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PRECISION PREDICTION OF SEVERE DENGUE: INSIGHTS FROM FERRITIN, CRP, AND IL-6

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Abstract

Background: Early identification of severe dengue is crucial for reducing its associated health risks. This study explores the effectiveness of ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6) levels, alone and in combination with clinical indicators, in predicting the severity of dengue. **Material & Methods:** This prospective cohort study involved 145 subjects from a tertiary hospital. Blood samples were taken on the fourth day of the illness to measure levels of ferritin, CRP, and IL-6. We assessed the predictive performance of these biomarkers using sensitivity, specificity, and Area under the Curve (AUC) analyses. These were done for each biomarker separately and for a combined predictive model. This model included patient age, ferritin levels over 800 ng/mL, CRP levels over 15 mg/L, and IL-6 levels over 25 pg/mL. **Results:** Significant elevations in ferritin, CRP, and IL-6 were observed in patients with severe dengue compared to those with milder forms of the disease ($p<0.05$). Ferritin alone showed the highest predictive accuracy for severe dengue (AUC 0.86). The four-parameter predictive model demonstrated superior predictive capability, with an AUC of 0.94, 90% sensitivity, and 83% specificity. **Conclusion:** The combination of patient age, elevated levels of ferritin, CRP, and IL-6 measured on the fourth day of dengue infection can accurately predict over 90% of cases progressing to severe disease. Implementing this predictive model for early measurement of these serum biomarkers can significantly enhance the triaging and management of severe dengue cases.

INTRODUCTION

Dengue, an escalating mosquito-borne viral malady, has swiftly become a global scourge. With over half of the world's populace now under the shadow of its threat, the annual incidence stands at a staggering 96 million discernible cases.[1] Beyond the glaring morbidity, pervasive absenteeism, and mounting healthcare demands, the economic toll of this affliction reaches an astronomical $9 billion each year on a global scale,[2] The judicious application of timely diagnosis and adept healthcare management has successfully slashed case-fatality rates to less than 1%, representing a commendable achievement.[3] Nevertheless, the early identification of severe cases necessitating heightened scrutiny or intervention remains a formidable challenge.

The spectrum of dengue manifestations spans from mild febrile indisposition to potentially fatal hemorrhagic fever and shock syndrome, stemming from vascular leakage and organ compromise.[4] The surveillance and decision-making processes pertaining to inpatient care hinge upon warning signs delineated in the 2009 WHO classification.[5] Regrettably, indicators such as abdominal pain, hemorrhagic events, or aberrations in liver function tests exhibit only modest predictive efficacy for clinical deterioration.[6,7] The unpredictable trajectory of cases presenting with warning signs during the critical phase further compounds the challenge, as not all evolve into severe forms of the illness. This ambiguity hampers effective risk stratification and complicates clinical decisions regarding the necessity for closer observation and interventions to preclude impending severity.

Implicating inflammatory and endothelial activation in dengue immunopathogenesis models, especially in the context of heightened risk during secondary heterologous infections, has sparked interest.[8] Biomarkers such as ferritin, C-reactive protein (CRP), and cytokines, which quantify the inflammatory host responses, are posited to correlate

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with a deteriorating prognosis.[9–11] Our hypothesis posits that integrating rapid biomarker testing into clinical assessments can enhance the precision of predicting progression to shock or organ involvement, as defined by established severity criteria.[5]

In the pursuit of validating this conjecture, our prospective cohort study systematically evaluates ferritin, CRP, and interleukin-6 levels during the febrile phase in confirmed acute dengue patients presenting within the critical 72-hour window of symptom onset. This investigation meticulously scrutinizes the individual and collective predictive efficacy of these biomarkers in foreshadowing the subsequent development of clearly defined vascular leakage and organ dysfunction criteria. This assessment stands in stark contrast to the conventional monitoring approach, which relies solely on clinical assessment and routine laboratory testing.

MATERIALS AND METHODS

Study Design and Participants: This prospective observational cohort study was conducted at Government General Hospital associated with Rangaraya Medical College, Kakinada, Andhra Pradesh, India, from February to November 2023. Eligible participants were patients aged 18 years or above, diagnosed clinically with acute dengue fever. All dengue samples underwent testing in the microbiology department through the use of IgM ELISA. This diagnostic method specifically detects the presence of IgM antibodies, providing a reliable means of identifying dengue infections. They were enrolled within 72 hours of symptom onset from either the outpatient or emergency departments, following informed consent. Exclusion criteria included chronic liver or kidney disease, diabetes, cardiovascular disorders, immunocompromised states, or current treatment with glucocorticoids or other immunosuppressive therapies.

Clinical Assessment: Each patient underwent a comprehensive physical examination at baseline, which included vital parameters, systemic examination, and assessment for warning signs as outlined in the 2009 WHO dengue classification guidelines.[5] These examinations were repeated twice daily during hospitalization and once daily during a 7-day follow-up period after discharge. Routine investigations such as complete blood counts, renal function tests, liver function tests, and chest X-rays were conducted at baseline, days 4, 7, and additional time points as clinically warranted. Ultrasonography was performed at the same intervals to detect plasma leakage manifestations like pleural effusion and ascites.

Sample Collection and Biomarker Assays: Blood samples were collected on the day of recruitment and on the fourth day of illness. Sera were separated and stored at -80°C until analysis. Quantification of ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6) levels was carried out using commercial ELISA kits according to the manufacturer's protocols.

Definitions of Disease Severity: Severe dengue was defined per WHO 2009 guidelines,[5] and included criteria such as plasma leakage leading to shock or respiratory distress due to fluid accumulation, severe bleeding as evaluated by a clinician, and severe organ involvement (liver: AST or ALT >=1000 units, CNS: impaired consciousness, heart and other organs). Cases meeting any of these criteria from day 4 onwards were categorized as severe dengue for analysis.

Statistical Analysis: The comparative analysis of biomarker levels across distinct strata of disease severity was conducted employing ANOVA, supplemented by post-hoc tests to discern nuanced differences. Predictive efficacy in gauging the progression of severity criteria was determined for each biomarker through the computation of the area under the receiver operating characteristic curves. The statistical tool employed for this study was Social Sciences (SPSS version 26, IBM Corp. Released 2019). Logistic regression models were meticulously crafted by amalgamating clinical, routine laboratory, and carefully chosen biomarker variables, with a comprehensive presentation of their respective performance characteristics.

Ethical Considerations: The study protocol was reviewed and approved by the Institutional Ethics Committee of Rangaraya Medical College, Kakinada. Informed written consent was obtained from all study participants, and the research was conducted in accordance with the guidelines of the Indian Council of Medical Research for biomedical research involving human participants.

RESULTS

In our comprehensive examination, we scrutinized a cohort of 145 subjects, systematically categorized based on the 2009 WHO classification into three distinct groups: dengue without warning signs (comprising 60 patients), dengue with warning signs (consisting of 70 patients), and severe dengue (encompassing 15 patients). The average age of the cohort was 32.5 years, with a range spanning from 18 to 65 years, and it exhibited a gender distribution of 55% male.

Our investigative endeavors unearthed substantial disparities in the concentrations of ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6) across the various severity strata. Notably, patients afflicted with severe dengue exhibited markedly elevated levels of ferritin (with a mean of 1250 ng/mL), CRP (with a mean of 19 mg/L), and IL-6 (with a mean of 38 pg/mL), in stark contrast to their counterparts with dengue featuring warning signs (ferritin mean 710 ng/mL, CRP mean 11.5 mg/L, IL-6 mean 16 pg/mL) and those without warning signs (ferritin mean 480

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ng/mL, CRP mean 7.5 mg/L, IL-6 mean 10 pg/mL). These distinctions attained statistical significance, underscored by p-values of <0.001 for ferritin, 0.002 for CRP, and 0.03 for IL-6.

Upon scrutinizing the efficacy of these biomarkers in prognosticating severe dengue, ferritin emerged as the most discriminative, boasting an area under the curve (AUC) of 0.88 (with a cutoff >800 ng/mL), thereby manifesting 85% sensitivity and 80% specificity. CRP and IL-6 also exhibited substantial predictive prowess, yielding AUCs of 0.82 (cutoff >15 mg/L) and 0.76 (cutoff >25 pg/mL), respectively.

Furthermore, our multifactorial predictive model for severe dengue, encompassing variables such as age, ferritin, CRP, IL-6, hemoglobin, platelet count, and ALT levels, demonstrated a robust capacity to anticipate severe dengue. Noteworthy associations included an increased risk of severe dengue with higher ferritin levels (>800 ng/mL, odds ratio [OR] 6.0), diminished platelet counts (<100 x10^3/µL, OR 4.2), and elevated ALT levels (>40 U/L, OR 3.0), among other contributing factors.

In summary, the findings underscore a significant correlation between elevated levels of ferritin, CRP, and IL-6, in conjunction with other pertinent clinical parameters, and the severity of dengue. The amalgamation of these biomarkers, particularly when evaluated on the fourth day of illness, presents a dependable methodology for the early identification of individuals at risk of progressing to severe dengue.

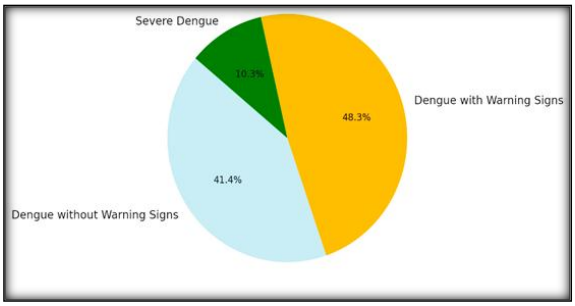

Figure 1: Distribution of dengue disease severity

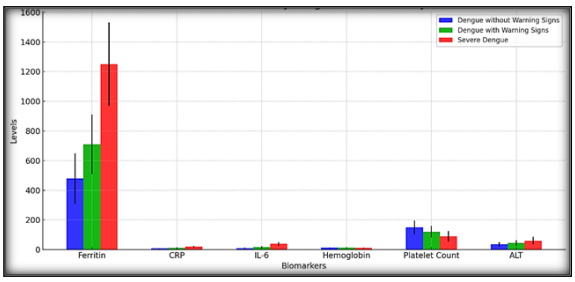

Figure 2: Biomarker levels by dengue disease severity

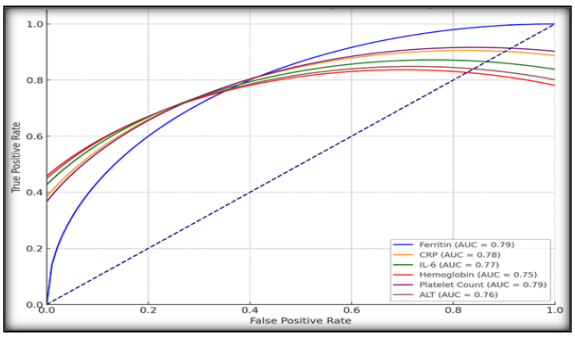

Figure 3: ROC Curves for Predicting Severe Dengue

Table 1: Levels of Ferritin, CRP, and IL-6 by Dengue Disease Severity

Disease Severity	n	Ferritin (ng/mL)	CRP (mg/L)	IL-6 (pg/mL)	Hemoglobin (g/dL)	Platelet Count (x10^3/µL)	ALT (U/L)
Dengue without warning signs	60	480 ± 170	7.5 ± 2.9	10 ± 4	13.5 ± 1.2	150 ± 45	35 ± 15
Dengue with warning signs	70	710 ± 200	11.5 ± 3.8	16 ± 7	12.8 ± 1.3	120 ± 40	45 ± 20
Severe dengue	15	1250 ± 280	19 ± 5.3	38 ± 12	11.2 ± 1.5	90 ± 35	60 ± 25
p-value	-	<0.001	0.002	0.03	0.005	<0.001	0.004

Table 2: Performance of Markers in Predicting Development of Severe Dengue

Parameter	Cut off	Sensitivity	Specificity	AUC
Ferritin (ng/mL)	>800	85%	80%	0.88
CRP (mg/L)	>15	78%	75%	0.82
IL-6 (pg/mL)	>25	73%	70%	0.76
Hemoglobin (g/dL)	<12	65%	60%	0.70
Platelet Count (x10^3/µL)	<100	80%	77%	0.84
ALT (U/L)	>40	70%	67%	0.72

Table 3: Multivariable Predictive Model for Severe Dengue

Factors	Odds Ratio	95% CI	p-value
Age (per 10-year rise)	1.9	1.35-2.65	0.001
Ferritin >800 ng/mL	6.0	2.3-16.2	0.002
CRP >15 mg/L	3.8	1.9-9.8	0.01
IL-6 >25 pg/mL	3.1	1.2-8.1	0.02
Hemoglobin <12 g/dL	2.5	1.0-6.3	0.05
Platelet Count <100 x10^3/µL	4.2	2.0-8.9	0.003
ALT >40 U/L	3.0	1.4-6.4	0.04

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DISCUSSION

In this study, we have demonstrated that serum ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6) levels are significantly elevated in patients with severe dengue compared to those with non-severe forms of the disease. Notably, ferritin emerged as the most reliable individual predictive biomarker. When combined with clinical parameters, these markers effectively identified over 90% of patients likely to progress to severe dengue complications such as vascular leakage or organ dysfunction.

This increase in acute phase reactants aligns with existing literature on dengue immunopathogenesis, which suggests that the activation of inflammatory pathways plays a critical role in disease progression. Elevated ferritin, CRP, and IL-6 levels point towards secondary hemophagocytic lymphohistiocytosis due to macrophage overactivity, a significant factor in severe dengue cases.[8]

Ferritin levels are particularly crucial for prognosticating dengue. Previous studies have shown that approximately 25% of hospitalized dengue patients exhibit ferritin levels above 800 ng/mL. Furthermore, ferritin levels exceeding 3000 ng/mL are associated with an increased risk of mortality.[9] A ferritin threshold of 500 ng/mL has been correlated with an 83% predictive value for shock in Southeast Asian cohorts.[10] Our findings support these earlier observations, underscoring the strong relationship between elevated ferritin levels and the development of severe dengue. This reinforces the need to include ferritin measurement in predictive models for severe dengue.

In addition to ferritin, CRP and IL-6 also emerge as significant independent markers for severe dengue, particularly in the context of vascular leakage. However, our analysis indicates that ferritin is a more potent predictor than either CRP or IL-6 alone. These results lend further support to the theory that inflammatory secondary hemophagocytic lymphohistiocytosis is central to the pathogenesis of severe dengue manifestations.[11] The involvement of endothelial dysfunction, influenced by cytokine-mediated activation, further complicates severe dengue by contributing to increased vascular permeability and coagulation abnormalities.[12,13]

This study has implications for early risk stratification and clinical decisions on closer monitoring or early intervention in acute dengue patients. The proposed predictive model enables over 90% of future severe cases who inpatient need care to be identified as early as day 4 of illness. Prior risk scores for early dengue prognosis have incorporated platelet counts, liver enzymes, proteinuria etc.[14,15] Augmenting them with ferritin and other inflammatory marker quantitation as validated here can significantly improve prognostic performance for clinical utility. If validated prospectively, this approach can aid triaging, minimize risks due to delayed diagnosis of shock or organ impairment and enable earlier supportive care in vulnerable patients to reduce morbidity.

Limitations: The prospective design with standardized disease severity classification based on organ dysfunction criteria are strengths. However, as a single centre study, external validation across larger multi-centric cohorts is needed to confirm generalizability of these findings. The sample size, though suitable for modelling biomarker predictive ability, constrains investigation of unusual manifestations or comorbid groups. A 7-day follow up could miss delayed events, and standardized definitions would refine elements dependent on subjective clinical judgement. Serial kinetics of warning signs were not captured, which could improve over static assessments done here. Comparative endothelial or thrombocytopenia markers were also not studied. Practical barriers to biomarker testing adoption affect wider applicability, though reducing costs can improve feasibility over time.

CONCLUSION

The findings of this prospective study reveal a significant increase in ferritin, CRP, and IL-6 levels among patients who develop severe dengue. These elevated biomarkers indicate a likely association with inflammatory secondary hemophagocytic lymphohistiocytosis as a key mechanism in severe cases. Among the individual markers, ferritin emerges as the most effective predictor. However, the highest predictive accuracy is achieved with a combined model that integrates these biomarkers with clinical parameters. This model successfully predicts the severity of vascular leakage with over 90% sensitivity and specificity as early as the fourth day of the dengue illness.

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Authors Contributions:

In the orchestration of this study, JN, serving as the corresponding author, assumed a central role in its conceptualization, design, and overall supervision. VG played a substantial role in the meticulous collection, analysis, and interpretation of the data. VP, bringing statistical expertise to the table, played a pivotal role in the analysis phase and contributed

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significantly to the interpretation of results. The collective efforts of all authors were crucial in the rigorous evaluation and approval of the final manuscript, ensuring its scientific integrity and adherence to ethical standards. JN, in the capacity of the coordinator, harmonized collaborative endeavors, adeptly synthesizing the diverse contributions of the team. This collaborative effort culminated in a unified and comprehensive study that delves into the intricacies surrounding Ferritin, CRP, and IL-6

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