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Validity of ferritin as a predictor of severe dengue in children

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ABSTRACT

Background: Dengue infection is a significant global health problem, particularly in tropical and subtropical regions, where severe dengue cases carry high morbidity and mortality. Ferritin levels, an acute-phase reactant and marker of iron storage, have been evaluated as a potential biomarker for predicting severe dengue infection. This study aimed to evaluate the validity of ferritin as a predictor of severe dengue in children.

Methods: This diagnostic study was conducted at Ngoerah Hospital, Denpasar, from January to June 2025, recruiting 69 children based on WHO criteria through consecutive sampling. Ferritin levels were measured using ELISA and analyzed using SPSS version 26 using bivariate tests, ROC analysis, and diagnostic parameter calculations.

Results: A total of 69 children with dengue infection were involved (mean age 10.09 years). The mean serum ferritin level in all patients was 4820.97±6327.75µg/L. In the severe dengue group, the mean ferritin level was 6973.75±9089.48 µg/L, while in the mild dengue group it was 3672.82±3849.37µg/L, with a mean difference of 3300.93 µg/L (95% CI: 187.10–6414.76; p=0.190). ROC curve analysis showed an AUC of 0.596 (95% CI: 0.454–0.739; p=0.190) with an optimal cut-off point at 880.9450 µg/L (sensitivity 83.33%; specificity 31.11%. Diagnostic evaluation showed an accuracy of 49.28%, a positive predictive value (PPV) of 39.22%, a negative predictive value (NPV) of 77.78%, a positive likelihood ratio (PLR) of 1.21, a negative likelihood ratio (NLR) of 0.54, and a DOR of 2.24 (0.65–7.85).

Conclusion: Ferritin levels can be a predictor of diagnosis in children infected with severe dengue.

Keywords: biomarker, children, ferritin, severe dengue.
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INTRODUCTION

Dengue fever is a significant global health problem, particularly in tropical and subtropical regions. According to the World Health Organization (WHO), dengue fever is a viral disease spread through mosquito bites, with an estimated 390 million infections per year, of which 96 million show clinical manifestations.[1,2] Dengue virus, a member of the *Flavivirus* family, is primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes.[3,4] Dengue fever can present with a wide range of clinical manifestations, ranging from asymptomatic infection or mild fever to severe clinical manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can cause significant morbidity and mortality, particularly in children.[2]

Dengue fever typically begins abruptly after an incubation period of 4–10 days following an infected mosquito bite. The febrile phase is characterized by high fever, severe headache, retro-orbital pain, muscle and joint pain, nausea, vomiting, and a rash lasting 2–7 days. In some patients, the disease progresses to a critical phase characterized by fever and plasma leakage, which can lead to DHF and DSS, accompanied by thrombocytopenia, increased vascular permeability, and the risk of hypovolemic shock.[5,6] Early identification of severe dengue fever is crucial to improve treatment outcomes and reduce mortality, especially in children who are susceptible to rapid fluid changes leading to shock. Difficulty distinguishing mild from severe dengue fever in the early stages remains common because symptoms overlap with other febrile illnesses, and there are no specific markers for the severe form in the early stages of infection.[7]

Biomarkers have been studied to predict disease severity, including platelet count, hematocrit, liver enzymes, and inflammatory markers such as cytokines in patients infected with dengue fever, but these biomarkers often have limited specificity and sensitivity in predicting severe dengue, especially in the early stages of the disease.[8] Ferritin, which can function as an acute-phase reactant and a marker of iron storage, has emerged as a potential biomarker because serum ferritin levels increase significantly during infection and inflammation and are associated with increased inflammation and immune system activation commonly seen in cases of severe dengue.[9] The pathogenesis of severe dengue involves an exaggerated

immune response with the release of pro-inflammatory cytokines that contribute to increased vascular permeability, plasma leakage, and shock. Ferritin is produced in response to inflammatory signals such as interleukin-1 (IL-1) and interleukin-6 (IL-6), which are increased in severe dengue fever, making serum ferritin levels a potential indirect marker of the severity of the inflammatory response.[10–12]

Previous clinical study has reported that ferritin levels greater than 500 mcg/mL correlate with progression to dengue with warning signs or severe dengue, especially on days 4 and 5 of fever, and threshold values greater than 500 ng/dL and greater than 1200 ng/mL on days 4 or later after illness onset can predict clinical deterioration.[13] Evidence evaluating serum ferritin levels as a single predictor of severe dengue in the pediatric population is still limited. This study aims to evaluate the validity of serum ferritin levels as a predictor of severe dengue in pediatric patients.

METHODS

Study Design

This study is a diagnostic test conducted at Ngoerah General Hospital, Denpasar, from January to June 2025. Research approval was obtained from the Head of the Pediatric Health Science Department, Faculty of Medicine, Udayana University/Ngoerah Hospital, Denpasar, the Research Ethics Committee, and the hospital director.

Population and Sample

The research subjects were recruited using consecutive sampling from all pediatric patients who met the inclusion criteria, namely a diagnosis of dengue infection based on World Health Organization (WHO) criteria, an age of ≤18 years, and the absence of comorbidities. Comorbidities referred to included concomitant diseases such as congenital heart disease, bronchial asthma, diabetes mellitus, chronic kidney disease, immune disorders, severe malnutrition, or other conditions that could affect the clinical course of dengue infection. Out of 95 patients recruited within the accessible population, 10 patients were excluded for not meeting the WHO diagnostic criteria for dengue infection. Furthermore, 16 other patients were excluded due to comorbid diseases, resulting in a total of 69 patients who met the inclusion criteria and were included as research subjects.

Variables and Data

The variables analyzed include serum ferritin levels as the independent variable, dengue infection severity as the dependent variable, and confounding variables such as age, sex, and nutritional status. Dengue infection severity is classified into two main categories according to the World Health Organization (WHO) 2011 criteria, namely mild dengue infection and severe dengue. Mild dengue infection includes dengue with or without warning signs, while severe dengue is characterized by signs of hemodynamic disturbance (shock), including severe dengue with compensated and decompensated shock.

Data Collection Procedure

Data were collected through demographic information, physical examination, and laboratory results, including serum ferritin measurement using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The examination process began with the collection of a peripheral blood sample, which was then centrifuged at 15,000 rpm for 15 minutes to obtain serum, which was stored at -80°C until analysis. The ELISA procedure included preparing equipment and reagents, standard dilution, incubation at 37°C, washing the healthy plate, adding substrate and stop solution, and reading absorbance at a wavelength of 450 nm to determine ferritin concentration.

Data Analysis

Data analysis was performed using SPSS version 26. Numerical data, such as age and serum ferritin levels, were presented as means with standard deviations, or medians if the data were not normally distributed. Categorical data, such as sex and dengue infection severity, were presented as frequencies and percentages. Normality testing was conducted using the Shapiro-Wilk test, and the relationship between serum ferritin levels and dengue infection severity was analyzed using comparative analysis of means through the Mann-Whitney test. ROC analysis was performed to determine the optimal cut-off point for categorizing serum ferritin levels into binary groups. The diagnostic value of serum ferritin levels in predicting severe dengue infection was evaluated through calculations of sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios.

RESULTS

Characteristics of Research Subjects

A total of 69 children infected with *dengue* at Ngoerah Hospital, Denpasar, were included as research subjects. The mean age of the children was 10.09 years with a standard deviation of 4.23 years. The gender distribution was relatively balanced, with 52.2% male (36 children) and 47.8% female (33 children). Nutritional status was divided into three categories: 29.0% were undernourished, 58.0% had good nutritional status, and 13.0% were overnourished. Based on disease severity, 65.2% of the children were classified as having mild cases, while 34.8% were classified as having severe cases. Laboratory parameters examined included serum ferritin and hematocrit levels. The mean serum ferritin level was 4820.97 µg/L with a standard deviation of 6327.75 µg/L. There were no mortalities among the subjects. All subject characteristics are detailed in **Table 1**.

Comparison of Mean Serum Ferritin Levels Between Mild and Severe *Dengue*

The normality of data distribution was tested using the Shapiro-Wilk test. Based on the analysis results, the serum ferritin variable was found to have a non-normal distribution ($p < 0.001$). Thus, the comparison of means was conducted using the non-parametric Mann-Whitney test. The analysis results are presented in **Table 2**.

High Serum Ferritin Levels as a Predictor of Severe *Dengue* Infection

The ROC curve analysis is shown in **Figure 1**. The serum ferritin variable demonstrated an Area Under the Curve (AUC) of 0.596 (95%CI 0.454-0.739; p-value = 0.190). At a cut-off point of 880.9450 µg/L, ferritin showed a sensitivity of 83.3% and specificity of 31.1%.

Diagnostic analysis of serum ferritin levels as a predictor of severe *dengue*

Table 1. Characteristics of Research Subjects

Variable	n (%)
Age (Mean ± SD) (years)	10.09 ± 4.23
Gender	
Male	36 (52.2)
Female	33 (47.8)
Nutritional Status	
Malnutrition	20 (29.0)
Good nutrition	40 (58.0)
Overnutrition	9 (13.0)
Disease Severity	
Mild	45 (65.2)
Severe	24 (34.8)
Serum Ferritin Level (µg/L) (Mean ± SD)	4820.97 ± 6327.75
Total	**69 (100.0)**

Table 2. Comparison of Mean Serum Ferritin Levels Between Mild and *Severe Dengue*

Dengue Severity Level	N	Mean Serum Ferritin Level (µg/L)	Mean Difference (95%CI)	p-value
Severe	24	6973.75 ± 9089.48	3300.93 (187.10–6414.76)	0.190
Mild	45	3672.82 ± 3849.37		

*Analysis was conducted using the Mann-Whitney Test. Significant if p-value ≤ 0.05.

Table 3. Confusion Matrix of Serum Ferritin Levels as a Predictor of Severe Dengue Infection

Serum Ferritin Level	Severe *Dengue*	Mild *Dengue*	Total
High	20 (TP)	31 (FP)	51
Low	4 (FN)	14 (TN)	18
Total	24	45	**69**

*Explanation: TP (True Positive): Patients with high ferritin levels who have severe *dengue*. FP (False Positive): Patients with high ferritin levels who have mild *dengue*. FN (False Negative): Patients with low ferritin levels who have severe *dengue*. TN (True Negative): Patients with low ferritin levels who have mild *dengue*.

infection was then conducted (Table 3). A total of 24 patients had severe *dengue,* and 45 patients had mild *dengue*. In the severe *dengue* group, 20 patients had high ferritin levels (true positives, TP) while 4 patients had low ferritin levels (false negatives, FN). Conversely, in the mild *dengue* group, 31 patients showed high ferritin levels (false positives, FP) and 14 patients had low ferritin levels (true negatives, TN). High ferritin levels detected severe *dengue* with a sensitivity of 83.3% (20/24).

Sensitivity reached 83.33%, indicating that the test successfully identified most patients with severe conditions. However, specificity was only 31.11%, indicating a high number of false-positive cases. The overall accuracy of the test was 49.28%, meaning that only about half of the total patients were correctly classified based on the test results. The PPV of 39.22% indicates that the actual probability of patients having severe *dengue* is still relatively low. Contrary, the NPV of 77.78% suggests that low ferritin levels are quite effective in identifying patients who do not have severe *dengue*. The PLR was 1.21, and the NLR was 0.54, supporting the finding that although ferritin has high sensitivity, the use of serum ferritin levels as a sole predictor for severe *dengue* infection requires further evaluation (Table 4).

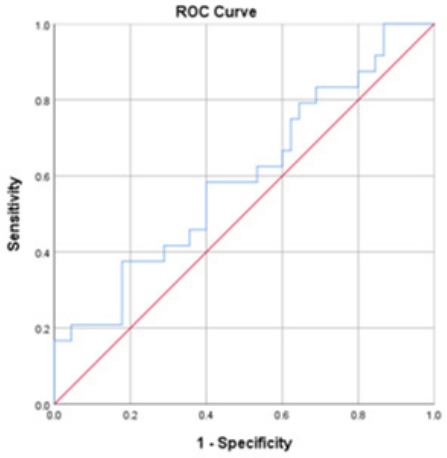


Figure 1. ROC Curve of Serum Ferritin Levels Against *Dengue* Infection Severity in Children

DISCUSSION

This study involved 69 pediatric patients diagnosed with dengue fever. The mean age of the subjects was 10.09 ± 4.23 years, indicating that the study population mainly consisted of school-aged children and early adolescents. A relatively balanced gender distribution was observed, and the majority of patients had good nutritional status. A small proportion of patients had severe dengue, while the majority were classified as having mild dengue. The mean serum ferritin level in pediatric patients with severe dengue was higher than in those with mild dengue, but the statistical test yielded a p-value of 0.190. The ROC curve analysis yielded an AUC value of 0.596, indicating a low discriminative ability of ferritin as a predictor of severe dengue, with relatively high sensitivity and low specificity.

Table 4. Diagnostic Values of Serum Ferritin Levels as a Predictor of Severe *Dengue Infection*

Diagnostic Parameter	Value
Sensitivity (Sn)	83.33%
Specificity (Sp)	31.11%
Accuracy	49.28%
Positive Predictive Value (PPV)	39.22%
Negative Predictive Value (NPV)	77.78%
Positive Likelihood Ratio (PLR)	1.21
Negative Likelihood Ratio (NLR)	0.54

These results are consistent with various earlier studies that evaluated the role of ferritin levels in predicting severe dengue in children. A previous study Valero et al. reported that ferritin levels significantly increased in dengue patients compared to controls, accompanied by an increase in IL-18, supporting the relationship between high ferritin levels and the occurrence of severe dengue infection in the pediatric age group.[14] Evidence from a prospective observational study also reported significantly higher mean serum ferritin levels in severe dengue than in non-

severe dengue and concluded that serum ferritin can help differentiate disease severity.[15] Findings from a severe dengue biomarker study further support this association, showing that serum ferritin was significantly increased in severe dengue compared to dengue fever patients and that ferritin tracked with macrophage activation, since soluble CD163 and ferritin were both elevated and correlated in severe cases.[16] Ferritin is an iron storage protein that also acts as an acute-phase reactant, increasing during systemic inflammation, such as in dengue infection, and severe dengue-associated hemorrhage and hemolysis can contribute to ferritin accumulation alongside secretion from activated macrophages.[14,16] Additionally, a pediatric-focused summary within the prospective study literature reported that serum ferritin levels greater than or equal to 1,200 ng/mL may predict dengue hemorrhagic fever in children, supporting the rationale for ferritin-based risk stratification in pediatric dengue.[15]

A previous study by Lakshmanan et al. reported that hyperferritinemia is associated with severe dengue infection using a retrospective study design on pediatric patients in an intensive care unit, and the age of subjects was comparable to the population included in this study. The similarity in results between Lakshmanan et al. and this study is likely due to similar age characteristics and disease phases at the time of laboratory data collection, since in children, the inflammatory response to dengue infection tends to be higher, thereby increasing ferritin levels as part of the acute phase response. Lakshmanan et al. reported that the majority of patients with severe hyperferritinemia were male, while this study found a relatively balanced gender distribution, and several previous studies also showed no significant difference between genders regarding the risk of developing severe dengue.[17] The theoretical explanation related to immunity and inflammatory response has been linked to sex hormones, with estrogen potentially providing a protective effect against infection and inflammation,[18] while other findings did not report a dominance of any particular gender among dengue patients with shock or severe dengue,[19] and no significant difference in ferritin levels between male and female children has also been reported.[20]

This study found that the distribution of nutritional status among children with dengue infection showed that the majority of patients had good nutritional status. Children with malnutrition tend to experience immune dysregulation, leading to prolonged inflammation, which can trigger increased ferritin levels as an acute-phase protein.[17] Additionally, another study by Evalda et al. found that ferritin levels in children with dengue shock were higher compared to those with uncomplicated dengue fever, and patients with poor nutritional status may experience more rapid clinical deterioration due to limited essential nutrient reserves that help control oxidative stress and inflammation.[19] Moreover, a study by Ruslianti et al. reported that patients with metabolic disorders or overweight may experience hyperferritinemia that does not necessarily reflect infection severity but rather a state of chronic inflammation.[21] Children with overnutrition may have elevated ferritin levels even without severe infection due to low-grade metabolic inflammation.[22]

The proportion of severe dengue cases and the diagnostic performance in this study showed variation compared to previous studies. A previous study by Manchala reported an AUC value of 0.83 for ferritin, indicating a better predictive performance, and ferritin levels greater than 500 ng/mL significantly correlated with severe dengue in children.[20] Another study by Vuong et al. reported that a combination of inflammatory and vascular biomarkers improved the predictive value for dengue severity, supporting the notion that the use of ferritin alone may not be sufficiently accurate.[23] In contrast, a study by Valero et al. did not explicitly evaluate diagnostic performance metrics such as AUC, sensitivity, or specificity, and their study focused more on the relationship between ferritin and IL-18 as markers of systemic inflammation contributing to disease severity.[14] Differences in results across studies may be caused by differences in population characteristics, sample size, and the disease phase at the time of sample collection, and a relatively high proportion of severe dengue cases may be associated with delayed diagnosis or limited clinical awareness regarding early warning signs of severe dengue.[24]

This study has limitations due to several confounding factors that potentially affect serum ferritin and hematocrit levels in children with dengue infection, such as environmental factors that were not analyzed. The data were obtained from only one hospital, which may introduce selection bias and limit the generalizability of the findings to a broader population.

CONCLUSION

This study showed that high serum ferritin levels can serve as a diagnostic predictor in children with severe *dengue* infection. Although ferritin has high sensitivity in detecting severe *dengue*, the low specificity indicates limitations in using ferritin as a sole predictor. Further research with a multi-center design and larger sample size, as well as more comprehensive control of confounding factors, is needed to strengthen the validity of ferritin as a biomarker for predicting severe *dengue* infection in children.

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CONFLICT OF INTEREST

None declared.

ETHICAL CLEARANCE

This research has obtained an ethical certificate from the Ethics Committee of the Faculty of Medicine, Udayana University (Ethical exception No: 2441/UN14.2.2.VII.14/LT/2024).

AUTHOR CONTRIBUTIONS

INBH, KAS initiated the study, collected and curated the data, performed the analysis, and wrote the initial draft; IWG designed the methodology, supervised the study, validated the findings, and edited the manuscript; KAS supervised the study, validated the findings, and edited the manuscript; IBGS, DKW, IMGDLU, SPS, IMYS, and PAS contributed to data

interpretation and critically revised the manuscript. All authors reviewed and approved the final manuscript and take responsibility for the integrity and accuracy of the work.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The authors used an AI-assisted language tool solely for grammar correction and language polishing. No AI tool was used to generate research ideas, data, statistical analyses, results, interpretation, or scientific conclusions. The authors reviewed and approved the final manuscript and take full responsibility for its content.

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