

# SERUM FERRITIN LEVELS IN CHILDREN WITH DENGUE INFECTION

Wathanee Chaiyaratana<sup>1</sup>, Ampaiwan Chuansumrit<sup>2</sup>,  
Kalayanee Atamasirikul<sup>3</sup> and Kanchana Tangnaratchakit<sup>2</sup>

<sup>1</sup>Research Center, <sup>2</sup>Department of Pediatrics, <sup>3</sup>Department of Pathology,  
Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

**Abstract.** The purpose of this study was to evaluate the levels of ferritin, an acute-phase reactant, in predicting the risk of dengue hemorrhagic fever (DHF) in patients with dengue infection. One hundred seventy-seven Thai children (100 males, 77 females) 4-16 years old (median age 11 years) with DF ( $n=44$ ) and DHF ( $n=133$ ) were enrolled in the study. All patients had serologic confirmation of dengue infection. Each had a venous blood sample drawn daily during hospitalization and at the outpatient clinic 2-4 weeks after discharge from the hospital, to determine serum ferritin levels. The median serum ferritin levels (ng/ml) in children with DHF (Day 2, 974; Day 3, 624; Day 4, 1,136; Day 5, 1,912; Day 6, 2,105; Day 7, 1,840; Day 8, 1,478 and Day 9, 1,144 of illness) were higher than those with DF (Day 2, 25.4; Day 3, 45.6; Day 4, 655; Day 5, 1,050; Day 6, 1,075; Day 7, 615; Day 8, 764 and Day 9, 600 of illness) with  $p$ -values of 0.013, 0.001 and 0.013 on Days 5, 6 and 7 of illness, respectively. A cutoff level of serum ferritin of 1,200 ng/ml was used to calculate sensitivity and specificity for DHF. The results reveal the sensitivities on Days 5, 6 and 7 of illness were 81.5, 84.4 and 89.9%, respectively, and the specificities were 42.4, 39.0 and 36.4%, respectively. High serum ferritin levels  $\geq 1,200$  ng/ml may be a predictor of dengue hemorrhagic fever.

## INTRODUCTION

Dengue infection, one of the most common mosquito-borne viral diseases of public health significance, is caused by any of four serotypes (DEN-1, DEN-2, DEN-3, or DEN-4). The clinical manifestations include asymptomatic infection, nonspecific fever, influenza-like symptoms known as dengue fever (DF) and the more severe manifestations of dengue hemorrhagic fever (DHF). DHF is comprised of 3 stages: febrile, toxic, and convalescent. The febrile stage lasts 2-7 days followed by an abrupt fall to a normal or subnormal tem-

perature. The toxic stage lasts 24-48 hours, with a final rapid clinical recovery without sequelae during the convalescent stage. The toxic stage is the most critical period. Shortly after the rapid drop in temperature, varying degrees of circulatory disturbance develop due to plasma leakage from increased vascular permeability.

Ferritin is an iron storage protein complex of isoferritins produced by the reticuloendothelial (RE) system. The RE system plays a critical role in iron metabolism by processing hemoglobin from senescent red blood cells. Acute inflammation and infection induce the blockade of iron release resulting in a decreased serum iron, a virulence factor for many microorganisms. Elevated levels of serum ferritin, an acute-phase reactant, reflect the clinical response to deprive microorganisms of serum iron (Worwood, 1990; Griffiths, 1991;

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Correspondence: Wathanee Chaiyaratana, Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

Tel: 66 (0) 2201-1618; Fax: 66 (0) 2201-1611

E-mail: rawcy@mahidol.ac.th

Wooldridge and Williams, 1993; Krol and Cunha, 2003). Elevated serum ferritin levels have been demonstrated in patients with West Nile encephalitis beyond those attributable to the acute-phase reaction of infection, correlating with severity of disease (Cunha *et al*, 2004). Since DHF is more severe than DF, the levels of serum ferritin in patients with DHF may be higher than those with DF.

In this study, we assessed serum ferritin levels during the entire clinical course, the febrile, toxic and convalescent stages, in children with dengue infection to predict the risk of developing DHF.

MATERIALS AND METHODS

Patients

One hundred seventy-seven Thai chil-

dren with dengue infection 4 to 16 years of age admitted to the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand from 2002 to 2005 were included in the study. All the patients survived. Ethical approval was obtained from the Faculty Ethics Committee, and informed consent was obtained from the parents. The details of the diagnosis, gender, type of dengue antibody response, median age and number of patients are shown in Table 1. Children had a venous blood sample drawn daily during hospitalization and at the outpatient clinic at 3-4 weeks after discharge from the hospital (Table 2).

D0 was the day of defervescence, when the temperature dropped below 37.5°C without a subsequent elevation. One day and two days before defervescence were designated

Table 1  
Demographic data of the studied patients.

Diagnosis	Number of patients					Median age (yrs) <sup>b</sup>
	Total	Male	Female	Primary <sup>a</sup>	Secondary <sup>a</sup>	
Dengue fever	44	26	18	2	42	10.7 (8.0-12.9)
DHF grade I	49	32	17	5	44	11.0 (8.7-13.0)
DHF grade II	62	33	29	3	59	12.2 (10.0-14.0)
DHF grades III and IV	22	9	13	0	22	10.1 (7.7-12.0)
Total	177	100	77	10	167	11.0 (9.0-13.0)

<sup>a</sup>type of dengue antibody response; <sup>b</sup>number in parentheses, interquartile range

Table 2  
Distribution of samples obtained from studied patients by day of illness after onset of fever.

Day of defervescence	D-4	D-3	D-2	D-1	D0	D+1	D+2	D+3		
Day of illness	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Follow-up	Total
DF	1	1	11	24	30	19	7	1	10	104
DHF grade I	-	1	8	24	41	29	7	-	14	124
DHF grade II	2	3	18	34	46	37	14	3	18	175
DHF grades III and IV	1	1	4	5	14	17	10	1	4	57
Total	4	6	41	87	131	102	38	5	46	460

as D-1, D-2, respectively, and so on. One day and two days after defervescence were designated as D+1 and D+2, respectively.

#### Diagnostic criteria

The clinical diagnosis of DHF is based on four major characteristic manifestations defined by the WHO (1997): 1) sustained high fever lasting 2 to 7 days; 2) a hemorrhagic tendency, such as a positive tourniquet test or petechiae; 3) thrombocytopenia (platelets  $\leq 100,000/\mu\text{l}$ ); and 4) evidence of plasma leakage manifested by hemoconcentration (an increase in hematocrit of  $\geq 20\%$ ) or a pleural effusion. The severity of DHF is categorized into four grades (WHO, 1997): grade I, without overt bleeding but having a positive tourniquet test; grade II, with clinical bleeding diathesis, such as epistaxis and ecchymosis; grade III, circulatory failure manifested by a rapid, weak pulse and narrowing pulse pressure or hypotension with the presence of cold, clammy skin and restlessness; and grade IV, profound shock in which pulse and blood pressure are not detected. In this study, patients without evidence of hemoconcentration had a right lateral decubitus chest radiograph taken 12-24 hours after defervescence. All patients with DF and DHF had serologic confirmation of dengue infection.

#### Serologic assay

Dengue-specific IgM and IgG antibodies were determined by capture ELISA technique. Dengue infection was defined as a primary infection when the ratio of IgM to IgG was  $>1.8:1$  and a ratio of  $<1.8:1$  was defined as a secondary infection.

#### Laboratory testing

A total of 460 serum specimens were included. The number of consecutive specimens from each patient was as follows: one ( $n=30$ ), two ( $n=58$ ), three ( $n=53$ ), four ( $n=26$ ), five ( $n=9$ ) and six ( $n=1$ ). A total of 436 specimens were collected from 167 patients with secondary infection (42 DF, 125 DHF) and 24

specimens were collected from 10 patients with primary infection (2 DF, 8 DHF). All serum specimens were aliquoted and stored at  $-70^{\circ}\text{C}$  until testing. Serum ferritin was determined by Vitros Ferritin Assay purchased from Ortho-Clinical Diagnostics Johnson & Johnson Company, UK.

#### Statistical analysis

The Mann-Whitney *U* test was used to calculate statistical significance for the differences in serum ferritin levels between patients with DF and DHF. A *p*-value  $<0.05$  was considered significant.

## RESULTS

A total of 177 patients were included in the study (Table 1). The median age of the patients was 11 years (interquartile range 9.0-13.0) and 56.5% were male. According to WHO criteria, 44 children were classified as having DF and 133 as having DHF (49 grade I, 62 grade II and 22 grades III and IV). Ten children (5.6%) were diagnosed with primary and 167 children (94.4%) with secondary dengue antibody response.

The median duration of the febrile stage was five days (minimum 1 day, maximum 10 days) with an interquartile range of 4 to 6 days. By analyzing the number of days in relation to defervescence with the day of illness after the onset of fever among 177 patients with dengue infection, results revealed that D-4 was the second day of illness (Day 2), D-3 was the third day (Day 3), D-2 was the fourth day (Day 4) and D-1 was the fifth day (Day 5) of illness with fever. D0, the day of defervescence, was the sixth day (Day 6) of illness. D+1, D+2, and D+3 were the seventh (Day 7), eighth day (Day 8) and ninth days (Day 9) of illness.

The median levels of serum ferritin among the patients with DHF were higher than those with DF with *p*-values of 0.013, 0.001 and 0.013 on Day 5 of illness (one day before defervescence), Day 6 of illness (day of defer-

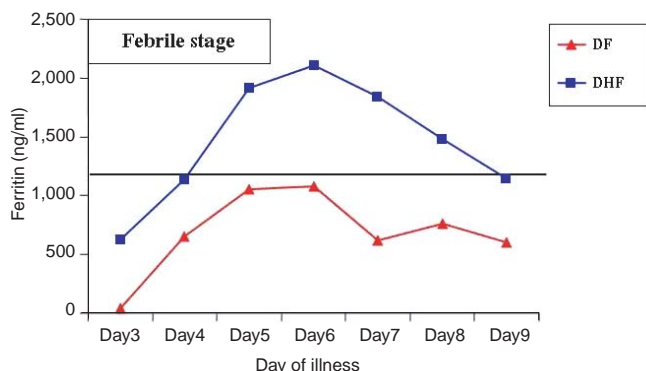


Fig 1—Comparison of median serum ferritin levels between patients with DF and DHF by day of illness after the onset of fever.

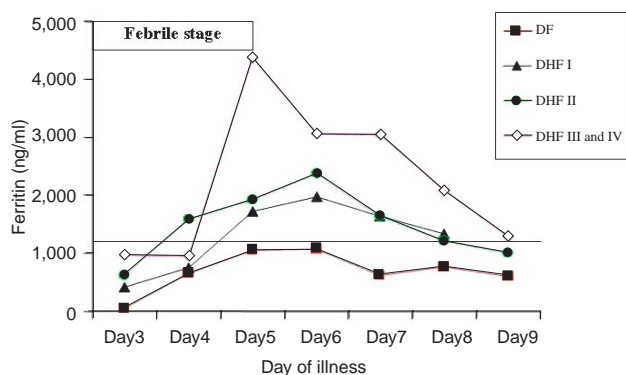


Fig 2—Comparison of median serum ferritin levels among patients with DF and DHF grades I, II, III and IV by day of illness after the onset of fever.

versence) and Day 7 of illness (one day after defervescence), respectively (Fig 1). The serum ferritin levels of patients with DF were less than 1,200 ng/ml during the entire course of illness, the febrile, toxic and convalescent stages, while the patients with DHF trended to have higher levels of serum ferritin during the febrile stages and the highest level was found on the day of defervescence. The median levels of serum ferritin on Days 5, 6 and 7 of illness were significantly higher in patients with DHF grades III and IV compared to those with DHF grades I, II and DF (Fig 2). However,

the levels of serum ferritin at follow-up 3-4 weeks after discharge from the hospital in both the patients with DF and DHF were similar, and in the normal range.

A cut-off level for serum ferritin of  $\geq 1,200$  ng/ml was used for predicting the clinical manifestation of DHF. The results for sensitivity during the Days 5, 6 and 7 of illness after the onset of fever were 81.5, 84.4 and 89.9%, respectively, while the specificities were 42.4, 39.0 and 36.4%, respectively.

### DISCUSSION

The designated dates of D-4, D-3, D-2, D-1, D0, D+1, D+2 and D+3 in relation to defervescence were essential for including patients with the same pathophysiological status in the same group since the febrile stage lasts 2 to 7 days. If the days of illness, such as Day 1 to Day 9, without relating to defervescence comparisons could not be made. For example, on Day 5 of illness, some patients had fever, but others patients had no fever. Converting the number of days in relation to defervescence to the days of illness starting from the onset of fever can be usefully applied to clinical practice.

Our data show that both patients with DF and DHF had higher serum ferritin levels during the febrile, toxic and convalescent stages than they did at follow-up. Patients with DHF had higher levels of serum ferritin than those with DF throughout the course of the illness. These findings are in concordance with the study of patients with West Nile encephalitis (Cunha *et al*, 2004). West Nile virus and dengue virus are in the same family of Flaviviridae. Those with DHF grades III and IV had higher

serum ferritin levels than those with DF and DHF grades I and II. This may be explained by the increased vascular permeability found in DHF, patients especially with DHF grades III and IV (Halstead, 1988).

The cut-off serum ferritin level of  $\geq 1,200$  ng/ml for predicting the progression to DHF has a high sensitivity but a rather low specificity. This may be limited by the small number of the patients who presented on Days 2 or 3 of illness, and in patients with DHF grades III and IV. The early diagnosis of DHF is helpful so appropriate patient care may be given to achieve a favorable outcome.

In conclusion, the use of a serum ferritin level  $\geq 1,200$  ng/ml should be considered as a tool to predict progression to DHF beginning on Day 4 of the illness and continuing into the convalescent stage.

## REFERENCES

- Cunha BA, Sachdev B, Canario D. Serum ferritin levels in West Nile encephalitis. *Clin Microbiol Infect* 2004; 10: 184-6.
- Griffiths E. Iron and bacterial virulence: a brief overview. *Biol Met* 1991; 4: 7-13.
- Halstead SB. The pathogenesis of dengue: challenges to molecular biology. *Science* 1988; 239: 476-81.
- Krol V, Cunha BA. Diagnostic significance of serum ferritin levels in infectious and non-infectious diseases. *Infect Dis Pract* 2003; 27: 199-200.
- Wooldrige KG, Williams PH. Iron uptake mechanisms of pathogenic bacteria. *Microbiol Rev* 1993; 12: 325-48.
- World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2<sup>nd</sup> ed. Geneva: WHO, 1997: 12-23.
- Worwood M. Ferritin. *Blood Rev* 1990; 4: 259-69.