

## Original Research Article

# Can serum ferritin levels predict the severity of dengue early?: an observational study

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### ABSTRACT

**Background:** Dengue infection is a major public health threat; early recognition is crucial to improve the survival in severe dengue. Although there are various biomarkers to predict the severity of dengue, they are not routinely used in clinical practice for prognostication. We analyzed whether serum ferritin can be used to predict the severity at an earlier stage.

**Methods:** A hospital based prospective observational study was done involving 119 dengue cases diagnosed by positive NS1 antigen or dengue specific serology (capture ELISA). Serum ferritin was measured in all at the time of diagnosis. Clinical and platelet count monitoring was done daily; classified as severe and non-severe according to 2009 WHO criteria.

**Results:** Out of 119, 5 developed severe dengue; patients with severe dengue had significantly lower median platelet count ( $p < 0.0001$ ); higher ferritin levels ( $p = 0.03$ ) and hospital stay ( $p < 0.0001$ ) than non-severe group. Age had a significant negative co-relation with platelet count ( $r = -0.427$ ;  $p < 0.0001$ ); positive correlation with ferritin levels ( $r = 0.16$ ;  $p = 0.08$ ) and hospital stay ( $r = 0.26$ ;  $p = 0.004$ ) indicating that elderly subjects are at risk of severe disease. Serum ferritin levels negatively correlated with the platelet count ( $r = -0.51$   $p < 0.001$ ). High ferritin levels in severe cases are noted from day 4 of clinical illness.

**Conclusions:** Elevated serum ferritin levels can be used as a potential early prognostic marker to predict the severity of dengue infection in clinical practice.

**Keywords:** Predictor of severity, Serum ferritin, Early, Severe dengue

### INTRODUCTION

Dengue infection poses a major health problem in tropical and subtropical countries.<sup>1</sup> 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.<sup>2</sup> 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”.<sup>3</sup> Dengue is caused by dengue virus serotypes I-IV and transmitted to human beings by the bite of *Aedes* mosquitoes.<sup>4</sup>

The clinical spectrum of dengue infection is variable from mild fever to severe forms of dengue such as dengue hemorrhagic fever and dengue shock syndrome. Severe dengue is characterized by severe thrombocytopenia with major bleeding, plasma leakage resulting in fluid accumulation, respiratory distress and

multi-organ dysfunction.<sup>5</sup> The presence of certain clinical warning symptoms which help to predict the severity are bleeding, skin rash, nausea, vomiting, abdominal pain and hepatosplenomegaly.<sup>6</sup>

Clinically severe dengue results from interplay between virus related virulence factors and host factors which include inflammatory response of the host to infection with exuberant T and B cell activation, release of cytokines (cytokine storm), altered endothelial function with increased vascular permeability and nutritional status of the host.<sup>7</sup>

In clinical practice, patients with dengue infection are grouped as 1) those with warning signs that need intensive monitoring and aggressive management 2) those without warning signs. Non-severe cases without warning signs can develop into severe dengue later during the course of illness. They are at risk of developing complications related to plasma leakage and thrombocytopenia often at the end of febrile stage of illness (third to fifth day of illness) which if unrecognized can be fatal.

Studies had identified various biomarkers for immune and endothelial cell activation, biochemical and genetic markers to predict the severe dengue.<sup>8</sup> The clinical utility of these markers is limited since measurement of these markers like soluble receptors, cytokines, growth factors, vWF; genetic profiling etc. is technically difficult and not widely available. Acute phase reactants like alpha1 anti-trypsin, ceruloplasmin and ferritin levels are elevated during dengue infection; they have a longer half-life unlike the cytokines, and it is feasible to measure their levels.<sup>9</sup>

Increased serum ferritin has been associated with severe dengue in children.<sup>10</sup> Hyperferritinemia in dengue infected subjects is associated with intense immune activation and coagulation disturbances as noted in Aruba Dengue outbreak.<sup>11</sup> An Indian study had shown that serum ferritin levels are significantly elevated in dengue infected cases compared to the controls; On group analysis, cases with severe dengue had higher ferritin levels than milder forms which was noted both during the febrile and defervescence stages of the illness.<sup>9</sup>

Results from the previous studies had shown that hyperferritinemia is associated with severe disease which is noted throughout the disease course. In a Malaysian study, a large proportion of patients developed severe dengue early during febrile stage.<sup>12</sup> 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.

## METHODS

This is a hospital based prospective observational study conducted from April 2015 to June 2016 in a tertiary care centre, PSG Institute of Medical Sciences and Research Centre (PSGIMS and R), Coimbatore, South India. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Human Ethics Committee, PSGIMS and R. All adult patients who were hospitalized and diagnosed with dengue infection either by dengue NS1 antigen or positive dengue specific serology were included in the study after obtaining their informed consent for participation in the study. Patients with chronic inflammatory conditions or diseases; iron overload status; sideroblastic anemia; thalassemia; malignancy; liver disease were excluded from the study.

### *Study methodology*

The study was based on prospective collection of data in dengue infected patients who fulfill the criteria as mentioned above. Diagnosis was based on NS1 antigen test (done with J. Mitra dengue NS1 Ag Microlisa kit) when the presentation was less than 5 days of illness or positive dengue specific serology (dengue IgM and dengue IgG done by Panbio dengue IgM; dengue IgG capture ELISA) if presented after 5 days of illness. Serum ferritin levels were measured if the presentation was less than 7 days from the onset of fever. After informed consent, 2ml of blood was collected from the study subjects; ferritin assay was done (by electrochemiluminescence immunoassay -COBAS e411) at the time of diagnosis if the diagnosis was made in the study center; or at the time of admission if referred from an outside hospital with positive test results (NS1 or dengue serology positive).

The clinical course of the disease and platelet count were monitored carefully on a daily basis and patients were classified as having severe and non-severe infection as per WHO 2009 criteria; classified as severe dengue when shock, respiratory distress, severe bleeding; organ impairment occur.<sup>5</sup> Mean lowest platelet count was calculated in both the subgroups from all the platelet count values obtained on a daily basis during the hospital stay. Mean ferritin levels were calculated and compared in both the subgroups.

### *Statistical analysis*

Descriptive statistics were reported as mean±Standard deviation (SD), median with 25<sup>th</sup> and 75<sup>th</sup> percentiles. Mann Whitney U test was used to compare platelet count and serum ferritin between severe and non-severe dengue groups. Spearman's Rank correlation was used to assess the correlation between age with platelet count, serum ferritin and hospital stay. All analyses were done using SPSS version 21.0. P value less than 5% was considered statistically significant.

**RESULTS**

A total of 119 subjects were analyzed, of which there were 68 males (57.14%) and 51 (42.85%) females. The mean age of the study population was 29.1±11.4 years. About 50% of the study population was less than 25 years. There was no significant difference in the mean age between male and female. Diagnosis of dengue infection was established by positive NS1 antigen in 61 subjects while in the remaining 58, it was diagnosed by positive dengue serology.

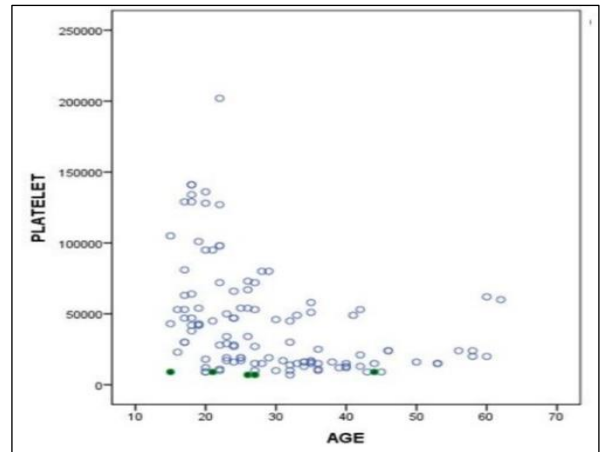
Among the total 119, only 5 (4.2%) subjects had clinical severe dengue, of which 4 were males and one female. The mean lowest platelet count was 40426.47×10<sup>9</sup>/L in males, 45000×10<sup>9</sup>/L in females. Around 75% of the study population stayed in the hospital for ≤ 3 days; 16% stayed for 4 days; 5% stayed for 5 days. Around 5% had a hospital stay for ≥9 days. Serum ferritin levels were measured in 14 subjects on day 3; 19 on day 4; 36 on day 5; 29 on day 6 and for the remainder 21 subjects it was measured on day 7. The mean ferritin levels measured was 1399.53±690.68ng/ml; (median-1856). The mean hospital stay was 3.11 days.

**Table 1: Comparison of serum ferritin, platelet count and hospital stay in severe and non-severe dengue.**

	Severe dengue	Non-severe dengue	P value
Serum ferritin ng/ml	2000 (2000, 2000)	1593 (757, 2000)	0.03*
Platelet count ×10 <sup>9</sup> /l	9000 (7000, 9000)	29500 (15750, 55000)	<0.0001*
Hospital stay days	16 (11, 21)	3 (2, 3)	<0.0001*

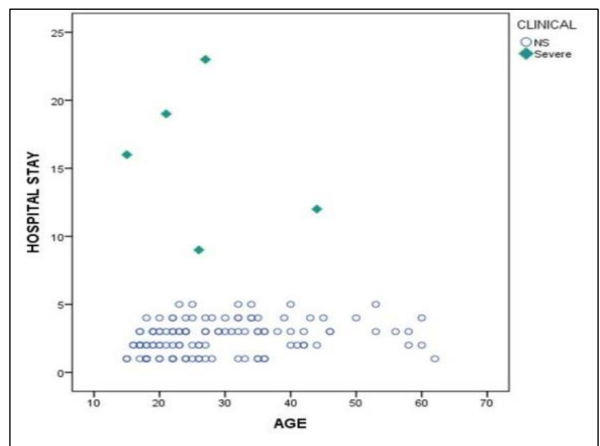
Median platelet count was significantly lower among patients who had severe dengue as compared to non-severe dengue group (Table 1). The median platelet count recorded during daily monitoring of platelets throughout the hospital stay were 9000 ×10<sup>9</sup>/L (7000, 9000) in severe and 29500×10<sup>9</sup>/L (15750, 55000) in non-severe dengue respectively. The median hospital stays, and serum ferritin levels were significantly higher among severe dengue subjects as compared to non-severe group (Table 1). The median serum ferritin levels and hospital stay among severe and non-severe dengue patients were 2000ng/ml (2000, 2000) vs. 1593ng/ml (757, 2000) and 16 (11, 21) vs. 3 days (2, 3) respectively.

In the present study, higher age showed a significant negative correlation with platelet count (Pearson co-efficient r= -0.427; p<0.0001). Also, higher age positively correlated with hospital stay (r= 0.26; p=0.004) and ferritin levels (r=0.16; p=0.08). The correlation with hospital stay is significant. Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient r= -0.51; p<0.001). The results are depicted in Figures 1-4.



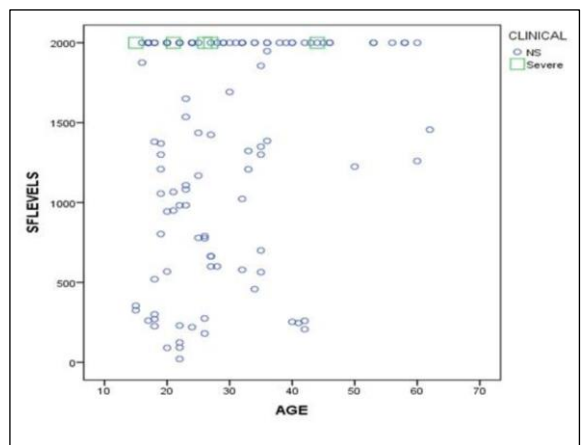
Age negatively correlated with platelet count which is significant (r= -0.427; p<0.0001\*)

**Figure 1: The co-relation co-efficient between age (years) and platelet count(x10<sup>9</sup>/L).**



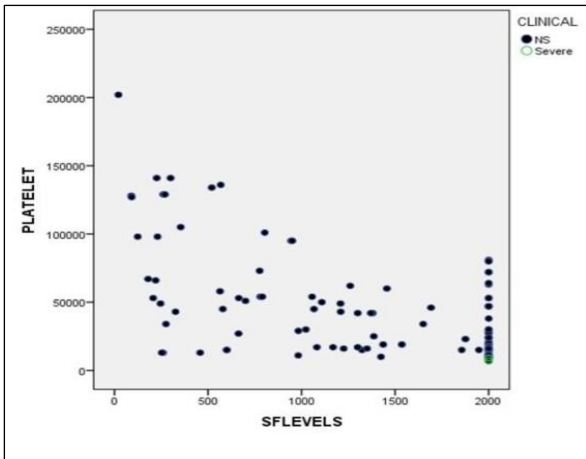
Age positively correlated with hospital stay which is significant (Pearson co-efficient r= 0.26; p=0.004\*)

**Figure 2: The co-relation co-efficient between age (years) and hospital stay (days).**



Age positively correlated with serum ferritin levels (non significant r=0.16; p=0.08)

**Figure 3: The co-relation co-efficient between age (years) and SF levels (serum ferritin in ng/ml).**



Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient  $r = -0.51$   $p < 0.001^*$ )

**Figure 4: The co-relation co-efficient between SF levels (serum ferritin in ng/ml) and platelet count.**

Serum ferritin measured from day 3 to day 7 of illness was compared in both the severe and non-severe groups and reported in Table 2. It was observed that non-significantly, patients who had severe dengue had higher median serum ferritin levels from day 4 onwards as compared to non-severe dengue cases.

**Table 2: Comparison of serum ferritin levels in severe and non-severe dengue measured from the day 3 to day 7 of illness.**

Serum ferritin measurement	Ferritin level in ng/ml		P value
	Severe dengue	Non-severe dengue	
3 day	-	n=14 1486 (1013, 2000)	-
4 day	n=1 2000±0 2000 (2000, 2000)	n=18 1001±811 859 (242, 2000)	0.421
5 day	n=2 2000±0 2000 (2000, 2000)	n=34 1549±587 2000 (1183, 2000)	0.349
6 day	n=1 2000±0 2000 (2000, 2000)	n=28 1222±727 1315 (565, 2000)	0.414
7 day	n=1 2000±0 2000 (2000, 2000)	n=20 1512±722 1974 (1103, 2000)	0.571

Serum ferritin measured from day 4 to 7 of illness was high in severe dengue compared to non-severe cases, although not statistically significant

**DISCUSSION**

Currently there is no specific drug or preventive vaccine available for dengue infection. The mainstay of

management of dengue infected subjects is supportive care and close monitoring for complications.<sup>13</sup> Most of these patients recover within 5 to 7 days from onset of the febrile illness while only a small percentage progress to severe dengue, with a study quoting 5%.<sup>14</sup> Severe dengue often occurs at the end of febrile or during convalescent stage and carries a very high mortality. It is very crucial to predict the severe form well in advance even prior to the appearance of warning signs by a simple diagnostic marker so that early identification and appropriate management would improve the outcome in these patients.

Increased expression of acute phase reactants is observed in patients with severe dengue infection when compared to non-severe cases.<sup>9</sup> 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.<sup>11</sup> 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.<sup>15</sup> while in severe cases, raised ferritin may assume a pathogenic role by activating immune cells resulting in cytokine storm.<sup>9</sup>

Males constitute 57.14% of the study population in the present study which was in accordance with other studies. The mean hospital stay was comparable with other studies. The mean duration of hospital stay was 2.73±1.41 days as observed by Ahmed et al, and 3.7±1.02 days in a study conducted in Rawalpindi.<sup>16,17</sup>

Results from the present study showed that hyperferritinemia is associated with severe dengue which is noted in other studies as well.<sup>9-11, 17</sup> 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.

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.<sup>11</sup> Results from the present study also had shown that hyperferritinemia (2000ng/ml) is seen in all cases with severe dengue and this increase in serum

ferritin levels is noted from day 4 of clinical illness in severe cases when compared to non-severe cases.

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.<sup>10</sup> 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. Patients with dengue hemorrhagic fever (DHF) grade III and IV had significantly higher median ferritin levels measured on day 5,6 and 7 of illness than those with grade I, II DHF and dengue fever. In that study it was concluded that serum ferritin level  $\geq 1,200$ ng/ml from day 4 of the illness predicts the occurrence of dengue hemorrhagic fever.

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).<sup>9</sup> 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 the febrile and defervescence stages of illness (940.09 $\pm$ 568.31 in non-severe cases vs. 1264.71 $\pm$ 492.59 in severe dengue on day of admission ; 418.19 $\pm$ 404.59 vs. 1490.74 $\pm$ 359.40 on day of defervescence) The study concluded that raised serum ferritin levels could predict the severity of dengue with a sensitivity and specificity of 76.9% and 83.3% respectively. The present study also showed similar results; hyperferritinemia was noted in severe cases compared to non-severe group from day 4 to day 7 of illness.

One another study showed significant association between raised serum ferritin levels on the day of admission and the development of severe dengue.<sup>16</sup> 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 100 $\mu$ g/dl) and b) Group B with high serum ferritin levels ; both the groups were followed up for the occurrence of severe dengue. 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.

Another significant observation made out in the present study was that age had a positive co-relation with the serum ferritin levels and the duration of hospital stay

while it negatively co-related with the platelet count. This implies that age is an important predictor of severity of dengue infection. As age advances, there is increased risk of developing severe disease with low platelet count, high ferritin levels and prolonged hospital stay. Studies had shown that elderly individuals are at risk of developing severe disease. In a study conducted in Southern Taiwan during a dengue outbreak with 5336 dengue cases, the incidence of dengue hemorrhagic fever and dengue shock syndrome is significantly higher in persons aged 60 years and above when compared with younger age groups.<sup>18</sup> Similar observation was made in a Singapore study; persons aged more than 60 years had more occurrence of severe dengue (20.3% vs. 14.6%, P = 0.006) as compared to younger adults.<sup>19</sup>

Our study is unique in that serum ferritin was measured once at the time diagnosis whereas in most of the earlier studies serum ferritin levels were done at multiple times during various stages of clinical illness.<sup>9-11</sup> The results of the present study in which serum ferritin was measured either at the time of diagnosis (or at the time of admission if referred from outside) within 7 days from onset of fever, correlated with the findings of the previous studies . It would be simpler, easier and convenient to measure serum ferritin on a single day, which is practically feasible in clinical setting catering to a large population.

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. Second, the upper limit of serum ferritin measured in the present study was 2000ng/ml due to laboratory limitations; so higher values could not be observed which if available can enable us to better understand about its correlation with both the severity of thrombocytopenia and clinical severity with more accuracy.

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

The present study had shown that elevated serum ferritin levels done early during the febrile stage of the illness (3<sup>rd</sup> to 7<sup>th</sup> day of illness) predict the severity of dengue.

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