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ORIGINAL ARTICLE

Serum Ferritin as a Marker to Prognosticate The Severity of Dengue Fever

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ABSTRACT
Dengue fever is classified as dengue fever with or without warning signs and severe dengue. This study demonstrated significant association between elevated ferritin levels and severe dengue. Thrombocytopenia and leukopenia were also significantlyassociated with severe dengue. The study is to determine Serum Ferritin as a marker to assess severity of Dengue and to determine the association between Serum Ferritin Levelsand Platelet Levels in Dengue. Itprovidesthe Socio-demographic profile of the study population. The serum ferritin can be used `as an adjunct marker to delineate cases of severe dengue and may serve as a marker for early prediction of severe disease.
Keywords: Serum Ferritin, Dengue Fever, leukopenia

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INTRODUCTION

Dengue, being a unique entity, presents with a myriad of symptoms that may differaccording to severity and age. [1] Infection occurring from any of the four dengue serotypes may be without symptoms or lead to classic DF or moreserious forms of the disease, DHF and DSS. [2] Dengue infection maybe detected by confirming NS-1 antigen present in the sera of patients in the acute phase. [3] The illness begins abruptly, initially preceded by an incubation period, comprising of 3 phases: febrile, critical followed by recovery. [6] DF may present with a mild fever or a in more debilitating form, seen more often in adolescents. The latter, severe form may present with by the sudden onset of highgrade fever, severe headache, retro-orbital pain, myalgia, arthralgia and a rash. [1,3] Symptoms generally occur predominantly in the early febrile stage. [5] In the critical phase, flushed appearance of the skin may be visible,with the presence of a petechial rash. This is generally is witnessed close to defervescence, around days 3-7, and is accompanied by the presence capillary leakage along with haemorrhage. [2] Children with age less than 15 years generally suffer from DHF or severe dengue.[6] DHF transiently leads to an increase in the permeability of the vessels, which results in plasma leakage, along with high grade fever, bleeding, accompanied by thrombocytopenia and haemoconcentration, which can ultimately result in shock (DSS). [5] However, in the primary phase of the illness, it might be challenging to delineate DHF from DF and other viral diseases. [4]

For a small subset of patients, dengue infection is life threatening with severe cytopenias and significant systemic response. The appearanceof macrophage activation in cases of dengue fever has been reported, possibly owing to increased morbidity and rising mortality in DF. [7] MAS consists of a life-threatening haematological syndrome, often occurring inconjunction with fever as well as cytopenias. This comprises of a complex situation that results from uncontrolled stimulation of tissue macrophages causing T-cell immunosuppression, hypercytokinemia along with histiocytic proliferation. Some patients will develop symptoms like bruising, purpura, petechiae, nose bleeding and gum bleeding. Rarely, there may be life threatening symptoms like CNS bleeding, GIT bleeding. Thrombocytopenia, occasionally seen in DF, is a regular feature forming one of the diagnostic criteria of DHF.

MATERIAL AND METHODS

STUDY DESIGN

The study is a Cross-Sectional Study.

STUDY POPULATION

The study population consists of children admitted at Sree BalajiMedical Medical College and Hospital.

SAMPLE SIZE

130 patients.

INCLUSION CRITERIA

1. Children from 1-14 years of age, with Dengue confirmed by NS1 positivity and/or IgM positive for Dengue Virus.

The cases were divided into the following categories-

a. Dengue without warning signs
b. Dengue with warning signs
c. Severe dengue

For the ease of statistical analysis the cases were grouped as Non-severe dengue and Severe Dengue. Non-severe dengue group consisted of patients from the category of dengue without warning signs (a) and dengue with warning signs (b).

EXCLUSION CRITERIA

I. Children with only IgG positivity for Dengue Virus.
II. Known cases of Thalassemia and Haemochromatosis.
III. Children who have received recent blood transfusions.
IV. Children receiving Iron supplementation.

STUDY PERIOD

One and half year, from December 2018 to June 2020.

STUDY PLACE

The study was conducted in the Department of Paediatrics at Sree Balaji Medical College and Hospital, Chennai.

METHODS

One hundred and thirty children, aged 1-14 years, who were suffering from Dengue fever, those satisfying the inclusion and exclusion criteria were included as participants in the study. Dengue fever positivity was detected by the NS1 ELISA-based antigen assay (generally positivity is observed in the initial three days of illness) and IgM-capture enzyme- linked immunosorbent assay. The principle of the test is detection of the IgM antibodies specific for dengue infection in the sera of patients, which are captured by the use of anti-human IgM, which was initially bound to the solid phase. Following this, dengue antigen is added and provided that the IgM antibody, present in the patient's serum is anti-dengue, it will result in binding to the dengue antigen. To ensure a colour reaction occurs, an enzyme substrate is then. In order to enable easy detection. The occurrence of anti-dengue IgM antibody is earlier than the IgG antibody, which can be detected by the 5th day of illness. However, the development of the IgM antibody may vary considerably among patients. Few patients may have evidence of the IgM antibody even on 2nd to 4th day of illness, whereas in others, the occurrence of IgM antibody may be delayed till 7th or 8th day of illness. The individual participant or their parents/guardian were explained about the study and they were also assured that, their identity would be kept strictly confidential. Written informed consent was obtained from the study participants or from their parents/guardian in both the English and Tamil, formats which are enclosed in Annexure section. After taking consent, using a proforma, history regarding the present illness was collected and entered, following which venous blood samples were collected, under strict aseptic precautions from all the participants to assess the basic haematological parameters like Complete Blood Count (CBC) and Serum Ferritin levels from Day 2 to day 7 of illness. Serum Ferritin levels were estimated by using Chemiluminescence Immunoassay (CLIA). Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies. The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The Ferritin (Human) CLIA Kit provides a rapid, sensitive and reliable assay. The antibodies developed for the test will determine a minimal concentration of human ferritin of 5 ng/ml.

RESULT

A cross-sectional study was conducted with 130 children at Sree Balaji Medical College and Hospital to evaluate if serum ferritin could be used to prognosticate the severity of dengue fever.

Table 1: Association of Serum Ferritin with Dengue

	Severe Dengue	NON- SEVERE DENGUE	CHI- SQUARE TEST
HYPERFERRITINEMIA	Frequency(%)	Frequency(%)	
YES	76 (90.47%)	11 (23.91%)	**X^2=59.49**
NO	8 (9.52%)	35 (76.08%)	**p≤0.001**

Table 1 suggest that 90.47% (76) cases of severe dengue manifested with the presence of hyperferritinemia and 9.52% (8) cases did not have any evidence of raised ferritin levels. However, in non-severe dengue cases only 23.91% (11) cases had hyperferritinemia, while 76.08% (35) cases consisted of ferritin levels within the normal range. Thus, there was a significant positive correlation between hyperferritinemia and severe dengue, as demonstrated by p≤0.001.

Table 2: Association of Thrombocytopenia with Dengue

	SEVERE DENGUE	NON- SEVERE DENGUE	CHI- SQUARETEST
THROMBOCYTOPENIA	Frequency (%)	Frequency (%)	
YES	77 (91.66%)	14 (38.88%)	**X^2=53.06**
NO	7 (8.33%)	32(88.88%)	**p≤0.001**

Table 2 illustrates that 91.66% (77) cases of severe dengue manifested with the presence of thrombocytopenia and 8.33% (7) cases had platelet counts > 1,00,000/cumm. While, in non-severe dengue cases 38.88% (14) cases had thrombocytopenia, while 88.88 % (32) cases consisted of platelet counts within the normal range. Thus, there was a significant positive correlation between thrombocytopenia and severe dengue, as demonstrated by p≤0.001.

Table 3: Association of Leukopenia with Dengue

	Severe Dengue	NON- SEVERE DENGUE	CHI- SQUARETEST
LEUKOPENIA	Frequency(%)	Frequency(%)	
YES	70 (83.33%)	3 (6.52%)	**X^2=71.22**
NO	14 (16.66%)	43 (93.47%)	**p≤0.001**

Table 3 illustrates that 83.33% (70) cases of severe dengue manifested with the presence of leukoopenia and 16.66% (14) cases had normal TLC. Whereas, in non-severe dengue cases 6.52% (3) cases had leukopenia, while 93.47% (43) cases consisted of TLC within the normal range. Thus, there was a significant positive correlation between leukopenia and severe dengue, as demonstrated by p≤0.001.

FIG 1: Correlation between thrombocytopenia and serum ferritin levels

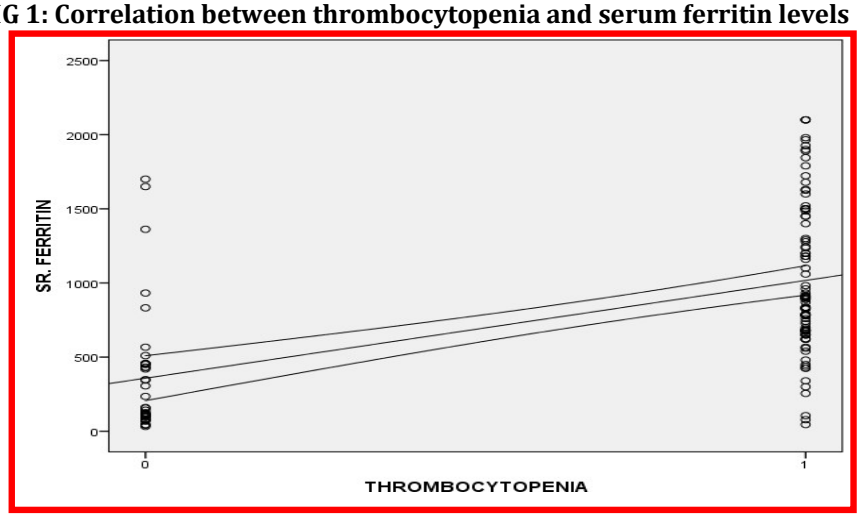
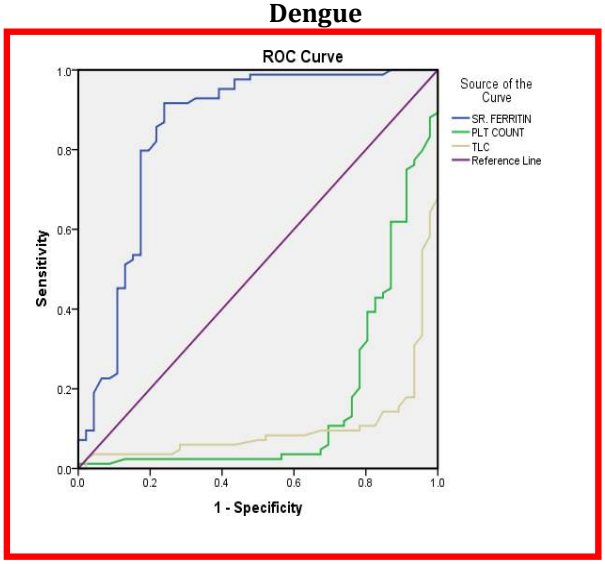


Fig 2 comprises of a scatter plot with regression estimate demonstrating a significant, positive, fair correlation between thrombocytopenia and hyperferritinemia, indicating that an association exists between thrombocytopenia and serum ferritin levels.

FIG 2: Receiver operating characteristic curve for identifying discriminating predictors of Severe Dengue



Area under the Curve:

Test ResultVariable(s)	Area	Std. Error [a]	Asymptotic Sig. [b]	Asymptotic 95% Confidence Interval	
				LowerBound	UpperBound
SR. FERRITIN	.846	.042	.000	.764	.928
PLT COUNT	.160	.041	.000	.080	.241
TLC	.102	.030	.000	.044	.160

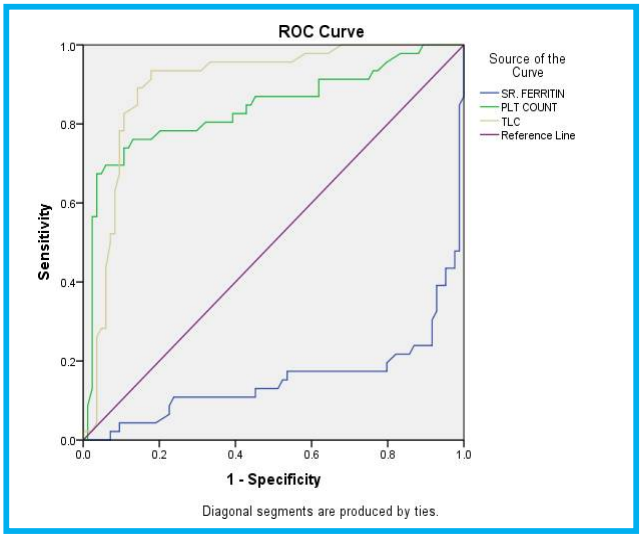


Fig 3: Receiver operating characteristic curve for identifying discriminating predictors in Non-Severe Dengue

Area Under the Curve:

Test ResultVariable(s)	Area	Std. Error [a]	Asymptotic Sig [b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
SR. FERRITIN	.154	.042	.000	.072	.236
PLT COUNT	.840	.041	.000	.759	.920
TLC	**.898**	.030	.000	.840	.956

DISCUSSION

Ferritin is an acute phase reactant that is produced in our body as response to any inflammation by reticuloendothelial cells. In the present study we could observe that dengue infected patients had an increase in serum ferritin. Also, it was observed that increased levels of ferritin can be a marker of the disease severity.

The present study results showed hyperferritinemia among severe dengue cases was {90.47%}. However, amongst the non-severe there was only 23.91% cases demonstrating increased ferritin levels. Similarly, a study by VAN de Weg **[**7] concluded that ferritin level were significantly increased in severe dengue compared to non – severe dengue. They observed that severe dengue cases had ferritin levels significantly higher than other febrile illness. In non – severe dengue increase in serum ferritin in comparison to other febrile illness was noticed at day 4-5 of illness.

The present study observed that 99.1% of severe dengue cases had thrombocytopenia, while in non-severe dengue cases 38.8% had low platelet counts. A statistically significant relation was established between dengue severity and platelet drop. Similarly, a study conducted by Praveen A Shinde et al [8] in 2017 observed that 79% of all dengue cases had thrombocytopenia while 21% had normal platelet counts. Their study results showed that 100 % cases with severe dengue had thrombocytopenia, while 44.2% cases comprised of normal platelet counts. Whereas, in the present study 91.66% of severe dengue cases had thrombocytopenia. The present study demonstrated a significant positive correlation between thrombocytopenia and increased ferritin, similar results noted by Petchiappan *et al* **[**9] in 2019 where a significant negative correlation was noted for serum ferritin with platelet counts. ($r=-0.51$, $p<0.001$). Another study conducted by Ahmed F et al demonstrated negative correlation between serum ferritin and lowest platelet count. [14, 8] However, the present study results were not in accordance with a similar study performed by Surendran et al. [10] They assessed the relationship between serum ferritin and thrombocytopenia using Pearson coefficient and this showed a negative correlation

CONCLUSION

Dengue fever is classified as dengue fever with or without warning signs and severe dengue. This study demonstrated significant association between elevated ferritin levels and severe dengue. Thrombocytopenia and leukopenia were also significantly associated with severe dengue. Thus, serum ferritin can be used as an adjunct marker to delineate cases of severe dengue and may serve as a marker for early prediction of severe disease. There was a positive correlation existing between rising ferritin levels and thrombocytopenia, indicating higher levels of serum ferritin was associated with decreased platelet counts. Severe dengue cases required a longer duration of hospital stay. The distinctive characteristic of this study was that serum ferritin was performed only once (between 3rd and 7th day of illness), compared to various other previous studies where serum ferritin levels were serially monitored. Thus, it is easier, simpler, and convenient and causes less discomfort to the patients, when serum ferritin levels are measured on a single day, which is practically more feasible. Further studies are required to evaluate whether serum ferritin levels can be used to differentiate between dengue fever and other febrile illnesses.

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