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C-reactive protein in children with dengue fever in Vietnam

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ABSTRACT

Precise diagnosis of the severity of dengue fever (DF) and its timely treatment reduce its mortality, but the sensitivity of clinical presentations is low for the classification of severity. Research on biomarkers is ongoing, and some studies in adults have shown the utility of C reactive protein (CRP) in the early diagnosis of dengue severity. However, the role of CRP in children with DF is still unknown. The aim of this study was to determine the value of CRP in distinguishing between DF and severe DF. A cross-sectional study was conducted from May 2016 to April 2017 at Children's Hospital 1 in Ho Chi Minh City, Vietnam. Serum CRP was tested on days 4 and 5 of the disease. Laboratory tests for DF were based on either a positivity for non-structured glycoprotein-1 antigen (NS1Ag) or enzyme-linked immunosorbent assay (ELISA) for immunoglobulin (Ig) M in acute phase serum. Among 270 patients, 29.6% had DF, 26.7% had DF with warning signs, and 43.7% had severe DF. The CRP level was measured in 123 patients on day 4 and 147 patients on day 5 of the illness. The median CRP level for DF was 2.4 mg/L, for DF with warning signs was 6.7 mg/L, and for severe DF was 7.3 mg/L. The CRP level was higher on day 4 than on day 5. The CRP level showed a statistically significant difference between the group with circulatory dysfunctions (p=0.02) or liver dysfunction (p= 0.04) and the other patients. The CRP cut-off point on day 4 that distinguished DF and severe DF was 5.8 mg/L and the area under the receiver operating characteristic curve (AUC) was 0.89, with a sensitivity of 82.9% and specificity of 80%. The CRP concentration in the early stage of illness may, therefore, help to distinguish between DF and severe DF.

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INTRODUCTION

Dengue fever (DF) is an important worldwide health problem, especially in South-East Asia and the Western Pacific regions, where 70% of the population experiences the epidemiology of DF (WHO, 2009). The disease has no specific drugs, nor has a preventive vaccine been effective. Therefore, early diagnosis and prediction of severity are critical for physicians in patient screening and management. In recent years, the hypothesis of immunization in the pathophysiology of dengue has prompted some studies on dengue-related biomarkers, such as interleukin (IL) and tumor necrosis factor-alpha

(TNFα), as DF has been associated with an increase in TNF-α, IL-I, and IL-6, which are cytokines involved in acute inflammatory responses (Veerman *et al.*, 2001; Villar-Centeno *et al.*, 2013). However, the half-life of these cytokines is very short, so their clinical use in evaluating DF is difficult. However, these cytokines are contributors to increases in C-Reactive Protein (CRP) (Gewurz, 1982).

Clinically, CRP is often used to diagnose and monitor the response to treatment of infection (Jaye and Waites, 1997). In addition, CRP is also considered to be a biomarker in the prognosis of the pathologies of several diseases, such as sepsis, malaria, acute pancreatitis, and chronic renal failure. With regard to DF, A study on 191 adults with DF in Taiwan found that CRP levels of 24.2 mg/L had 70% sensitivity and 71.3% specificity in predicting severe DF in adult patients, especially in the first 3 days of the disease (Chen *et al.*, 2015).

In Vietnam, DF has a high incidence in children, and the number of severe pediatric cases admitted to hospital is high all year round. Early diagnosis and classification of severity are critical, but the clinical presentations have low sensitivity. The present study was conducted at a central specialized hospital to determine the value of CRP measurements in children for the differential diagnosis of DF severities.

MATERIALS AND METHODS

Study design and Ethics consideration

This cross-sectional study was conducted at Children's Hospital 1 in Ho Chi Minh City, Vietnam, between May 2016 and April 2017. The research project was approved by the Research Ethics Council of Children's Hospital 1. Patients participated in this study entirely voluntarily and written informed consent was obtained in each case.

Participants and Sampling

A total sampling technique was applied to select patients with DF admitted to the pediatric intensive care unit (ICU) and the Dengue department. The patients were recruited with the following inclusion criteria: i) a maximum age of 15 years; ii) a diagnosis of DF according to 2009 WHO classification; and iii) positivity for non-structured glycoprotein-1 antigen (NS1Ag) or an enzyme-linked immunosorbent assay (ELISA) confirmation of dengue immunoglobulin (Ig) M. Exclusion criteria were: i) DF suggestive of bacterial coinfection and use of antibiotics during treatment; ii) experience of inflammations or other infections; and iii) unwillingness to participate in the study. All children were diagnosed, managed, and monitored according to guidelines for diagnosis and treatment issued by WHO (2009) and updated by the Vietnam Minister of Health in 2011 (WHO, 2009). Each patient was categorized into one of three groups: mild DF (MDF), DF with warning signs (WDF), and severe DF (SDF).

Laboratory tests

CRP was tested on day 4 (if the children were admitted before or on day 4) and day 5 of the illness to determine the likelihood of progressing to severe DF. CRP was measured at the Biochemistry department using a spectrophotometry and potentiometry analyzer (Olympus AU 680B chemistry analyzer). Laboratory diagnosis of DF was based on either a positive NS1Ag reading or ELISA detection of IgM in acute-phase serum. NS1Ag was found by an immunochromatographic assay using the Dengue NS1 antigen test (Humasis Co., Ltd, Korea). IgM was assessed by an IgM-capture ELISA (NovaTec, USA).

Statistical analysis

Data were analyzed using SPSS 24.0. A p-value of less than 0.05 was considered statistically significant. Categorical variables were presented as n (%), using the chi-square test for determination of statistical significance; whereas numerical variables were presented as medians (interquartile range) because of a skewed distribution. The Mann-Whitney U test and Kruskal-Wallis H test were used to determine statistical significance. We also used the Receiver Operator Characteristics (ROC) curve to determine the importance and threshold value of CRP in predicting the severity of DF.

RESULTS AND DISCUSSION

Patient characteristics

The mean age of the 270 patients was 8.1$\pm$4.2 years. Of these 270 patients, 209 (77.4%) were at least 5 years old, 23 (8.5%) patients were overweight, 80 (29.6%) had MDF, 72 (26.7%) had WDF, and 118 (43.7%) had SDF.

CRP concentration in DF

The CRP level was measured in 123 patients on day 4 and in 147 patients on day 5 of the disease. The median CRP level was 5.5 (2.4–9.5) mg/L. In total, 23% of the patients had CRP levels $\geq$10 mg/L. The median CRP level in the MDF, WDF, and SDF cases were 2.4 (1.4–4.2), 6.7 (3.5–12.6), and 7.3 (3.6–13.7) mg/L, respectively. The CRP levels in the SDF and WDF groups were significantly different from the levels in the MDF group ($p<0.001$). The CRP levels in the 3 groups also differed significantly between day 4 and day 5 (Table 1).

Table 1: CRP levels by day of disease and severity in children with dengue fever

Day of disease	Total (N=270)	MDF (n=80)	WDF (n=72)	SDF (n=118)	P-value**
Day 4 (n = 123)	7.4 (3.7-14.8)	3.2 (2.0-4.4)	10.5 (7.3-17.8)	11.8 (5.6-21.9)	<0.001
Day 5 (n = 147)	3.6 (1.9-7.0)	2.1 (1.4-3.2)	3.4 (1.7-6.5)	5.5 (2.7-8.3)	<0.001
P-value*	<0.001	0.04	<0.001	<0.001	

Data are presented as median (interquartile); *Mann-Whitney U test of the median between day 4 and day 5; **Kruskal-Wallis H test of the medians between the MDF, WDF and SDF groups; CRP, C-Reactive protein; MDF, mild dengue fever; WDF, dengue fever with warning signs; SDF, severe dengue fever

The median CRP levels by age were 3.2 (1.0–7.3) mg/L for patients <5 years old and 6.0 (2.8–9.8) mg/L for patients ≥5 years old. The CRP level in cases ≥5 years old was significantly higher (p<0.001). In the overweight children, 23 had median CRP levels of 9.3 (4.2–31.0) mg/L, which was significantly higher than the normal status, where the median CRP value was 4.9 (2.3–8.5) mg/L. The median CRP level showed no significant difference according to organ failure (Table 2).

CRP threshold value for the prediction of DF

We used the ROC curve to determine the meaning and threshold value of CRP in predicting the severity of DF. For the 270 cases, the AUC was 0.78 and the CRP level was 4.3 mg/L, with a sensitivity of 70% and specificity of 78%. The AUC on day 4 was 0.89, and the CRP level was 5.8 mg/L, with a sensitivity of 82.9% and specificity of 80%. The AUC on day 5 was 0.73, and the CRP level was 2.9 mg/L, with a sensitivity of 70% and specificity of 72% (Figure 1).

CRP concentration

The median of CRP was 5.5 (2.4–9.5) mg/L. The rate of DF patients with CRP <10 mg/L was 77%. Other studies did not show high CRP values in DF. For example, T-S Ho in Taiwan reported a CRP concentration of 6±11 mg/L (Ho *et al.*, 2013). Kutsuna in Venezuela also reported CRP in dairy products at 5.1 mg/L (Ohmagari *et al.*, 2014). Other studies have reported higher levels of CRP, such as those reported for patients in Canada (12.1±6.8 mg/L) (Conroy *et al.*, 2015; Salazar *et al.*, 2014) , in Brazil (14.5±5.4 mg/L) (Bethell *et al.*, 1998), and in Taiwan (19.3 mg/L) (Chen *et al.*, 2015), but the overall value is still low.

These previous values were obtained from adults at the acute stage of the disease. DF is a viral infection, and CRP levels in viral infections are often less than <10 mg/L, as with other viral infections (Ansar and Ghosh, 2013). CRP levels in DF are not high and vary between studies, which may reflect the heterogeneity in the age range and the time at which CRP is measured. The results of previous studies are summarized in Table 3.

Association between CRP levels and dengue severity

Serum CRP concentration can increase according to the severity of the disease. In the present study, the values for CRP differed between the MDF (2.4 mg/L, range 1.4–4.2), WDF (6.7 mg/L, range 3.5–12.6), and SDF (7.3 mg/L, range 3.6–13.7) groups, and this difference was statistically significant between the SDF group and the WDF group when compared to the MDF group (p<0.001). Table 3 shows that the CRP levels in DF are not particularly high, although a difference is evident between severe and non-severe disease. This indicates that the CRP value, a factor that reflects the inflammation of the body, is likely a component of the mechanism of immune disease in dengue.

Association between CRP levels and day of illness

A difference in CRP values was also evident between days 4 and 5 of the DF illness in terms of general illness, as well as in terms of the severity of the disease. The CRP values for dengue were higher at day 4 (CRP = 7.4 mg/L) than at day 5 (CRP = 3.6 mg/L). When considering severity, the CRP level on days 4 and 5 were 3.2 mg/L and 2.1 mg/L, respectively, in the MDF group, 10.5 mg/L and 3.4 mg/L in the WDF group, and 11.8 mg/L and 5.5 mg/L in the SDF group (p<0.05). Eppy *et al.* (2016) in Indonesia, reported that CRP levels were higher on day 3 than on day 5 of the disease and that CRP levels were also higher in a group with plasma leakage than in a group without leakage. The authors argued that the reactions of the cytokine "cyclone" occur during the onset of febrile illness (from day 1 to day 3) and that these reactions are resolved during the severe period (day 4–6) with the expression of loss plasma at that stage (Eppy *et al.*, 2016). Similarly, studies by Chen et al. on adults also reported that the CRP values were higher when collected on day 3 than on day 5 Chen *et al.* (2015). These results are shown in Table 4 and compared with the present study findings.

Table 2: CRP levels by age,body weight, and organ failure in children with dengue fever

Organ failure	N (%)	CRP concentration (mg/l)*	P-value
Age group			<0.001†
<5 years	61 (22.6)	3.2 (1.0-7.3)	
≥5 years	209 (77.4)	6.0 (2.8-9.8)	
Body weight**			<0.001†
Normal nutritional status	247 (91.5)	9.3 (4.2-31.0)	
Overweight	23 (8.5)	4.9 (2.3-8.5)	
Circulatory dysfunction			0.002†
Yes	114 (42.2)	6.6 (3.4-12.3)	
No	156 (57.8)	4.2 (2.0-8.1)	
Liver dysfunction			0.040†
Yes	21 (7.8)	10.5 (4.2-16.8)	
No	249 (92.2)	6.0 (2.5-10.0)	
Respiratory dysfunction			0.128†
Yes	76 (28.1)	5.9 (3.2-10.7)	
No	194 (71.9)	4.8 (2.1-9.2)	
Severe hemorrhage			0.620†
Yes	19 (7.0)	4.4 (1.1-18.0)	
No	251 (93.0)	5.6 (2.4-9.3)	
Number of organ failure (organ(s)			0.775‡
0	147 (54.4)	5.7 (2.1-10.3)	
1	50 (18.5)	7.6 (3.7-10.8)	
2	58 (21.5)	6.7 (2.8-14.1)	
3	15 (5.6)	6.7 (4.2-9.8)	

*CPR concentration is presented as median (interquartile); **Overweight was based on a body mass index >23 kg/m^2; †Mann-Whitney U test of the median; ‡Kruskal-Wallis H test of the median; CRP, C-Reactive Protein

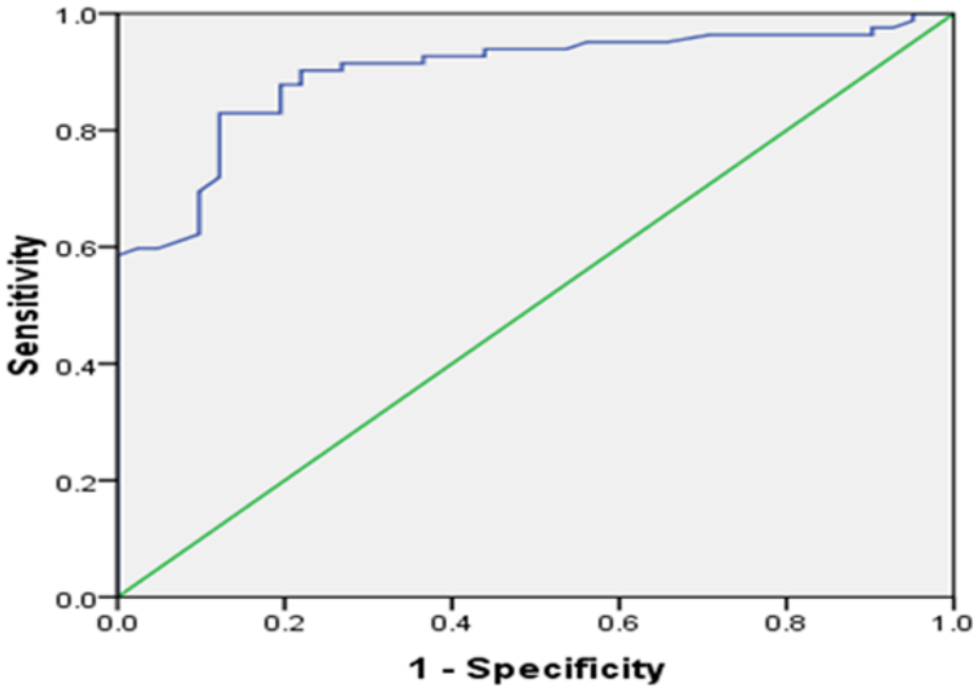

Figure 1: CRP threshold values for the prediction of dengue fever (DF) in children on day 4 of illness

Table 3: CRP concentrations reported in previous studies

Study	Number of participants	CRP concentration (mg/l)*	
		MDF/WDF	SDF
Malaysia (Bethell *et al.*, 1998)	443	4.0 (3.0-4.2)	6 (2.0-29.5)
Colombia (Simmons *et al.*, 2015)	245	4.2 (1.2-10.3)	12.2 (10.6-15.9)
Taiwan Chen *et al.* (2015)	191	9.8 (0.5-215.5)	30.7 (1.2-205.5)
Canada (Conroy *et al.*, 2015)	126	18.7 (9.2-41.3)	21 (6.4-41.4)
Venezuela (Ohmagari *et al.*, 2014)	70	10.5±8.3	11.7±1.9
This study	270	5.5 (2.3-9.5)	7.3 (3.6 – 13.7)

*CRP concentration was presented as mean ± SD or median (interquartile); CRP, C-Reactive protein; MDF, mild dengue fever; WDF, dengue fever with warning signs; SDF, severe dengue fever

Table 4: CRP level with day of illness

	DF with plasma leakage		DF without plasma leakage	
	Day 3	Day 5	Day 3	Day 5
Indonesia (Eppy *et al.*, 2016)	10 (4.3–36.5)	5 (2–20.1)	6.8 (3–21.6)	2.9 (0.1–9.9)
Taiwan (Chen *et al.*, 2015)	36.2 (3.3–205.5)	29 (6.9–144)	14.4 (0.6–69)	8 (0.5–215.5)
This study		5.5 (2.7–8.3)		2.1 (1.4–3.2)

*CRP concentration was presented as median (interquartile; : CRP, C-Reactive protein; DF, dengue fever

In our pediatric patients, we saw that the daily CRP levels in patients with dengue hemorrhagic fever were also consistent with the kinetic dynamics during inflammation, as described during the progression of infectious diseases. Serum CRP levels rapidly increased over 5 mg/L within 6 hours and peaked after about 48 hours, but the CRP levels remained elevated for 24–48 hours after the onset of infection. The serum half-life of CRP is about 19 hours, and CRP undergoes a 50% reduction in daily concentrations after acute inflammatory stimulation has been resolved, and unless there is new infection, the CRP levels return to normal at 5–7 days after inflammation, despite the fact that the disease is ongoing (Hoffmann, 1999).

Association between CRP concentration and age

Age-related CRP levels showed statistically significant differences between children <5 years of age (3.2 mg/L, range 1.0–7.3) and children >5 years of age (6.0 mg/L, range 2.8–9.8). This is similar to the findings of Winston et al in normal subjects, where CRP levels were found to increase with age, with levels about 1 mg/L in young adults (25–30 years old) and about 2 mg/L in the elderly (70–74 years old) (Ho *et al.*, 2013).

Association between CRP concentration and nutritional status

Serum CRP levels were also affected by overweight. In the present study, serum CRP levels were higher in overweight children, at 9.3 (4.2–31.0) mg/L, than in normal-weight children, at 4.9 (2.3 to 8.5) mg/L ($p<0.001$).

Association between CRP levels and organ failure

We also examined the increases in CRP levels in DF patients in terms of organ damage due to DF effects on circulation, respiration, liver function, and coagulation. We found that CRP levels were higher when the disease was worse, but the difference was only statistically significant in the groups with circulatory failure or liver failure. Specifically, patients with circulatory failure had CRP levels of 6.6 (3.49–12.39) mg/L, while patients without circulatory failure had CRP levels of 4.2 (2.0–8.1) mg/L. Patients with hepatic impairment had CRP levels of 10.5 (4.2–16.8) mg/L, while patients without liver impairment had CRP values of less than 6.2 (2.6–10.4) mg/L. In cases of circulatory failure, the increased production of CRP is influenced by some cytokines, such as IL-1, IL-6, and TNF-α, that are produced during inflammation. These cytokines are thought to play a role in increasing blood vessel permeability, thereby causing plasma leakage that results in circulatory failure, which is a major mech-

anism of DF (Eppy *et al.*, 2016). Regarding the value of CRP in liver failure, the definition of liver failure in DF is a liver enzyme level of 1,000 UI/L or higher. In this study, patients with severe hepatic impairment were in the shock group, and CRP values were higher in patients with hepatic insufficiency. Other research has found that other tissues of the body, such as vascular smooth muscle cells, endothelial cells, renal fat cells, alveoli macrophages, and atherosclerotic lesions, also participate in CRP synthesis (Salazar *et al.*, 2014). A recent hypothesis proposed to explain the pathogenesis of DF involves a cross-reaction of NS1 antibodies with endothelial cells and induction of injury through activation of endothelial cell inflammation (Screaton *et al.*, 2015; Simmons *et al.*, 2015), but this hypothesis still needs to be verified.

CRP level in the prognosis of severe DF

This study used the ROC curve to determine the CRP significance and threshold values for predicting the degree of DF in children from DF with warning signs to severe DF. Analysis on day 4 of the disease revealed a CRP value of 5.8 mg/L, and patients with a high risk of DF at the warning level had a sensitivity and specificity of 82.9% and 80%, respectively. The AUC of 0.89 shows that CRP levels predict the disease prognosis at a fairly good level. Chen et al. showed that the threshold value of CRP in adult dengue patients was 24.2 mg/L for 70% sensitivity and 71.3% specificity for the prognosis of DF severity, with an AUC of 0.7 (Chen *et al.*, 2015). The threshold value was lower in our study than in the study by Chen *et al.* (2015) because of age differences, as CRP increases with age. Moreover, in adults, a number of underlying chronic conditions may increase CRP levels. In addition, the date of specimen collection can change the CRP value, and most of the samples collected by Chen *et al.* (2015). Were taken during the first 3 days of the disease.

CONCLUSION

In children, a CRP concentration with a cut-off point of 5.8 mg/L in the early stage of illness may help to distinguish between DF and severe DF.

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Conflicts of Interests

The authors have no conflicts of interests to declare.

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