Serum Ferritin: A Biomarker to predict Disease Severity in Patients with Dengue Infection

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Abstract
Background: *Each year, up to 400 million people are infected and 22,000 die from severe dengue globally. An important cause of acute febrile illness especially in tropics and subtropics in the world is dengue fever. It is approximated that one million dengue cases are reported to WHO from these regions. Aim of the study was early detection of dengue severity using ferritin as a biomarker in dengue patients.*
Methods: *A retrospective study was conducted on 50 patients in Department of Medicine from November 2018 to December 2019. Data of all patients admitted with dengue were collected and analysed with special reference to serum ferritin.*
Results: *In 50 patients, majority were in the age group of 20-40 years. Mean ferritin level was much higher in severe dengue patients i.e. 861.28 ± 183.17 as compared to patients with non severe dengue i.e. 400 ± 151.7. Serum ferritin levels was high in patients with severe dengue as compared to non severe forms.*
Conclusion: *Serum ferritin levels may serve as a biomarker to predict dengue disease severity*
Keywords: *severe dengue, serum ferritin, dengue fever.*

Introduction

Dengue, a mosquito borne viral infection has become a significant public health problem with an estimated 3.9 billion people in 128 countries being at risk of infection.[1,2] Each year, up to 400 million people are infected and 22,000 die from severe dengue globally.[3] An important cause of acute febrile illness especially in tropics and subtropics in the world is dengue fever. It is approximated that one million dengue cases are reported to WHO from these regions.[4]The

dengue virus which has four serotypes is transmitted to humans by Aedes mosquitoes especially Aedes aegypti. The four serotypes of dengue virus cause anywhere from asymptomatic infection to severe dengue.[5]

The illness is divided into 3 distinct phases {1}A nearly febrile phase was lasting from day 1 to day 7{2}A critical phase from day 3 to day 8{3} Convalescent-phase.

The dengue illness can proceed to potential complications like dengue haemorrhagic fever

(DHF) and dengue shock syndrome (DSS), both defined as severe dengue by WHO. The commonly used investigations to identify the illness are NS1 ANTIGEN (non-structural protein 1), IgM and IgG by MAC-ELISA/RAPID KIT TEST. [6,7] Clinically dengue fever is suspected when acute febrile illness of 2–7 days presents with two or more than two of the following, namely, headache, retroorbital pain, myalgia, arthralgia, rash, and haemorrhagic manifestations[8] Ferritin is an acute phase reactant released by the reticuloendothelial cells. Hyperferritinemia is associated with immune activation and coagulation disturbances.[9] Currently, no tests are available to monitor or predict severity and outcome of dengue, present study was aimed to validate serum ferritin level as a marker of severe dengue as compared to non-severe dengue.

Methodology

This was a retrospective study conducted on 50 patients in Department of Medicine, Dr. D.Y.Patil Medical and Research Centre, Pune from November 2018 to December 2019. The data of all patients admitted with dengue at Dr D.Y.Patil Medical and Research Centre, Pune were collected and analysed. Patients admitted were evaluated for CBC, Ferritin, TROP T, CPKMB, SGOT, SGPT, Albumin levels, Chest X-Ray, USG, ECG and 2D Echo.

Inclusion Criteria

Patients admitted with the diagnosis of Dengue fever on the basis of history, clinical examination and positive non-structural protein 1 (NS1) antigen or positive IgM antibodies for Dengue were included in this study.

Exclusion Criteria

Patients having microcytic anaemia, presenting with DHF or DSS were excluded, Patients other than dengue presenting as acute febrile illness were excluded and pregnant women were also excluded.

Statistical Analysis

The data was entered in Microsoft Excel 2010 version spreadsheet and analysis was done by EPI INFO (version 7) provided by CDC Atlanta. Tables were prepared for the variables. Pearson chi-square test was used for categorical variables in the study. Results on continuous measurements are presented on mean ± SD. In our study 'p' value less than 0.05 was considered to be significant.

The ethical clearance was obtained from the Institutional Ethical committee.

Procedure

Patients were divided into two groups on the basis of ferritin levels.

Normal levels of serum ferritin were considered between 24-300ng/dl.

Patients having levels more than 600ng/dl were considered high serum ferritin levels.

Group A consisted of patients with ferritin level<600ng/dl and those >600ng/dl were included in Group B. Severe dengue was defined as patients having warning signs like mucosal bleeding, increasing haematocrit, decreasing platelet count, fluid accumulation, abdominal pain, severe plasma leakage, severe bleeding and organ failure.

Discharge from the hospital without complications or developing severe dengue during the hospital stay was taken as end point of study

Dengue IgG,IgM and NS1 was estimated by BENESPHERA ELISA Microplate Washer W21,BENESPHERA ELISA Microplate Reader and E21 LISAWASH 4000 ELISA Microplate Washer.

Serum ferritin level of all the patients was sent to pathology department and was measured by ELISA on the day of admission.
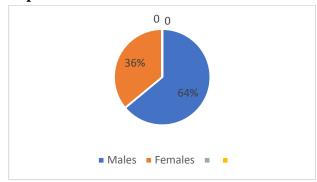
Results

This study was conducted on 50 patients admitted with diagnosis of dengue fever

Following Graph and Tables show the observations of the study

Graph 1: Gender distribution

Out of the total 50 patients, 64% were males i.e.32 patients and 36% were femalesi.e.18 patients.

Table 1: Age wise distribution

Age group	<20yrs	20-40yrs	>40yrs
Male	8	20	4
Female	1	13	4

Of 50 patients, majority were in the age group of 20-40 years followed by less than 20 years and more than 40 years respectively.

66% (33) patients were in 20- 40 years,18% (9) patients in <20 years and 16% (8) patients in >40 years.

In this study, Mean patient age was 31.5 ± 11.2 years.

Mean duration of hospital stay was 4 ± 1.12 days.

Mean duration of fever was 5.3 ± 1.81 days.

Table 2: Gender wise distribution of serum ferritin levels

Serum ferritin(ng/dl)	male	female	Total
<600	17	13	30
>600	15	5	20
Total	32	18	50

In Patients with serum ferritin levels <600ng/dl, 17 were males and 13 were females and serum ferritin levels >600ng/dl, 15 were males and 5 were females.

Table 3: Patient wise distribution of serum ferritin levels

Serum ferritin levels	Number of patients
<300	2
300-400	16
401-500	7
501-600	5
601-700	4
701-800	8
801-1000	6
>1000	2

This table shows serum ferritin levels ranging from <300 to >1000ng/dl in the study subjects.

Mean ferritin level was much higher in severe dengue patients i.e. 861.28 ± 183.17 as compared to patients with non severe dengue fever i.e. 400 ± 151.7.

Table 4: Association between serum ferritin level and severe dengue illness

Severe dengue	Serum ferritin (<600ng/dl)	Serum ferritin (>600ng/dl)	P value
No	28	4	
Yes	2	16	0.001

Chi square test was used to compare categorical variables and to predict the correlation between serum ferritin and dengue severity.

This table signifies that serum ferritin levels was high in patients with severe dengue at the time of admission compared to non severe forms which was statistically significant with a p value of <0.05.

In group A of 30 patients, 2 had severe dengue with serum ferritin levels of <600 ng/dl; whereas in group B,out of 20 patients 16 had severe dengue with serum ferritin levels of >600 ng/dl.

Out of total 18 severe dengue patients, all of them had plasma leakage in the form of ascites, pleural effusion and pseudo gall bladder wall thickening.

Platelet count levels dropped to 20,000 in 13 patients, remaining 5 patients platelet count dropped till 30,000.

2 patients had dengue myocarditis.15 patients had dengue hepatitis.3 patients had mucosal bleeding and no one had DSS.

Discussion

In a study conducted by Selvamuthukumaran S, the majority of patients belonged to the male gender 59.19 %. Mean hospital stay duration was 5 ± 2 days. 12 patients out of 30 with ferritin levels 200-300ng/dl developed severe dengue, whereas 30 patients out of 49 patients developed severe dengue with ferritin levels 300– 400ng/dl and 46 out of 62 patients developed severe dengue with ferritin levels 400– 500ng/dl and 6 patients who

had ferritin levels 500–600ng/dl had severe dengue.[4]

In my study, out of the total 50 patients, 64% were males (majority) and 36% were females. Mean duration of hospital stay was 4 ± 1.12 days and out of 30 patients, 2 developed severe dengue with serum ferritin levels of <600 ng/dl and 16 out of 20 patients developed severe dengue with serum ferritin levels of >600 ng/dl.

In a study by Nadeem M et al. Mean patient age was 30.7 ± 13.8 years. Mean duration of fever was 5.6 ± 1.3 days. Mean duration of hospital stay was 3.7 ± 1.02 days. In 70% patient's serum ferritin level was found >100 µg/dl, whereas only 30% had ferritin level ≤ 100µg/dl. Mean ferritin levels were much higher in patients with severe dengue i.e. 317.54 ± 109.52 as compared to the patients with uncomplicated dengue fever i.e. 168.69 ± 130.7.[10]

In my study, mean patient age was 31.5± 11.2 years. Mean duration of fever was 5.3 ± 1.81 days. Mean ferritin level was much higher in severe dengue patients i.e. 861.28 ± 183.17 as compared to patients with non severe dengue i.e. 400 ± 151.7.60% patients had serum ferritin values more than 600ng/dl and 40% patients had less than 600ng/dl.

In a study by Ahmed A et al. showed mean duration of fever at presentation was 5.49 ± 2.65 days and mean duration of hospital stay was 2.73 ± 1.41 days. [11]

Study conducted by Soundravally R et al. concluded that raised ferritin levels could predict the dengue severity with sensitivity of 76.9 % and specificity of 83.3% on the day of admission. [12]

Conclusion

Raised serum ferritin levels are significantly associated with severe dengue. Mean ferritin levels are also high in patients with severe dengue as compared to dengue fever.

Serum ferritin levels on the day of admission may serve as biomarker for an early prediction of disease severity in dengue virus infection.

Limitation

A major limitation of this study was small sample size.

Abbreviations

WHO- World Health Organization

DHF- Dengue Haemorrhagic Fever

DSS- Dengue Shock Syndrome

CBC- Complete Blood Count

USG- Ultra Sonography

ECG- Electro Cardio Gram

SGOT- Serum Glutamic Oxalo Acetic Transaminase

SGPT- Serum Glutamic Pyruvic Transaminase

SD- Standard Deviation

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