SERUM FERRITIN AND C-REACTIVE PROTEIN AS POTENTIAL PREDICTIVE MARKERS FOR DISEASE SEVERITY IN ACUTE DENGUE

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INTRODUCTION

Dengue fever is a mosquito-borne viral disease caused by infection with any of four serotypes of dengue viruses (DENV 1-4) that predominantly spreaded throughout the tropical and subtropical regions worldwide. Approximately 390 million people across 128 dengue epidemic countries are at risk of acquiring dengue viral infection annually, of which 96 million manifest as symptomatic dengue cases (World Health Organization (WHO, 2022).

While the majority of cases are asymptomatic, symptomatic dengue can manifest a wide spectrum of clinical outcomes ranging from self-limiting febrile illness to more severe forms, occasionally accompanied by life-threatening complications of bleeding, organ impairment, and vascular leakage leading to shock. These severe outcomes occur late during the disease course, usually after the febrile phase, between days 04 -06 of illness onset. This allows a potential window of opportunity to identify patients at risk of developing the severe disease (Vuong et al., 2020). At present no therapeutic is available for dengue and management of dengue cases continues to rely on supportive therapy following constant examination and round-the-clock nursing care. Early recognition of severe dengue cases during the febrile phase is therefore important to minify fatal complications of severe dengue, thus reducing the mortality rate and health care burden (Thach et al., 2022).

Although the mechanism is poorly understood, recent evidence postulates that immune-mediated mechanisms play a key role in the pathogenesis of dengue infection. Therefore, host immune response mediators can serve as predictive markers of severe dengue. Ferritin and C-reactive protein (CRP) are two acute-phase proteins synthesized by the cells of the reticuloendothelial system in response to infection and inflammation. In dengue fever, ferritin and CRP levels disproportionately increase compared to other febrile illnesses. Recent studies have shown that hyperferritinemia and elevated levels of CRP collaborate with an increased risk of developing complications, suggesting these candidates could serve as biomarkers of dengue disease severity (Chaudhuri et al., 2017). This study sought to investigate the significance of serum ferritin and CRP levels measured early in the febrile phase to predict the disease severity of dengue virus-infected patients.

METHODOLOGY

A descriptive cross-sectional study was performed involving a total of 106 subjects, aged more than 12 years who have been clinically diagnosed as having dengue viral infection and admitted to the Colombo South Teaching Hospital (CSTH), Sri Lanka from January 2020 to June 2021. Pregnant women and patients having any other infections or inflammatory conditions were excluded from this study. All the subjects were serologically confirmed for having dengue viral infection on admission either by demonstrating DENV–specific non-structural glycoprotein 1 (NS1) antigen or by dengue specific IgM antibody. Serum ferritin and CRP assays were performed on patients' samples collected on admission, within the first 03 days of the febrile phase. Reference ranges of serum ferritin and CRP were adopted from

the CSTH laboratory protocols (ferritin: 21.81-274.66 ng/mL for males and 4.63-204.0 ng/mL for females, CRP: <0.5 mg/dL). All the subjects were followed up until the day of discharge and assigned into three severity groups: dengue fever without warning signs, dengue fever with warning signs, and severe dengue as per the WHO 2009 revised classification guidelines (WHO, 2009). Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 24. Mann-Whitney-u test and binary logistic regression model were performed to find the significance of study parameters and severity groups, and the significance of predictors of dengue. The significance level was assessed at a level of 5% ($p<0.05$). The study was approved by the ethics review committee of CSTH under protocol 876.

RESULTS AND DISCUSSION

All the 106 laboratory confirmed dengue cases were identified as the non-severe dengue with or without warning signs (Figure 01) and none of the subjects were diagnosed with severe dengue during the study period. The study consisted of 72.65% males and 27.35% females, with an age distribution ranging from 15 to 79 years (mean ±SD: 31 ±13.4 years).

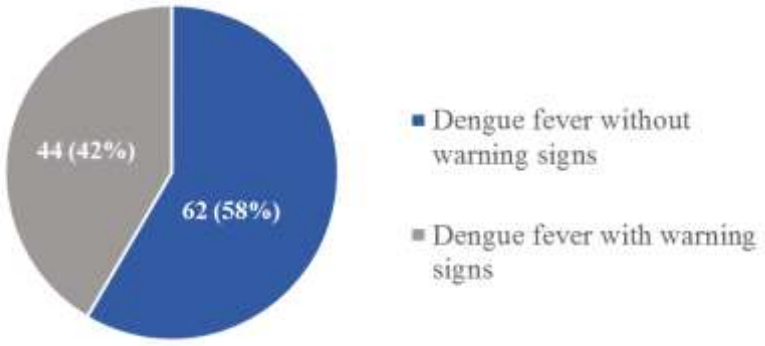

Figure 01: Number of cases with non-severe dengue without warning signs and cases with non-severe dengue with warning signs from all the confirmed dengue cases (n=106).

In the present study, serum ferritin level (median: 753.73 ng/mL, interquartile range (IQR): 1658.6) and CRP level (median: 10.8 mg/L, IQR: 12.75) measured within the first 03 days of illness was higher than the expected reference range in all the dengue cases. This agrees with the fact that ferritin and CRP are early immune markers of dengue viral infection. Several small-scale studies have found that ferritin and CRP levels in patients with dengue are higher than in other potential viral infections. Furthermore, a CRP value of approximately 30 mg/L and a ferritin value of 1291 ng/mL was proposed as the best cut-off values for differentiating dengue from other febrile illnesses of infective or inflammatory etiology (Vuong et al, 2020; Chaudhuri et al., 2017). However, these cut-off values are controversial with our findings as most of dengue cases in our cohort had ferritin and CRP levels less than the aforementioned cut-off values. Further large scale multi-centered studies are warranted to confirm the validity of these cut-offs in the diagnosis of dengue from other febrile illnesses.

In the comparison of dengue severity groups, serum ferritin values in patients with warning signs (median: 2055.6 ng/mL, IQR: 4634.8) were significantly higher compared to those who did not manifest any warning signs of severe dengue (median: 471.4 ng/mL, IQR: 615.5) ($p<0.001$) (Table 01). Binary logistic regression analysis for assessing the validity of ferritin level as a predictive candidate of the severity of dengue, our findings revealed that elevated ferritin level in the first 03 days of illness onset was associated with an increased likelihood of developing warning signs in dengue ($p=0.009$, adjusted odds ratio (AOR): 1.001, model sensitivity: 82.5%.). Though the findings of the present study are limited to two dengue severity forms, dengue fever with warning signs and dengue fever without warning signs due

to the lack of severe dengue cases, the strong differences in ferritin levels between these two non-severe dengue sub-groups profound that elevated ferritin level at the early phase of dengue may serve as a predictive marker of severe dengue. In agreement with the given fact, Suresh et al. (2020) have shown that a ferritin level of 895 ng/mL on day 04 is an excellent predictor of dengue warning signs. It was also a useful prognostic marker of dengue, with a level of approximately 1,380 ng/mL associated with the highest risk of developing severe dengue. In fact, many studies suggested that the ferritin level measured within 3-4 days of illness onset has the highest sensitivity and specificity for prediction of severity (Suresh et al. 2020; Murmu & Mubarak, 2021). Though the majority of dengue cases with warning signs in our study had ferritin levels above 1,380 ng/mL at the early stage, none of them progressed to severe dengue. The reason for this could be close monitoring and good supportive care. However, the findings of our study imply that dengue patients with elevated levels of serum ferritin have an increased likelihood of developing warning signs, hence patients who are at risk of progression of severe dengue. Thus, serum ferritin level would be an important biomarker for clinicians to prioritize the patients who need early intervention with supportive therapy, especially in resource-limited settings where potentially thousands of patients review daily.

Table 01: Comparison of serum ferritin and CRP with disease severity of dengue (n=106)

Laboratory parameter	Disease severity				p-value
	DF with warning signs (n=44)		DF without warning signs (n=62)		
	Median	IQR	Median	IQR	
Serum ferritin (ng/mL)	2055.6	4634.8	471.4	615.5	<0.001
CRP (mg/L)	11.7	10.9	8.9	13.9	0.116

However, comparing the CRP values among severity groups; DF with warning signs (median: 11.7, IQR-10.9 mg/L) and DF without warning signs (median: 8.9, IQR-13.9 mg/L) yielded no significant differences (p=0.116). Our findings are consistent with several other studies which found no significant variation in CRP levels and subsequent progression of severe dengue (Thach et al., 2020). However recent studies have yielded conflicting results. By contrast, Chen et al. (2015) reported a significantly higher CRP level early in the febrile phase of illness in severe dengue than in non-severe dengue, with a CRP threshold level of 30.1 mg/L (100% sensitivity, 76.3% specificity). Unfortunately, the lack of severe dengue cases in our study cohort compromises the statistical power of our study findings. The disagreement of our results with others' findings may also be influenced by the inconsistency in timing of the specimen collection, heterogeneity of laboratory methods, and variation of age groups.

CONCLUSIONS/RECOMMENDATIONS

In conclusion, ferritin measured in the first 03 days of illness onset can be a useful biomarker for the early prediction of patients who are at risk of developing warning signs of severe dengue. However, our results require validation in a large sample set including severe dengue cases to explore the association between ferritin level and severe dengue progression, and to evaluate the most effective threshold value for predicting severe dengue.

There was no difference in variation of CRP levels between dengue with warning signs versus non-severe dengue during the first 03 days of illness. To provide a definitive answer as to the value of CRP level for severity prediction in dengue, further studies are needed in a large population including a full spectrum of dengue severity groups.

ACKNOWLEDGMENTS

We would like to acknowledge all the patients who took part in this study and the medical and nursing staff at CSTH, Kalubowila, Sri Lanka who supported to accomplish this endeavour.

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