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**Original Research Article**

# Correlation between serum ferritin level and severity of dengue fever in a tertiary care center: an observational study

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## ABSTRACT

**Background:** Serum ferritin is an acute phase protein and elevated levels of ferritin have been associated with the pathogenesis of many inflammatory infectious viral diseases. Dengue is a mosquito-borne tropical infection that caused by the dengue virus. TNFα and interleukin 1α, another proinflammatory cytokine, transcriptionally induce the H-chain of ferritin. Therefore, serum ferritin can be used as a prognostic marker for dengue severity.
**Methods:** This is a case control study conducted between July 2017 to December 2018.
**Results:** On the 3rd day of fever, the median values of serum ferritin in dengue, fever without warning signs, with warning signs, and severe dengue were 513.5 ng/ml, 1002 ng/ml and 2352.4 ng/ml respectively. On the 7th day of fever, the median values were 474 ng/ml, 900 ng/ml, and 2949 ng/ml respectively. Serum ferritin 1247 ng/ml on day 3 has a sensitivity of 96.4% and specificity of 91% for prediction of severity. Area under the curve for serum ferritin on day 3 was 0.963 (95% confidence limit: 0.934-0.991). Serum ferritin 1050 ng/ml on day 7 has a sensitivity of 98.2 % and specificity of 93% for prediction of severity. Area under the curve for serum ferritin on day 7 was 0.977 (95% confidence limit: 0.957-0.998).
**Conclusions:** Elevation of serum ferritin was significantly seen in those with severe dengue. Serum ferritin can be used as a prognostic marker for dengue severity. Day 3 serum ferritin can be used as a prognostic marker for dengue severity.

**Keywords:** Dengue fever, Ferritin, TNFα, ECLIA, Cytokine

## INTRODUCTION

Dengue is a systemic and dynamic disease. It has a wide clinical spectrum that includes both severe and non-severe clinical manifestations.[1] After the incubation period, the illness begins abruptly and is followed by three phases; febrile, critical, and recovery.[2] Dengue virus transmitted from human to human through bite of *Aedes* mosquitoes. DENV circulation occur in two cycles: an endemic/epidemic cycle between human and peridomestic mosquitoes, *Aedes aegypti* and *Aedes albopictus* and a sylvatic enzootic cycle between non-human primates and several arboreal *Aedes* species. At present, five genotypes have been identified in DEN-1; five in DEN-2 (one of which is only found in non-human host); four in DEN-3; four in DEN-4.[3,4] Halstead in 1970s proposed the 'antibody dependent immune enhancement theory' (ADE) based on in vitro and primate studies.[5] This association of sequential dengue infections being a risk factor severity has been confirmed repeatedly in epidemiological studies.[6,7] A particular sequence of infection by dengue serotype have been linked to severity of disease, suggesting severe dengue is more common in a secondary infection with DENV 2.[8,9] During second infection with different serotype, pre-existing antibody from the first infection fail to neutralise and may instead enhance viral uptake and replication in mononuclear cell.[10] Other factors like more virulent strain, host genetic

factor, age and comorbidities may play role in severity.[11-14] According to WHO in 2009 it is classified as dengue with or without warning signs and severe dengue. The warning signs abdominal pain tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy/restlessness, liver enlargement >2 cm and laboratory increase in hematocrit concurrent with a rapid decrease in platelet count. Severe dengue is defined as severe plasma leakage, leading to fluid accumulation with respiratory distress or shock, severe organ impairment (including cardiac, liver: ALT>1000 IU/l and CNS: altered consciousness) and severe bleeding. Infection is accompanied by an acute phase response, non-specific process that includes the production of acute phase proteins (APP) prior to full activation of the immune response. The main purpose of acute phase response is to protect tissue from damage by pathogens and harmful molecules. During this response, some protein rise known as "positive APPs ", and some protein decrease known as " negative APPs". The changes in the concentration of APPs are due largely to the change in their production by hepatocytes, which in turn are regulated by cytokines such as IL-1, IL-6, TNFα.[15] TNFα and interleukin 1α, another proinflammatory cytokine, transcriptionally induce the H chain of ferritin, which suggests that inflammation and stress can regulate cellular ferritin.[16,17] The roles of positive APPs include host- adaptive and host-defense mechanisms, which act by binding to foreign substances and by modulating phagocytic cell functions. The positive APPs include CRP, ACT, also known as orosomucoid, serum amyloid a, fibrinogen, haptoglobin, ceruloplasmin, and ferritin. An increase in serum ferritin concentration occurs in response to any infection or inflammatory process, but serum ferritin may also reflects the total body store of iron, hence the low ferritin can only reflect depleted iron stores in the absence of infection.[18] Negative APPs include transferrin, albumin, transthyretin, and retinol binding protein. The magnitude of the change in the concentration of the APPs during an acute phase response varies considerably: ceruloplasmin can increase by about 50%, whereas CRP can increase by as much as 1000 fold and ferritin by 3000 folds.[19] Therefore serum ferritin makes positive APPs that can be used as the predictor of the severity of disease.

### *Objectives*

Objectives of current study were; to study serum ferritin in dengue fever and to correlate between serum ferritin level and severity of dengue fever.

## METHODS

Current study was a case control study involving 200 cases and 200 controls (expected proportion in controls: 0.02, assumed odds ratio: 4, confidence level: 0.95, power: 0.8). Patients admitted to department of medicine, SCB medical college and hospital, Odisha, India from between July 2017 To December 2018 with dengue fever based on dengue non-structural 1 antigen positive with ELISA were selected. Among them 200 patients were selected. Similar number of age and sex matched healthy people without any fever was taken as controls. Patients with the following illness like iron deficiency anemia, collagen vascular disease, immune-compromised state, requiring multiple blood transfusions, liver diseases, diabetes, chronic kidney disease, malignancy are excluded. Patients with fever, arthralgia, myalgia, headache, rash, and haemorrhagic manifestations are subjected to dengue NS-1 antigen test. Dengue NS-1 Ag test was done by enzyme linked immunosorbent assay method. It is an analytical biochemistry assay. Those patients with positive dengue NS-1 Ag were taken as cases. The severity of dengue fever is classified by clinical features and blood parameters according to WHO classification 2009 of dengue fever. Serum ferritin was measured by electrochemiluminescence-immnuno assay (ECLIA) on the Roche diagnostic cobas e 411 analyzer on day 3 and day 7 of fever. Serum ferritin was then compared with the controls and how it varied with the severity of disease. Statistical analysis was done using statistical package for social (SPSS) version 25.

## RESULTS

All patients were presented with fever, arthralgia, and myalgia and minimum were presented with altered sensorium. Out of 200 cases, the number of cases of dengue fever without, with warning signs and severe dengue were 84 (42%), 60 (30%), and 56 (28%), respectively. Median serum ferritin in cases and controls were 915 ng/ml and 164 ng/ml, respectively.

**Table 1: Different presentations of dengue fever.**

| Presentation | N (%) |
|---|---|
| **Fever** | 200 (100) |
| **Arthralgia** | 200 (100) |
| **Rash** | 108 (54) |
| **Nausea** | 58 (29) |
| **Abdominal pain** | 46 (23) |
| **Persistent vomiting** | 59 (29.5) |
| **Mucosal bleeding** | 32 (16) |
| **Liver enlargement >2 cm** | 19 (9.5) |
| **Clinical fluid accumulation** | 13 (6.5) |
| **Dengue haemorrhagic fever** | 9 (4.5) |
| **Dengue shock syndrome** | 35 (17.5) |
| **Altered sensorium** | 2 (1) |
| **ALT >1000** | 3 (1.5) |
| **Severe plasma leakage** | 12 (6) |

Mann Whitney U test shows there was a statistically significant rise in serum ferritin among dengue fever than normal people. Independent samples Kruskal Wallis test, $p<0.001$. It show that serum ferritin was significantly high in severe dengue than dengue fever with or without warning signs. Kruskal Wallis test $p<0.001$, there was a significant difference

Median serum ferritin was maximum in the age group >45 years. Serum ferritin day 3 observation were; area under curve=0.963, confidence limit of 95% with lower bound is 0.934 and the upper bound is 0.991and 1247 ng/ml has a sensitivity of 96.4% and specificity of 91%. Serum ferritin day 7 observation were; area under curve=0.977, confidence limit of 95 % with lower bound is 0.957 and the upper bound is 0.998 and 1050 ng/ml has a sensitivity of 98.2% and specificity of 93%.

**Table 2: Distribution of severity of dengue fever.**

| Types of fever | N (%) |
|---|---|
| **Dengue fever without warning signs** | 84 (42) |
| **Dengue fever with warning signs** | 60 (30) |
| **Severe dengue fever** | 56 (28) |

**Table 3: Serum ferritin in cases and controls.**

| Serum ferritin (n=200) | Cases | Controls |
|---|---|---|
| **Mean±SD** | 1465.9±1593 | 167±25.9 |
| **Median (Q1-Q3)** | 915 (568-1900) | 164 (149-185) |

**Table 4: Comparison between serum ferritin of cases and controls.**

| Gender | Median of cases (Q1-Q3) | Median of controls (Q1-Q3) | Mann Whitney U Test |
|---|---|---|---|
| **Males** | 938 (552-2000 ) | 163 (149-184) | p< 0.001 |
| **Females** | 877 (626-1305 ) | 171 (153-185.7) | p<0.001 |

Total numbers of 200 patients were studied. Males were 136 (68%) and females were 64 (32%). Most of the patients lied between 26-35 years of age in both males and females. Out of the 200 cases, 84 (42%) (51 males & 33 females) belonged to dengue fever without warning signs, 60 (30%) (44 males and 16 females) to dengue fever with warning signs and 56 (28%) (41 males and 15 females) belonged to severe dengue. In our study, (Table 1) it was found that all patients with dengue fever were having fever (100%), arthralgia, and myalgia (100%). Along with fever arthralgia, 108 (54%) patients presented with rash, 58 (29%) with nausea, 46 (23%) with abdominal pain, 59 (29.5%) with persistent vomiting, 32 (16%) with mucosal bleeding, 2 (1%) with altered sensorium. On physical examination, hepatomegaly 19 (9.5%), clinical fluid accumulation 13 (6.5%), and severe plasma leakage 12 (6%) was seen. High ALT (>1000 IU/l) was present in 3 (1.5%) cases. There were 9 (4.5%) and 35 (17.5%) cases of dengue haemorrhagic fever and dengue shock syndrome, respectively. Out of 200 cases, the number of cases of dengue fever without, with warning signs and severe dengue were 84 (42%), 60 (30%), and 56 (28%), respectively (Table 2). Median serum ferritin in cases and controls were 915 ng/ml and 164 ng/ml, respectively. Mean serum ferritin on the 3rd day of fever of cases was 1465.9±1593 ng/ml and control was 167.2±25.9 ng/ml (Table 3). The median values of serum ferritin on the 3rd day among male cases and female cases were 938 with an interquartile range (Q1-Q3) of 552 to 2000 and 877 with interquartile range (Q1-Q3) of 626 to 1305 respectively. Mann Whitney U test shows there is a statistically significant rise in serum ferritin among dengue fever than normal people (Table 4).Independent samples Kruskal Wallis Test, p<0.001. It shows that serum ferritin is significantly high in severe dengue than dengue fever with or without warning signs on day 3 of illness (Table 5).

**Table 5: Serum ferritin among cases on day 3.**

| Day 3 serum ferritin | Dengue fever without warning signs | Dengue fever with warning signs | Severe dengue |
|---|---|---|---|
| **Mean±SD** | 531±174.4 | 1146±503.4 | 3210±2078.4 |
| **Median (Q1-Q3)** | 513.5 (435-648.7) | 1002 (913-1184.7) | 2352.4 (1991-3770 ) |

**Table 6: Serum ferritin among cases on day 7.**

| Day 7 serum ferritin | Dengue fever without warning signs | Dengue fever with warning signs | Severe dengue |
|---|---|---|---|
| **Mean±SD** | 453.3±141.4 | 1004±429.1 | 3410±2018 |
| **Median (Q1-Q3)** | 474 (370-549) | 900 (814-1000) | 2949 (2003-4125 ) |

Kruskal Wallis test p<0.001, there is a significant difference in severe dengue and non severe dengue on day 7 of illness (Table 6). Elevation of serum ferritin was significantly seen in those with severe dengue (p<0.001) rather than non-severe dengue. Serum ferritin can be used as a prognostic marker for dengue severity. Therefore raised in serum ferritin among dengue fever was statistically significant. Median serum ferritin is maximum in the age group >45 years (Table 7).

**Table 7: Median values of serum ferritin in different age groups.**

| Median serum ferritin | Age group (years) | | | |
|---|---|---|---|---|
| | **15-25** | **26-35** | **36-45** | **>45** |
| **Day 3** | 650 (500-1488) | 820 (522-1488) | 999 (757-2193) | 1135 (662.5-2022) |
| **Day 7** | 573 (450-1100) | 760 (465-1450) | 950 (618.5-2564.5) | 1000 (588.2-2205) |

Therefore the increase in serum ferritin as a result of vascular leakage in dengue fever is associated with the severity of the disease. On 3rd day of illness ferritin value of 1247 ng/ml has a sensitivity of 96.4% and specificity of 91% with an area under curve of 0.963. On 7th day of illness ferritin value of 1050 ng/ml has a sensitivity of 98.2% and specificity of 93% with an area under curve of 0.977 (Figure 1).

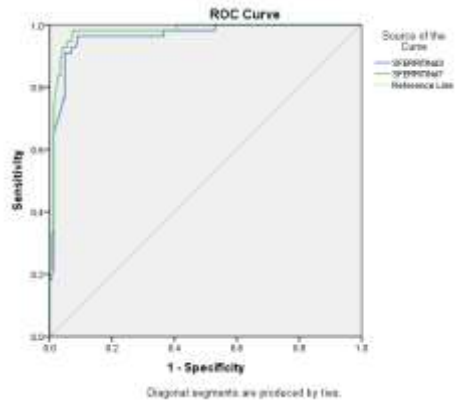


**Figure 1: ROC curve.**

## DISCUSSION

The total numbers of 400 people were participated in the study. Among them, 200 were cases and 200 were controls. There were 136 males and 64 females in our study. The sex distribution was also the same in controls. Therefore that there was no statistically significant difference in cases and controls and they were comparable. Maximum number of patients were in the age group of 26 to 35 years, in both males and females were 45 (33.1%) and 28 (43.8%), respectively. This indicates that most of the patients belonged to working age groups. Elderly patients (>45 years) were less affected. Thai et al in 2011 found that mostly young adults were affected with dengue fever. Our results show a similar results as obtained by Thai et al and Raza in 2012 showed that the maximum patients belonged to 16 to 35 years of age.

In male, 51 (37.5%) had dengue fever without warning signs, 44 (32.4%) with warning signs, and 41 (30.1%) had severe dengue. In female cases, 33 (51.6%) had dengue fever without warning signs, 16 (25%) with warning signs, and 15 (23.4%) had severe dengue (Table 5). It was seen that maximum patients had dengue fever without warning signs. The severity of dengue fever was not related to gender ($\chi^2$= 3.533, p=0.17). Most patients were having only fever, aches, nausea, and rashes and they did not progress to the severe disease.

In our study, it was found that all patients with dengue had fever (100%), arthralgia, and myalgia (100%). Along with fever arthralgia, 108 (54%) patients presented with rash, 58 (29%) with nausea, 46 (23%) with abdominal pain, 59 (29.5%) with persistent vomiting, 32 (16%) with mucosal bleeding, 2 (1%) with altered sensorium. On physical examination hepatomegaly 19 (9.5%), clinical fluid accumulation 13 (6.5%), and severe plasma leakage 12 (6%) was seen. High ALT (>1000 IU/l) was present in 3 (1.5%) cases. There were 9 (4.5%) and 35 (17.5%) cases of dengue haemorrhagic fever and dengue shock syndrome, respectively (Table 1). These values showed the clinical profile of the dengue patients presented to this hospital. Maximum patients were having only fever, arthralgia, and myalgia. On the basis of symptoms, they were classified according to the WHO 2009 classification for dengue. It was seen that 84 patients (42%) came under dengue fever without warning signs, 60 patients (30%) with warning signs, and 56 patients(28%) came under severe dengue (Table 2). In current case, the mean±standard deviation value of serum ferritin was 1465.9±1593 and the median was 915 ng/ml with an interquartile range (Q1-Q3) of 568 to 1900 ng/ml. In controls, the mean±standard deviation value was 167±25.9 ng/ml and the median was 164 ng/ml with an interquartile range (Q1-Q3) of 149 to 185 ng/ml (Table 3). It was seen that among males the median value of serum ferritin in the case was 938 ng/ml, while among controls it was 164 ng/ml with an interquartile range (Q1-Q3) of 552 to 2000 ng/ml and 149 to 184 ng/ml respectively. In addition in females, the median in cases was 877 ng/ml and in controls was 171 ng/ml with an interquartile range (Q1-Q3) of 626 to 1305 ng/ml and 153 to 185.7 ng/ml respectively (Table 4). This clearly showed that the serum ferritin in dengue fever was significantly higher than in normal people as seen by Mann Whitney U test. This raise shows that serum ferritin is a fast-acting positive APPs in dengue fever. This rise is due to the increase sensitivity of ferritin mRNA to cytokines released. This showed similar results to the study conducted by Chaudhuri et al in West Bengal. The level of serum ferritin in different types of dengue in the 3rd and 7th day of fever, respectively were shown in (Table 5-6). On the 3rd day of fever, the median values of serum ferritin in dengue, fever without warning signs, with warning signs, and severe dengue were 513.5 ng/ml, 1002 ng/ml and 2352.4 ng/ml respectively. On the 7th day of fever, the median values were 474 ng/ml, 900 ng/ml, and 2949 ng/ml, respectively. There was a significantly high ferritin level in severe dengue than in nonsevere dengue as computed by Kruskal Wallis U test. As the severity increased, serum ferritin values also increased. These findings are in concordance with the study patients in Wathanee et al 2008 in Bangkok. Serum ferritin is an acute phase reactant and is frequently elevated during inflammatory or infectious conditions. Increased serum ferritins have been observed in some viral infections such as hepatitis C, West Nile fever, and H5N1. Ferritin was reported to be associated with disease severity in patients with West Nile encephalitis caused by West Nile virus, an arbovirus of Flaviviridae. Dengue virus, an arbovirus, was reported to produce increased ferritin in patients. Our study showed higher ferritin in severe dengue than the non-severe dengue. This may be explained by the increased vascular permeability in

severe disease. The cytokines released in severe form causes a raise in cellular ferritin by induction of the H chain of ferritin both transcriptionally and posttranscriptionally. Serum ferritin in different age groupss of cases showed that in patient >45 years of age had a median value of 1135 ng/ml on the 3rd day of fever and 1000 ng/ml on the 7th day of fever (Table 7). This showed that serum ferritin in elderly patients was higher than that in younger patients. A study done in 2014 by Ryan Oakley and Binu Tharakan showed vascular hyper permeability in an aging population is increased. With vascular hyper permeability state among elderly people with dengue, fever causes higher serum ferritin in these groups. There was an increase in serum ferritin in whom had warning signs. Those who had capillary leak syndrome had a median value of 2750 ng/ml (1925-3405.75) while those did not have had a value of 822.5 ng/ml (519.75-1272.25). This is explained by the maximum amount of cytokine released which in turn increased serum ferritin. Therefore if serum ferritin is raised, it is expected that the patient already has or may develop warning signs.

In our study (Figure 1), serum ferritin 1247 ng/ml on day 3 has a sensitivity of 96.4% and specificity of 91% for prediction of severity. Area under the curve for serum ferritin on day 3 was 0.963 (95% confidence limit: 0.934-0.991). Serum ferritin 1050 ng/ml on day 7 has a sensitivity of 98.2 % and specificity of 93% for prediction of severity. Area under the curve for serum ferritin on day 7 was 0.977 (95% confidence limit: 0.957-0.998). Day 3 serum ferritin carries predictive values in determining the severity of dengue fever. Serum ferritin maintains a high level throughout the course of fever with complications whereas it settles down in case of fever without complications. Complications of dengue fever arise from day 4 onwards. Therefore serum ferritin in day 7 has less predictive value for determination of complications, although day 7 serum ferritin has more area under the curve in ROC plot. We couldn't evaluate serum ferritin levels in those patients who recovered.

## CONCLUSION

Serum ferritin can be used to assess the severity of dengue fever. Day 3 serum ferritin carries more predictive value in determining the severity of dengue fever, which results in an early intervention that reduces hospital stay and mortality.

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## REFERENCES

1. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet. 1998;352:971-7.
2. Kalayanarooj S. Early clinical and laboratory indicators of acute dengue illness. J Infect Dis. 1997; 176(2):313-21.
3. Nisalak A. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. Am J Trop Med Hygiene. 2003;68(2):191-202.
4. Rico-Hesse R. Dengue virus evolution and virulence models. Clin Infect Dis. 2007;44(11):1462-6.
5. Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature. 1977;265:739-41.
6. Sangkawibha N, Rojanasuphot S, Ahandrik S. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. Am J Epidemiol. 1984;120:653-69.
7. Guzman MG, Kouri G, Valdes L, Bravo J, Alvarez M, Vazques S, et al. Epidemiologic studies on dengue in Santiago de Cuba, 1997. Am J Epidemiol. 2000; 152:793-9.
8. Thein S, Aung MM, Shwe TN. Risk factors in dengue shock syndrome. Am J Trop Med Hyg. 1997;56:566-72.
9. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg. 1988;38:172-80.
10. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science. 1988;239:476-81.
11. Rico-Hesse R. Dengue virus evolution and virulence models. Clin Infect Dis. 2007;44(11):1462-6.
12. Loke H, Bethell DB, Phuong CX, Dung M, Schneider J, White NJ, et al. Strong HLA class I-restricted T cell responses in dengue hemorrhagic fever: a double-edged sword. J Infect Dis. 2001;184(11):1369-73.
13. Coffey LL, Mertens E, Brehin AC, Fernandez-Garcia MD, Amara A, Despres P, et al. Human genetic determinants of dengue virus susceptibility. Microbes Infect. 2009;11:143-56.
14. Loke H, Bethell D, Phuong CX, Day N, White N, Farrar J, Hill A. Susceptibility to dengue hemorrhagic fever in Vietnam: evidence of an association with variation in the vitamin D receptor and Fc gamma receptor IIA genes. Am J Trop Med Hyg. 2002;67: 102-6.
15. Feelders, RA, Vreugdenhil, G, Eggermont, AM, Kuiper-Kramer, PA, van Eijk, HG, Swaak, AJ. Regulation of iron metabolism in the acute phase response: interferon γ- tumour necrosis factor α-induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest. 1988;28:520-7.
16. Torti SV. The molecular cloning and characterization of murine ferritin heavy chain, a tumor necrosis factor-inducible gene. J Biol Chem. 1988;263:12638-44.
17. Wei Y, Miller SC, Tsuji Y, Torti SV, Torti FM. Interleukin 1 induces ferritin heavy chain in human muscle cells. Biochem Biophys Res Comm. 1990; 169:289-96.

18. Gabay C, Kushner I. Acute phase proteins and other systemic responses to inflammation. New Eng J Med. 1999;340:448-54.
19. Fleck A, Myers MA. Diagnostic and prognostic significance of the acute phase proteins. In: Gordon AH, Koj A, eds. The acute phase response to injury and infection. Amsterdam: Elsevier Science Publishers; 1985:249-71.

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