

A Study to Access the Role of Serum Ferritin Levels as an Early Prognostic Marker to Predict the Severity of Dengue Viral Fever

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Abstract

Dengue fever is among the world's foremost viral hemorrhagic fevers, the most geographically widespread arthropod-borne viral illnesses. Compared to other bacterial and viral infections, the level of serum ferritin disproportionately raised in dengue fever and the raised level of serum ferritin corroborates with an increased risk of developing complications. Ferritin could be used as a marker to differentiate between dengue and other acute febrile illnesses.

Method: In this prospective study we included 100 patients with confirmed diagnosis of dengue fever conducted for eighteen months. The patients underwent all routine investigations and serum ferritin levels. The ferritin levels are recorded and analyzed and compared with severity of dengue illness.

Results: The mean age of study group was 37.8 years and the age of patients ranged from 21 to 56 years. 67% were males and 33% were females. Fever (100%) was the most common symptom. Only 12 cases were having severe dengue. 88 cases were not severe among 100 patients. AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) were higher in patients with severe dengue. P value=0.001. Serum ferritin levels during day 1 and day 4 were significantly higher in patients with severe dengue, as evident from the P value in the current study. Serum ferritin levels were more during day 4 of dengue compared to day 1.

Conclusion: The study concluded that ferritin level as a good to excellent predictor in the diagnosis of dengue. We also had the same opinion. The study identified that high serum ferritin levels of more than 1,200 ng/ml could predict dengue hemorrhagic fever.

Keywords: Dengue, Dengue hemorrhagic fever, Ferritin, Liver enzymes

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Introduction

Dengue fever is among the world's foremost viral hemorrhagic fevers, the most geographically widespread arthropod-borne viral illnesses, caused by Arbovirus of Flavivirus genus consists of 4 dengue serotypes [1,2] and affects an estimated 3.97 billion people across the 128 endemic countries comprising India [3]. It is transmitted by mosquitos *Aedes aegypti* and *Aedes albopictus*.

Dengue presents as 2-7 days fever with two or more of the associated manifestations including Headache, myalgia, arthralgia, retro-orbital pain, rash, and hemorrhagic manifestations [4]. It can cause hemorrhagic fever or shock syndrome. Dengue Haemorrhagic Fever (DHF) is defined as a case with clinical criteria of dengue Fever plus Thrombocytopenia (<100,000 cells per cumm) with plasma leakage and hemorrhagic tendency. Whereas Dengue Shock Syndrome (DSS) is defined as a patient having all the criteria for dengue hemorrhagic fever with evidence of circulatory failure exemplified by rapid and weak pulse and narrow pulse pressure (mm Hg) or hypotension for age, cold and clammy skin and restlessness [5].

According to WHO (World Health Organization), around 50 to 100 million new infections are estimated to occur every year in more than 100 endemic countries across the world, out of which around 500000 people with severe dengue require hospitalization each year and about 2.5% of those affected die [6] Previous studies had identified various biomarkers for immune and endothelial cell activation, biochemical and genetic markers to predict the severity of dengue fever [7]. The clinical utility of these markers is limited since measurement of these markers like cytokines, growth factors soluble receptors, Vwf (Von Willibrand Factor) genetic profiling etc. is technically difficult and not widely available. Compared to other bacterial and viral infections, the

level of serum ferritin disproportionately raised in dengue fever and the raised level of serum ferritin corroborates with an increased risk of developing complications [8,9]. Ferritin could be used as a marker to differentiate between dengue and other acute febrile illnesses. Correspondingly, the presence of hyperferritinaemia (ferritin levels ≥ 500 $\mu\text{g/L}$) was correlated with markers of immune activation and coagulation disturbances and clinical disease severity, suggesting that it could serve as a marker of disease activity [10] Clinical markers to define the presence and severity of dengue virus infection are important for diagnostic and treatment purposes [11]. We aimed this study to determine serum ferritin levels as an early prognostic marker to predict the severity of dengue viral fever.

Objective: To assess if serum ferritin levels act as an early prognostic marker to predict the severity of dengue viral fever.

Methods

Sample size: After taking informed written consent, this study was performed on 100 patients for Eighteen months admitted in different medical wards.

Study place: JLN Medical College, Ajmer, Rajasthan

Study design: Prospective, observational study

Study period: February 2020 to July 2021. Ethical considerations: Study was done after taking approval of ethical committee of this institute and obtaining consent from patients.

Inclusion criteria:

1. Patients diagnosed with dengue infection by dengue NS1 (Nonstructural) antigen (ELISA) -Enzyme Linked Immunosorbent Assay
2. Patients diagnosed with dengue infection by specific serology IgM antibody of ELISA.

Exclusion criteria:

1. Patients with chronic inflammatory conditions or diseases
2. Iron overload status; sideroblastic anemia; thalassemia; malignancy.
3. Liver disease
4. Pregnancy of any duration

Methodology

History was taken in detail and duration of fever and other associated signs and symptoms were noted. Necessary investigations were done. Patients were then followed throughout the clinical course of the disease. Diagnosis was based on NS1 antigen test (done with dengue NS1 Ag Microlisa kit) when the presentation was less than 5 days of illness or positive dengue specific serology (dengue IgM done by NIV Dengue MAC ELISA Kit, version 2.4 if presented after 5 days of illness. Serum ferritin levels were measured. After informed consent, 2ml of blood was collected from the study subjects; ferritin assay was done (by MAGLUMI Fully-auto chemiluminescence immunoassay (CLIA) analyzer) on the day 1 and day 4. The clinical course of the disease and platelet count were monitored carefully on day 1 and day 4 and patients were classified as having severe and non-severe infection as per WHO 2009 criteria; classified as severe dengue when shock, fluid accumulation with respiratory distress, severe bleeding; server organ impairment occur.

Serum ferritin estimation [10]

Ferritin is a high-molecular iron storage protein (MW approx. 450,000 Da) which can incorporate varying amounts of iron in the form of iron-(III)- hydroxide/phosphate (max. 4,500 iron ions/molecule). The Iron represents approximately 25% of the total molecular weight. Ferritin can mainly be found in the cytoplasm of the reticuloendothelial cells, in liver cells and to

a small extent in the precursors of the red cells in the bone marrow. It stores Iron in a physiologically active form. Thus, it gives a measure of the amount of iron available for haemoglobin synthesis. The determination of serum ferritin has its significance in the diagnosis and monitoring of Iron deficiency. Reduced ferritin serum levels Indicate Iron deficiency: iron overload or idiopathic haemochromatosis are associated with elevated ferritin serum concentrations. Elevated ferritin levels have also been found in a variety of malignancies, especially in Hodgkin- or Non-Hodgkin lymphoma, acute or chronic leukaemia as well as in breast or bronchial carcinoma

Statistical analysis

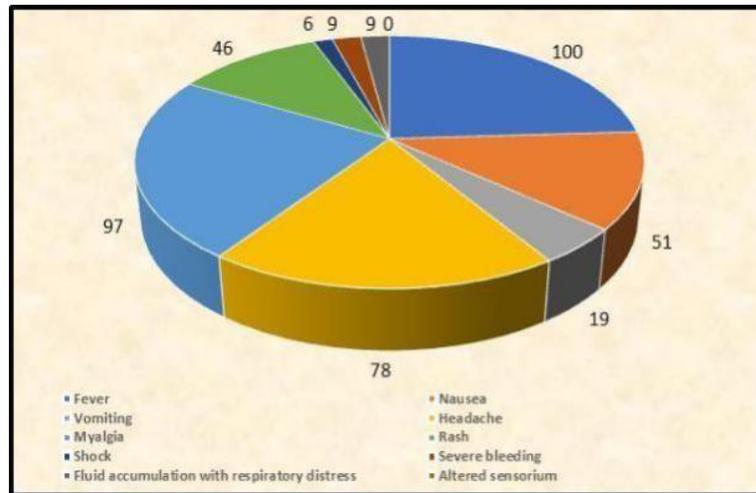
The data was coded and entered into Microsoft Excel spread sheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of percentages, means and standard deviations. The unpaired t test and Chi-square tests were used for qualitative data.

Results Demography

Out of 100 patients included in the current study, most of the patients (34%) belongs to 20 to 30 years age group, followed by 30 to 40 years. The mean age was 37.8 years and the age of patients ranged from 21 to 56 years. 67% were males and 33% were females.

Symptoms

Fever (100%) was the most common symptom, followed by myalgia (97%), headache (78%), nausea (51%), Rash (46%) and vomiting (19%), severe bleeding was seen in (9%) patients, fluid accumulation with respiratory distress in (9%), shock was seen in (6%) patients. No patient had altered sensorium.



Graph 1: Symptoms of patients

Dengue severity

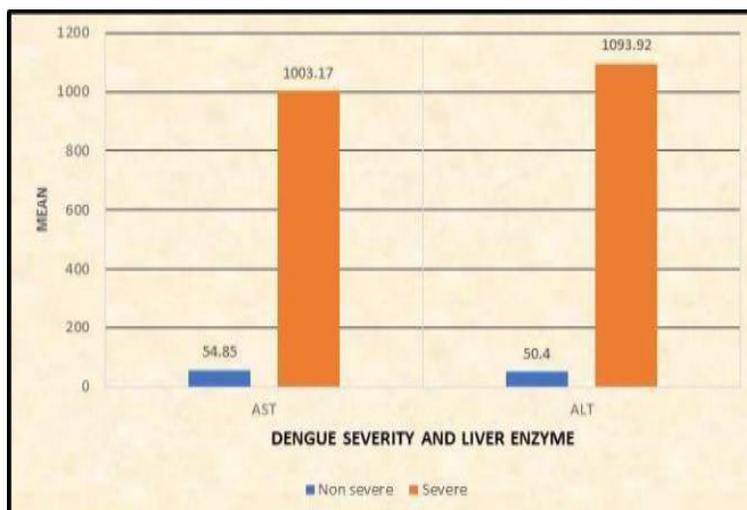
Only 12 cases were having severe dengue. 88 cases were not severe among 100 patients.

Table 1: Severity of Dengue

Dengue Severity	Frequency	Percent
Non severe	88	88.0
Severe	12	12.0
Total	100	100.0

Severity and liver enzymes:

AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) were higher in patients with severe dengue. P value=0.001.

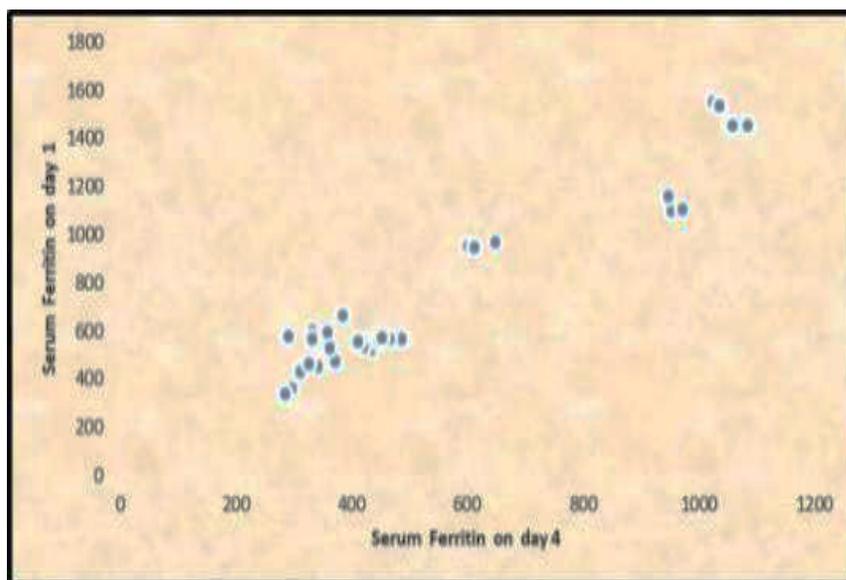


Graph 2: Severity of dengue fever with liver enzymes (AST/ALT)

Dengue severity and serum ferritin: Serum ferritin levels during day 1 and day 4 were significantly higher in patients with severe dengue, as evident from the P value in the current study. Serum ferritin levels were more during day 4 of dengue compared to day 1

Table 2: Serum ferritin and dengue severity

Serum Ferritin	Dengue Severity	N	Mean	Std. Deviation	P value
S ferritin at 1 day	Non severe	88	374.23	58.853	0.001 (S)
	Severe	12	919.00	184.872	
S ferritin at day 4	Non severe	88	524.22	72.545	0.001 (S)
	Severe	12	1269.75	250.057	



Graph 3: Correlation plot shows positive correlation between level of serum ferritin on day 1 with day 4

Table 3: Correlation between serum ferritin on day 1 with day 4

		Serum ferritin day 1	Serum ferritin day 4
Serum ferritin day 1	Pearson Correlation	1	860**
	P value		<0.01
	N	12	12
Serum ferritin day 4	Pearson Correlation	860**	1
	P value	<0.01	
	N	12	12

** . Correlation is significant at the 0.01 level (2-tailed).

There was strong correlation between serum ferritin at day 1 and day 4 which is also statistically significant (p<0.01).

Discussion

In the current study, 100 patients with dengue were analyzed. Serum ferritin levels and platelet counts were assessed during day 1 and day 4 and their role in severity was assessed.

Demographic variables

Most of the patients (34%) belong to 20 to 30 years age group, followed by 30 to 40 years. 67% were males. In the study published by Velammal *et al* (2019) [12]. 119 patients were included. The mean age was 29.1 ± 11.4

Symptoms and severity

Out of 100 patients included in the current study, fever was the most common symptom. Fever was the most common symptom in the study conducted by Nadeem *et al* [13], similar to the current study. In the study of Cornelia (2014) [10], the most sign observed was abdominal pain (45%) followed by vomiting (23%), followed by epistaxis (5%) and hepatomegaly (2%). In the current study, out of 100 patients included, severe bleeding was seen in 9% patients, fluid accumulation with respiratory distress in 9%, shock was seen in 6% patients.

No patients were observed to have altered sensorium. Twelve cases were having severe dengue. 88 cases were not severe among 100 patients in the current study. In Cornelia's study [10], 1 case was severe out of 44 patients.

In Velammal's study [12] 4.2% patients had severe dengue. In the study of Soundravally (2014) [15], 13 cases were having severe dengue out of 96 patients included. The mean AST and ALT levels for non-severe dengue patients were less compared to patients of Jyothi's study.[16] Sowmya brata *et al* [14] reported SGOT and SGPT to be significantly higher in dengue patients as compared to non- dengue patients.

years. The mean age was less compared to the current study. Around 50% of the study populations were aged less than 25 years. 57.14% were males. More males were having dengue, similar to the current study. In the study done by Nadeem *et al* [13] in Pakistan, the mean age was 30.7 years, and more males were affected similar to the current study. In the study done by Sowmya brata [14] in North India, the mean age was 30.7 years, and more males were affected similar to the current study

Serum Ferritin and Severity of dengue:

Serum ferritin levels during day 1 and day 4 were significantly higher in patients with severe dengue, as evident from the P value of <0.01 in the current study. Serum ferritin level was found to be a good predictor of severe dengue on day 1 and on day 4, it was an excellent predictor of severe dengue, as reported by Sumatha *et al* (2020) [17]. In the current study, Regression model showed that serum ferritin at day 4 was found to be a higher predictor for dengue severity followed by serum ferritin at Day 1.

There was significant difference ($P=0.03$) between serum ferritin levels between severe and non-severe cases of dengue by Velammal *et al*. [12] In Nadeem's study [13], in about 70% patients, serum ferritin level was below 100 $\mu\text{g/dl}$. Out of 31 patients with normal ferritin levels, only two patients developed severe dengue. The study concluded that serum ferritin levels may act as biomarker for an early prediction of disease severity in dengue virus infection. We also had the same opinion According to Soundra valley [15], among all alpha 1 antitrypsin, serum ferritin and ceruloplasmin, the elevated serum ferritin levels could predict the dengue severity with greatest sensitivity and specificity of 76.9 and 83.3 %, respectively. In the study of Sowmya brata [14], Ferritin level in the dengue patients was significantly

more compared to non- dengue patients. ($P < 0.01$).

Conclusion

The study concluded that ferritin level as a good to excellent predictor in the diagnosis of dengue. We also had the same opinion. In the study of Wathanee (2008) [18], the mean serum ferritin levels increased from day 2 to day 9 of illness with dengue fever compared to those without dengue fever. The study identified that high serum ferritin levels of more than 1,200 ng/ml could predict dengue haemorrhagic fever.

Limitations

In this study the sample size is 100 indicating that the study sample is small, and the primary limitation is the interpretation of results.

Further recommendations

Prospective observational study can be done for estimation of CRP and ESR and correlation with severity of dengue.

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