

ORIGINAL RESEARCH ARTICLE

A Study of Serum Ferritin Level in Patients of Dengue Fever and Its Correlation with the Severity of Dengue Fever in Tertiary Care Hospital of South Gujarat

Maulikkumar K Prajapati¹, Urvi C Patel², Pritesh G Patel³, Chintan M Kakadiya⁴, Kamalchandra P Naik⁵, Deepika Patel⁶

Authors' Affiliations: ¹⁻⁶Surat Municipal Institute of Medical Education and Research, Surat, India

Corresponding Author: Dr. Kamalchandra P Naik, Email: drkpnaik@gmail.com

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ABSTRACT

Introduction: In more than 100 endemic countries, there are 50 to 100 million new cases reported per year, according to the WHO. The dengue pandemic has caused significant death. There is usually only supportive care offered and no effective therapy. The purpose of the study was assess any effect on the level of serum ferritin in patients of dengue.

Methodology: This was an observational, Prospective study conducted at Surat Municipal Institute of Medical Education and Research General Medicine department among dengue fever cases.

Result: Serum ferritin level from on admission then on day 3 and then day 5 in non-severe and severe dengue cases showed p value <0.001 which was statically significant.

Conclusion: The use of a serum ferritin level alone with a cut-off value of more than 900 ng/ml is indicated.

Keywords: Dengue fever, serum ferritin, Dengue shock syndrome, Dengue hemorrhagic fever

INTRODUCTION

The flavi virus, which produces undifferentiated viral fever to serious consequences including haemorrhages, hypotensive shock, and involvement of several organ systems, is the source of dengue, the fastest spreading arthropod-borne viral illness known to humankind. It has recently spread throughout the entire world and is an epidemic.

According to the WHO, there are 50 to 100 million new cases each year in more than 100 endemic nations. Significant mortality has been caused by the dengue pandemic. There is no effective therapy and frequently just supportive care is provided.

While most dengue infections are asymptomatic or appear as an undifferentiated viral fever, some can cause bleeding and plasma leakage, leading to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Only a small percentage of instances might develop difficulties, which need for careful monitoring and assessment. Most patients heal without any issues. [1]

To start the necessary rigorous and supportive care, it is crucial to estimate the risk of development to severe dengue as soon as possible using straightforward, quantifiable assays. The term "severe dengue" refers to shock brought on by fluid plasma leakage, whether it is accompanied by respiratory difficulty, severe bleeding, or severe organ dysfunction. Less than in prior years, the earlier reported case mortality from dengue infection ranged from 2.5% to 5.4%.

The most significant causes contributing to dengue infection mortality have been demonstrated to be shocked and organ dysfunction. [2]

The only options currently are early identification of severe dengue fever patients, rigorous hydration management, and monitoring for sequelae as there is no approved vaccine or effective antiviral medication to prevent infection. We need a measure to gauge the severity of the same to anticipate problems and identify which cases need monitoring.

In the current investigation, we investigated whether serum ferritin measurements made early in the course of the disease may be utilized as a marker to identify the severity of the condition and could aid in accurately classifying and managing patients.

METHODOLOGY

Study was started in Department of General Medicine-IPD patients, at Tertiary care Hospital, Surat the study was carried out on 100 patients admitted during the period of June 2020- November 2021 the hospital. This was an observational, Prospective study. study was started After taking ethical approval, 12 months for enrolling the patients and 6 months for compiling, tabulating, analyzing and finalizing the results. Study was including all those cases who had follow this Inclusion criteria (for cases) Patients who meet all the following criteria will be included in the study Patients

>18 years admitted in SMIMER hospital medicine ward satisfying WHO Criteria for confirmatory diagnosis as DF, DHF & DSS. Patient willing to participate with informed consent, those cases who had follow this criterion were excluded from this study. Patients with fever of any other aetiology, Patients <18 years of age, Patients with known liver, kidney and coagulation disorders. Patients with chronic inflammatory disease. Patients of known case of anemia with Hb<10gm/dl. Patients with known iron overload status (hemochromatosis, multiple blood transfusions). Patients not willing to participate and doesn't give consent. Sample size was calculated by considering the proportion of Dengue fever in Surat city between 2020-2021 and data recorded is P=16.6%; (P value is the proportion of number of cases of dengue fever outdoor/indoor admitted in wards/ICU).

The sample size was calculated using formula $Z_{\alpha-1}^2 PQ/L^2$ where P is prevalence 16.6%, Q is 1-P, L is allowable error which was kept at 6% and $Z_{\alpha-1}$ is level of significance error which was 95%. The calculated sample size was 154.

A detailed history was taken followed by a detailed clinical examination to assess clinical severity and complications. All the patients in this study were proved to be cases of dengue by positive serological tests. All the investigations were sent before the treatment was started. Once the patient was diagnosed to have dengue, they were started on fluid therapy and symptomatic treatment according to the new WHO guidelines for treatment of Dengue. Other supportive treatment was given according to the patient's conditions. Patients diagnosed as dengue fever admitted in medicine wards. Serum ferritin levels was measured on the day of admission (Day1), 3rd and 5th day of admission in hospital which was done by ELISA. While collecting blood for serological studies from suspected Dengue cases, all universal precautions were taken considering all human specimens as bio-hazardous materials. A structured clinical proforma was used for data collection in which all the details of the patients were recorded. Information regarding clinical history, general examination along with demographic variables were recorded. While sending the samples for laboratory confirmation, the day of sample collection was mentioned to guide the laboratory for the test to be performed (NS1 for samples collected from day 1 to day 5 and IgM after day5). Patient evaluation was done date wise from the day of admission to the day of discharge.

Method of serum ferritin estimation

Immune enzymatic colorimetric method is used for quantitative determination of Serum Ferritin concentration in human serum or plasma. Ferritin ELISA Assay Kit which follows a 'sandwich type' of assay is used for the estimation of ferritin in human serum quantitatively. The principle of Ferritin ELISA test is based on consecutive binding of human Ferritin to two monoclonal antibodies, one monoclonal antibody specific to ferritin immobilized on micro well plates and the other conjugated with horse radish peroxidase (HRP). After incubation, they are allowed to bind simultaneously to the plate and HRP to get conjugated. The bound/free partition is performed by a simple solid phase washing which removes any unbound HRP conjugate. The reaction is terminated by adding the stopping solution. The enzyme HRP in the bound fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution

(H_2SO_4) is added. The absorbance is measured on a micro titre plate reader and the colour intensity is seen which is proportional to the Ferritin concentration in the sample. The Ferritin concentration in the sample is calculated based on a standard curve which is based on the set of standards used to plot the curve. For serum ferritin approximately around 0.2-0.5 ml of serum is required per duplication method. Collection of 3-5 ml of blood is preferred in a properly labelled tube and allowed it to get clotted.

It is then centrifuged, and upper layer of serum is removed carefully. The serum is then stored up to 4°C for 24 hours or at -10°C.

Method of blood collections well as advantage and disadvantage of study was explained to the patients. The personal identification of the patients like name, address etc. We're not entered during the preparation of the data spread sheet. Unique identifiers were allotted to all the patients and only the unique ID was entered during data entry and analysis. All the patients' Performa were kept securely under lock and key with the principal investigator. Patient were educated about clinical features of dengue fever. Patient were explained about results of dyslipidaemia in chronic kidney disease and appropriate management was advised. Individual written and informed consent was taken from the patients who were included in this study consent for allowing the clinical data to be used for study purpose. All data were entered in MS Excel 2007 and statistical analysis was performed using the statistical software SPSS 1.6.0. and Openepi version 3.01. The statistical analysis was done by appropriate statistical method.

Statistical method: Descriptive analysis was explained by frequency and percentage. Test of significance was applied using Pearson's chi-square test for categorical variables and Mann Whitney U-test for continuous variables to find the differences between two groups. Results were defined as statistically significant when the P value was less than 0.05.

RESULT

Table 1 shows that demographic variables of dengue cases, as per age distribution of cases 18 to 30 years group contributed 61 (61%) of total cases. While gender distribution of male group was contributed 61 (61%) of total cases. In addition to severity of dengue majority of cases were belong from non-severe dengue which was 72 (72%) of total cases while only 28 (28%) of cases were belong from severe dengue manifestation. Furthermore, duration of fever majority of cases had 3 to 5 days fever which contribute 63 (63%) of total cases. While most common complication of dengue was hepatitis which was noted in 28 (28%) of total cases.

Table 2 shows comparison of serum ferritin level in non-severe and severe dengue cases. The comparison from day 1, day 3 and day 5 were not show any statistically significant.

Table 3 shows that comparison of serum ferritin level from on admission then on day 3 and then day 5 in non-severe and severe dengue cases were show p value <0.001 which was statically significant.

DISCUSSION

Hepatic cells, monocytes, and macrophages all contain significant amounts of ferritin, an acute phase reactant.

Table 1 Demographic variables of cases

Demographic Variables	Cases (%)
Age	
18-30	61 (61 %)
31-40	19 (19 %)
41-50	12 (12 %)
51-60	6 (6 %)
>60	2 (2 %)
Gender	
Male	61 (61 %)
Female	39 (39 %)
According to dengue severity	
Non severe	72 (72 %)
Severe	28 (28 %)
Duration of fever	
<2 Days	10 (10 %)
3-5 Days	63 (63 %)
6-10 days	25 (25 %)
>11 days	2 (2 %)
According to severe dengue manifestations	
ARDS	15 (15 %)
Shock	23 (23 %)
Bleeding	26 (26 %)
Hepatitis	28 (28 %)
AKI	8 (8 %)
Encephalitis	1 (1 %)
Myocarditis	0 (0 %)

Table 2: Comparison of serum ferritin level in non-severe and severe dengue

Day of test	Serum Ferritin level		
	Non severe dengue	Severe dengue	P value
Day 1	694.3 ± 247.5	709.8 ± 245.01	0.988
Day 3	859 ± 235.3	884.2 ± 226.08	0.842
Day 5	763.5 ± 194.6	778.6 ± 181.04	0.691

Table 3: Comparison of serum ferritin level according to days of treatment in non-severe and severe dengue

Serum Ferritin	Severe dengue cases	Non severe dengue cases	P value
Cases	28	72	
On admission (Day 1)			
< 900 (ng/ml)	5 (17.85%)	66(91.66%)	<0.0001
> 900 (ng/ml)	23 (82.14%)	6(8.33%)	
Days of treatment (Day 3)			
< 900 (ng/ml)	4 (14.28%)	52(72.22%)	<0.0001
> 900 (ng/ml)	24 (85.71%)	20(27.77%)	
Days of treatment (Day 5)			
< 900 (ng/ml)	8 (28.57%)	60(83.33%)	<0.0001
> 900 (ng/ml)	20 (71.42%)	12(16.66%)	

It has been demonstrated that cytokines and iron can stimulate the creation of ferritin. We demonstrated that elevated ferritin levels have been linked to a proinflammatory cytokine profile. In our study gender distribution of male group was contributed 61(61%) while female was contributed 39(39%) of total cases.as per Yagnik H. Chhotala, Chetal M et al study 66% were male and 34% were females. While as per S. Selvamuthukumar et al study most patients belonged to the male gender 59.19 % (n = 87).[3]A research done at Singapore revealed that NS1Ag positive patients with fever due to dengue virus were male (71%).[8] This discrepancy can be explained by Surat's poor sex ratio and its greater than average population of young males. Males are more likely to get dengue infection than females are due

to increased exposure to dengue-carrying mosquitoes throughout the day, either at work or while travelling to and from work. Another possible explanation is a pattern of health-seeking behaviours that is present in both genders. As per our study age distribution of cases 18 to 30 years group was contribute 61(61%) of total cases This might be because of the large influx of young people moving to Surat from other regions of the state and country. In the working-age demographic of 18 to 30 years old, dengue was often observed. As per Keshva et al the mean age was 30 years, and the age range was 21 to 70 years.

As per our study duration of fever majority of cases had 3 to 5 days fever which contribute 63(63%) of total cases. As per Chaiyaratana w. et al (2008) [4] studies, 172 cases with dengue infection between 2002 - 2005 were included in their study., which showed median duration of febrile period as 5 days.

In our study at day 1 Non severe dengue mean and SD was 694 ± 247.5 severe dengue mean and SD was 709.8 ± 245.01 Similar study conducted in South India by Soundravally R et al, [5] included 48 dengue cases and 48 cases with other febrile illness as controls, serum ferritin levels were measured on the day of admission (which is a median of 4 days after the onset of fever) .Compared to other febrile illness, dengue cases established noteworthy rise in serum ferritin levels; also within clinical groups, severe dengue cases exhibit elevated ferritin levels compared to non-severe cases throughout the stage of illness (940.09 ± 568.31 in non-severe cases vs. 1264.71 ± 492.59 in severe dengue) on day of admission. Compared to our study, our study also showed significant higher levels of serum ferritin in severe dengue compared to non-severe dengue on the day of febrile stage of illness i.e (694 ± 247.5 in severe cases and 709.8 ± 245.01 in severe dengue) respectively.

In our study at day 5 Non severe dengue mean and SD was 763.5 ± 194.6 severe dengue mean, and SD was 778.6 ± 181.04 In a study conducted in South India by Soundravally R et al, [5] which included 48 dengue cases and 48 cases with other febrile illness as controls, serum ferritin levels were measured on day of 5 day of admission. Compared to other febrile illnesses, dengue cases demonstrated significant increase in serum ferritin levels; also, within clinical groups, severe disease exhibited higher ferritin levels compared to non-severe group throughout stage of illness on Day 5 i.e (418.19±404.59 vs. 1490.74±359.40 on day of 5 of admission). Our study also showed significant higher levels of serum ferritin in severe dengue compared to non-severe dengue on the day 5 of admission i.e (778.6 ± 181.04 in severe cases and 763.5 ± 194.6 in severe dengue) respectively.

Our study found that severe dengue cases had higher serum ferritin values on admission day (Day 1) compared to less severe cases, with a statistically significant p-value of 0.001. In contrast to non-severe cases, where only 6 individuals had high ferritin levels over 900 ng/ml, 23 out of 28 cases with severe dengue had serum ferritin levels of more than 900 ng/ml, which is statistically significant. There were 66 and 5 instances of mild and severe dengue, respectively, with serum ferritin levels below 900ng/ml.

Based on the cutoff value of 900 ng/ml for serum ferritin, our current study revealed that on Day 3 of admission, the greatest number of patients were classified as severe dengue

as opposed to non-severe cases, with a statistically significant p-value of 0.001. Compared to non-severe cases of dengue where only 20 had high ferritin levels more than 900ng/ml, 24 severe patients had serum ferritin levels of more than 900ng/ml. There were 52 and 4 instances of mild and severe dengue, respectively, with serum ferritin levels below 900ng/ml.

In severe dengue infections, 20 out of 28 individuals had serum ferritin levels greater than 900 ng/ml on day five after admission, according to the study. This was statistically significant compared to non-severe cases, where only 12 cases had high ferritin levels greater than 900 ng/ml. There were 60 and 8 instances of mild and severe dengue, respectively, with serum ferritin levels below 900ng/ml.

Chaiyaratana w. et al (2008)[4] study showed that both severe and non-severe dengue cases had higher serum ferritin levels during the febrile, toxic and convalescent stages than they did at follow-up. Patients with DHF had elevated levels of serum ferritin than those with DF all the way through the course of the admission. Individuals with DHF grades III and IV had high serum ferritin levels than those with DF and DHF grades I and II.

Cornelia AM Van de wag (2014)[6] conducted a study on the Caribbean Island Aruba, during the outbreak between Sep 2011 - April 2012. It confirmed that ferritin can be used as a clinical marker to classify between dengue and other febrile illnesses. The incidence of hyperferritinaemia in dengue virus infected patients is indicative for highly active disease resulting in immune activation and coagulation disturbances. Therefore, they suggested that patients with hyperferritinaemia should be monitored carefully, as there is a high risk for them to develop severe disease. Our study also showed hyperferritinaemia in severe dengue cases compared to dengue fever cases.

Muhammed Nadeem et al (2016)[7] conducted a study on 104 patients with dengue infection. In that study they concluded that high serum ferritin levels are notably linked with severe dengue. Mean ferritin levels are also high in patients with severe dengue as compared to dengue fever. And Serum ferritin levels on the day of admission may serve as biomarker for an early forecast of disease severity in dengue virus infection. Compared to other studies, our study also showed significant rise in serum ferritin levels during all hospitalized days of admission.

LIMITATION OF STUDY

The dengue infection spectrum is broad, and this study only included hospitalized dengue patients who had severe dengue as well as admitted patients who had non-severe dengue but had warning signals that were positive. This study does not include a sizable group of non-severe dengue patients who get OPD care. We have done the study with a relatively small number of samples, which is a significant restriction. However, this information augments the body of knowledge in the medical literature and suggests that we continue our investigation during subsequent dengue epidemics. In patients with various systemic diagnoses, such as anemia, iron overload states, chronic inflammatory illnesses, and chronic systemic diseases, we did not correlate the level of serum ferritin.

CONCLUSION

As a method of predicting progression to severe dengue during the earlier phase of the disease and continuing to recover, the use of a serum ferritin level alone with a cut off value of more than 900 ng/ml is indicated.

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