the hepatocytes themselves. Liver injury could have been caused by the cumulative effect of the three mechanisms mentioned above. Reports of dengue patients developing acute liver failure (Lawn *et al*, 2003) and encephalopathy (Sirivichayakul *et al*, 2000) have also been demonstrated. There has been a suggestion that one should search for coinfection in cases with unusual manifestations of dengue infection (Pancharoen and Thisyakorn, 1998). If this is the case, it is possible that superimposed infection or multiple organ dysfunction in dengue patients is triggered by intestinal mucosal injury via bacterial translocation or a systemic inflammatory response.

Intestinal fatty acid binding proteins are 13-14 kDa intracellular proteins with a high degree of tissue specificity. I-FABP is exclusively localized in the epithelium cells in the small intestine. Many studies have shown that serum I-FABP is a diagnostic marker for intestinal damage with high accuracy (Lieberman *et al*, 1997; Pelters *et al*, 2003; Niewold *et al*, 2004). The molecular size of I-FABP is much smaller than albumin. Therefore, the rise in serum I-FABP levels in dengue patients in this study is not due to hemodilution caused by capillary leakage but rather the release of I-FABP from injured

enterocytes into the circulation. Although our results strongly suggest that intestinal injury occurs in patients with dengue infection, especially in severe cases, the study did not address the mechanisms of mucosal injury towards liver injury, the severity of dengue infection, or superimposed infection via bacterial translocation. Since inflammatory cytokines play critical roles in intestinal ischemia and systemic inflammatory response, further investigation of the interactions between dengue infection and intestinal ischemia may answer the exact role of mucosal injury and lead to a better therapeutic strategy in the future.

Evidence of intestinal mucosal injury in patients with dengue infection was demonstrated. Patients with DHF grade IV had high serum I-FABP levels and liver injury. Since mucosal injury is associated with a systemic inflammatory response, the progression of the disease, including acute liver injury, may be partially caused by mucosal injury. This increases our understanding into the pathophysiology of dengue infection.

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REFERENCES

- Horie Y, Wolf R, Granger DN. Role of nitric oxide in gut ischemia-reperfusion-induced hepatic microvascular dysfunction. *Am J Physiol* 1997; 273: G1007-13.
- Kanda T, Fujii H, Tani T, *et al.* Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology* 1996;110: 339-43.
- Khanna S, Vij JC, Kumar A, Singal D, Tandon R. Dengue fever is a differential diagnosis in patients with fever and abdominal pain in an endemic area. *Ann Trop Med Parasitol* 2004; 98: 757-60.
- 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: e1-4.
- Lieberman JM, Marks WH, Cohn S, *et al.* Organ failure, infection, and the systemic inflammatory response syndrome are associated with elevated levels of urinary intestinal fatty acid binding protein: study of 100 consecutive patients in a surgical intensive care unit. *J Trauma* 1998; 45: 900-6.
- Lieberman JM, Sacchetti J, Marks C, Marks WH. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery* 1997; 121: 335-42.
- Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. *Postgrad Med J* 2004; 80: 588-601.
- Niewold TA, Meinen M, van der Meulen J. Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia in pigs. *Res Vet Sci* 2004; 77: 89-91.
- Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C. Dengue infection: a global concern. *J Med Assoc Thai* 2002; 85: S25-33.
- Pancharoen C, Mekmullica J, Thisyakorn U. Primary dengue infection: what are the clinical distinctions from secondary infection? *Southeast Asian J Trop Med Public Health* 2001; 32: 476-80.
- Pancharoen C, Thisyakorn U. Coinfection in dengue patients. *Pediatr Infect Dis J* 1998; 17: 81-2.
- Pelsters MM, Namiot Z, Kisielewski W, *et al.* Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. *Clin Biochem* 2003; 36: 529-35.
- 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.
- Thisyakorn U, Thisyakorn C, Limpitikul W, Nisalak A. Dengue infection with central nervous system manifestations. *Southeast Asian J Trop Med Public Health* 1999; 30: 504-6.
- Vejchapipat P, Williams SR, Proctor E, Lauro V, Spitz L, Pierro A. Moderate hypothermia ameliorates liver energy failure after intestinal ischaemia-reperfusion in anaesthetised rats. *J Pediatr Surg* 2001; 36: 269-75.
- Wang JY, Tseng CC, Lee CS, Cheng KP. Clinical and upper gastroendoscopic features of patients with dengue virus infection. *J Gastroenterol Hepatol* 1990; 5: 664-8.