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A prospective observational study of serum ferritin levels in dengue fever

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Abstract
Aim: To analyse whether serum ferritin measured during early disease course can be used as a marker to indicate the severity which helps to triage and manage them appropriately.
Material and Methods: The present hospital based prospective observational study comprised of 70 patients admitted in wards and ICU of Department of Medicine, Chattrapati Shivaji Subharti Hospital, Meerut satisfying inclusion criteria during the study period. Patients with NS1 positivity (Days 2–8) and/or positive IgM for dengue (Days 6–10) were considered to be dengue cases and those with other confirmed diagnoses were considered as 'Other febrile illness'. The outcomes was measured as the course of illness whether patients develop warning signs or detoriate to severe dengue, condition at the time of final outcome (discharged healthy or death) and the length of hospitalisation. The study outcome of this study was the correlation between serum ferritin levels and the patient's outcome.
Results: Mean serum ferritin (ng/ml), platelet count and hospital stay (in days) was 1431.82±657.18, 18500±9011.67 and 3.46±5.19 respectively. Mean serum ferritin level and hospital stay (in days) was comparatively more among subjects suffering from severe dengue as compared to subjects having non severe dengue with statistically significant difference as $p<0.05$. Significant negative correlation was found between serum ferritin level and platelet count while significant positive correlation was found between serum ferritin level and hospital stay.
Conclusion: Thus, in conclusion, serum ferritin level may be considered as a tool to predict the progress of dengue severity.

Keywords: dengue, severity, ferritin

Introduction

Dengue infection poses a major health problem in tropical and subtropical countries [1]. According to 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; of which around 500000 people with severe dengue require hospitalization each year and about 2.5% of those affected die[2]. The burden of the disease is so much that in the year 2012 World Health Organization classified the disease as "the most important viral disease that is transmitted by mosquitoes" [3]. Dengue is caused by dengue virus serotypes I-IV and transmitted to human beings by the bite of Aedes mosquitoes [4, 5].

The four serotypes of dengue virus cause anywhere from asymptomatic infection to severe dengue [6]. The illness is divided into 3 distinct phases i.e. an early febrile phase was lasting from day 1 to day 7, a critical phase from day 3 to day 8 and Convalescent-phase. The dengue illness can proceed to potential complications like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), both defined as severe dengue by WHO [7].

Dengue fever is diagnosed by NS1 antigen reactivity by ELISA method usually for the first 5 days of fever. After that IgM detection by MAC-ELISA is used to diagnose dengue fever but IgM appears usually within 5–7 days of fever but sometimes it may take more time, even up to 12 days, to appear [8]. Detection of NS1 antigen is a fair tool for diagnosing dengue virus infection (DVI). The sensitivity of NS1 for diagnosis is more than 90% within 2-3 days of illness. But the sensitivity gradually decreases after that period and it is even lower beyond 5th day [9]. Detection of dengue virus specific IgM can also diagnose DVI with a good sensitivity and specificity. In patients not previously infected with dengue virus, this IgM response is slow rising. It is 50% in 3–5 days, 80% in more than 5 days, and 99% in 10th day [10]. Furthermore, IgM dengue antibody may be nondetectable till 8th day of illness [11].

In dengue fever, serum ferritin is disproportionately raised compared to any bacterial or viral infection and this elevated level corroborates with an increased risk of developing complications [11]. Ferritin is an iron storage protein complex of isoferritins produced by the reticuloendothelial (RE) system. The RE system plays a critical role in iron metabolism by processing hemoglobin from senescent red blood cells. Acute inflammation and infection induce the blockade of iron release resulting in a decreased serum iron, a virulence factor for many microorganisms. Elevated levels of serum ferritin, an acute-phase reactant, reflect the clinical response to deprive microorganisms of serum iron [12-15]. Elevated serum ferritin levels have been demonstrated in patients

with West Nile encephalitis beyond those attributable to the acute-phase reaction of infection, correlating with severity of disease [16, 17].
Results from the previous studies had shown that hyperferritinemia is associated with severe disease which is noted throughout the disease course [18]. It is very crucial to predict the risk of progression to severe dengue at the earliest, by simple measurable tests to initiate appropriate intensive, supportive therapy. In the present study, we analysed whether serum ferritin measured during early disease course can be used as a marker to indicate the severity which helps to triage and manage them appropriately.

Material and Methods
The present hospital based prospective observational study was done among 70 patients admitted in wards and ICU in the Department of Medicine, Chattrapati Shivaji Subharti Hospital, Meerut. Patients with NS1 positivity (Days 2–8) and/or positive IgM for dengue (Days 6–10) were considered to be dengue cases.

Sample Size Calculation
Sample size was determined by using the effect sizes from the previously published study (Soundravally R *et al*., Infection 2014) and with the help of following formula:

$n=z^2pq/d^2$

p = 0.769 (76.9%) (Approximate estimate of sensitivity of elevated Serum ferritin levels to predict severe dengue).
q=0.231(23.1%) (Compliment of 'p').
Z=1.96 (scoreat95%confidenceinterval),
d^2= 0.10 (margin of error).
n=1.96^2*0.769*0.231/(0.10^2)=68.24.
Thus, the minimum sample size required according to this formula was 68.24 or 68.

Inclusion Criteria
1. All patients of age satisfying the definition of dengue fever according to WHO 2012 definition.
2. Both males and females
3. Febrile patients with serology positive dengue from outside labs were included in the study.

Exclusion Criteria
1. Patients who refuse to give consent for participation in the study,
2. Pregnant women.
3. Any patient who take leave against medical advice and transfer to other institute and whose final outcome cannot be known were excluded before the final statistical analysis.

Method of measurement of outcome of interest
All the patients hospitalized with the diagnosis of dengue fever underwent estimation of serum ferritin levels at the time of diagnosis. The course and outcome of illness for the entire duration of length of stay was monitored. The outcomes was measured as the course of illness whether patients develop warning signs or detoriate to severe dengue, condition at the time of final outcome (discharged healthy or death) and the length of hospitalisation. The study outcome of this study was the correlation between serum ferritin levels and the patient's outcome.

Methodology
Study was undertaken only after obtaining the approval of scientific committee and the ethics committee of the institute. Inpatients admitted with undiagnosed cause of fever, serum levels of Ferritin, TC of WBC, platelet count and haematocrit was measured. Dengue serology was followed up for positivity. Patients with NS1 positivity (Days2–8) and/or positive IgM for dengue (Days6–10) prior to discharge was considered as dengue cases. We excluded data of all the patients in whom other causes of fever were confirmed as the diagnosis.
Dengue IgG and IgM are estimated in the hospital laboratories by micro ELISA kit and NS1 positivity is also estimated by ELISA kit. Serum Ferritin levels are estimated by Immuno-luminescence method by Cobas-E411instrument. Hemoglobin, TC, DC, platelet and haematocrit are estimated by photometric evaluation with auto-analyzer LH750, 780.

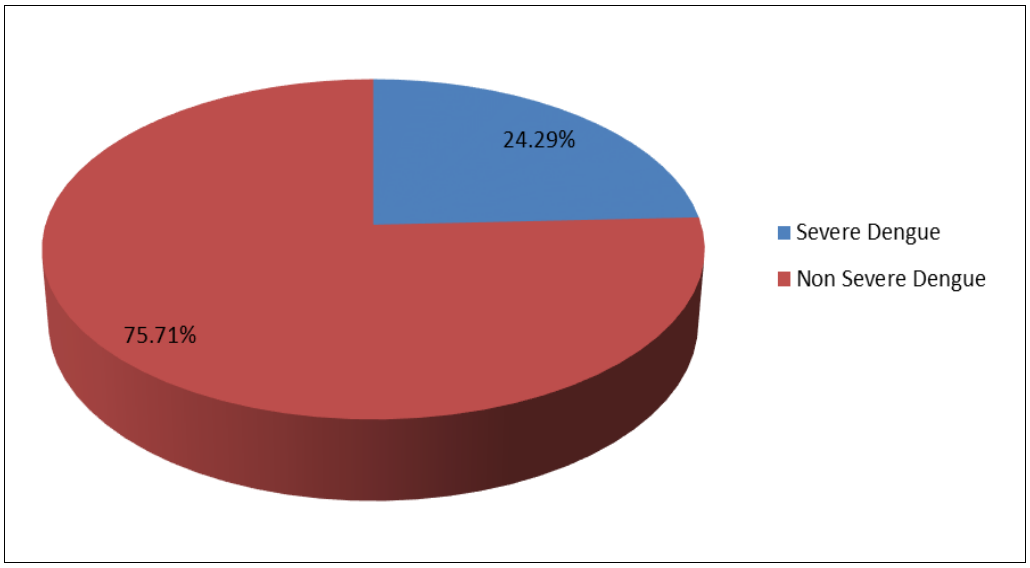
Statistical analysis
Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). Difference between two groups was determined using student t-test and the level of significance was set at $p < 0.05$.

Results
Out of 70 subjects, 65.71% and 34.29% were male and female respectively. 15.71%, 70.00% and 14.29% of the subjects belonged to <20 years, 21-40 years and >40 years of age respectively. Mean age among the study subjects was 34.61±12.79 years (table 1).

Table 1: Gender and age distribution among the study subjects

Gender	N	%
Male	46	65.71
Female	24	34.29
Age Group (in years)		
<20 Years	11	15.71
21-40 Years	49	70.00
>40 Years	10	14.29
Mean±SD	34.61±12.79	

Severe and non severe dengue was reported among 24.29% and 75.71% of the subjects respectively (graph 1). Bleeding, shock, organ dysfunction and respiratory distress were found among 82.35%, 58.82%, 41.18% and 17.65% of the subjects respectively.



Graph 1: Distribution of subjects according to dengue severity

Mean serum ferritin (ng/ml), platelet count and hospital stay (in days) was 1431.82±657.18, 18500±9011.67 and 3.46±5.19 respectively (table 2).

Table 2: Distribution of serum ferritin, platelet count and hospital stay among the study subjects

Parameters	Mean	SD	Median
Serum Ferritin (ng/ml)	1431.82	657.18	1922
Platelet Count*$10^{9/l}$	18500	9011.67	15650
Hospital Stay (in days)	3.46	5.19	7

Mean serum ferritin level and hospital stay (in days) was comparatively more among subjects suffering from severe dengue as compared to subjects having non severe dengue with statistically significant difference as $p<0.05$. Mean platelet count was comparatively more among subjects suffering from non severe dengue as compared to subjects having severe dengue with statistically significant difference as $p<0.05$ (table 3).

Table 3: Comparison of serum ferritin, platelet count and hospital stay among the study subjects according to illness.

Parameters	Severe Dengue		Non Severe Dengue		p value
	Mean	Median	Mean	Median	
Serum Ferritin (ng/ml)	1892.78	1950	1391.38	1540	0.02*
Platelet Count*$10^{9/l}$	8230.13	8000	27190.27	26500	<0.01*
Hospital Stay	12.80	14	5.62	4	<0.01*

*: statistically significant

Table 4 shows the correlation between serum ferritin and platelet count as well as and hospital stay. Significant negative correlation was found between serum ferritin level and platelet count while significant positive correlation was found between serum ferritin level and hospital stay.

Table 4: Correlation between serum ferritin and platelet count as well as and hospital stay

Parameters	Serum Ferritin (ng/ml)	
	r value	p value
Platelet Count*$10^{9/l}$	-0.39	0.03*
Hospital Stay	0.51	<0.01*

*: statistically significant

Discussion

Ferritin is an acute phase reactant released by the reticuloendothelial cells. Hyperferritinemia is associated with immune activation and coagulation disturbances.[19] 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.

In our study, out of 70 subjects, 65.71% and 34.29% were male and female respectively. Similar male dominance was reported by Selvamuthukumaran S[7] and SA Kanitkar *et al* [20] in their studies.

15.71%, 70% and 14.29% of the subjects belonged to <20 years, 21-40 years and >40 years of age respectively. Mean age among the study subjects was 34.61±12.79 years in our study. In a study by Nadeem M *et al* [21], mean patient age was 30.7 ± 13.8 years. SA Kanitkar *et al* [20] in their study found that majority were in the age group of 20-40 years followed by less than 20 years and more than 40 years respectively. Mean patient age was 31.5± 11.2 years.

Mean serum ferritin (ng/ml), platelet count and hospital stay (in days) was 1431.82±657.18, 18500±9011.67 and 3.46±5.19 respectively in this study. Mean serum ferritin level and hospital stay (in days) was comparatively more among subjects suffering from severe dengue as compared to subjects having non severe dengue with statistically significant difference as $p<0.05$. Mean platelet count was comparatively more among subjects suffering from non severe dengue as compared to subjects having severe dengue with statistically significant difference as $p<0.05$. Significant negative correlation was found between serum ferritin level and platelet count while significant positive correlation was found between serum ferritin level and hospital stay.

Similarly Petchiappan V[5] in their study reported that hyperferritinemia is associated with severe dengue which is noted in other studies as well. To analyze whether serum ferritin can predict the severity earlier in the course of illness, day wise serum ferritin levels was compared in both the sub-groups; the serum ferritin levels were higher in severe dengue cases when compared to non-severe group and this increase is noted from day 4 of illness onwards which clearly indicates that serum ferritin can predict the severity of dengue at an earlier stage.

In a study by Nadeem M *et al* [21], 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. SA Kanitkar *et al*[20] in their study showed 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. Study conducted by Soundravally R *et al* [8], 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. A study conducted during a dengue outbreak on the Caribbean island Aruba which compared the ferritin levels in dengue infected patients with those having other viral illnesses concluded that ferritin is a useful clinical marker to differentiate dengue from other febrile illnesses; serum ferritin levels >1500ng/ml is associated with severe disease; serum ferritin levels measured on day 4-5 better predicts the outcome of dengue infection and it concluded that these patients with hyperferritinemia should be monitored carefully for complications [22].

Another study conducted in one hundred seventy-seven Thai children which measured serum ferritin levels during the entire clinical course (febrile, toxic and convalescent stages) showed similar results. Increased serum ferritin was associated with severe dengue; patients with non-severe dengue had serum ferritin levels less than 1,200ng/ml during the entire course of illness, while severe forms recorded higher levels of serum ferritin during the febrile stage and the defervescence stage [17]. In a study conducted in South India by Soundravally R *et al*, which included 48 dengue infected 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) and day of defervescence (which is a median of 4 days after the day of admission) [8].

One another study showed significant association between raised serum ferritin levels on the day of admission and the development of severe dengue. Serum ferritin was measured on the day of admission in a total of 104 dengue positive cases and they were grouped into a) Group A with normal ferritin levels (up to 100µg/dl) and b) Group B with high serum ferritin levels; both the groups were followed up for the occurrence of severe dengue.[23] Severe dengue was noted only in 2 out of 31 patients with normal serum ferritin levels while more than half of the patients with severe dengue (35 out of 73) had raised serum ferritin levels. The study concluded that serum ferritin can be used as an early marker to predict the severity of the disease [5].

Increased expression of acute phase reactants is observed in patients with severe dengue infection when compared to non-severe cases. This serves to prognosticate the dengue infected patients well ahead of the appearance of clinical warning signs. One such acute phase reactant is ferritin which is produced by reticuloendothelial cells in response to infection and inflammation. Ferritin is highly elevated in dengue infected patients than in patients with other febrile illnesses. Hyperferritinemia seen in these patients exhibit two opposite functions; early in the phase of clinical illness, increased serum ferritin levels exert a protective effect by chelating the toxic free iron radicals at the site of inflammation, while in severe cases, raised ferritin may assume a pathogenic role by activating immune cells resulting in cytokine storm [5].

Limitations

The present study has few limitations as well. First, is the small number of severe dengue observed in the present study; hence we were unable to derive the cut-off value of day wise ferritin levels which can predict the risk of development of severe dengue. A larger sample size with more number of severe cases would be appropriate before arriving at a definite conclusion. Also, the nature of infection (primary or secondary); dengue serotypes were not taken into consideration. To address the limitations in the present study, future studies are needed to find out the cut off level of serum ferritin which can predict severe dengue at the earliest for effective disease management.

Conclusion

Thus, in conclusion, serum ferritin level may be considered as a tool to predict the progress of dengue severity. Considering the fact that measurement of serum ferritin level is a fast and low-cost lab test, biosensors may be developed to use ferritin as a marker in a clinical setup for effective disease management

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