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# Serum Ferritin as an Early Indicator of Severity of Dengue

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**Abstract**
**Background:** Dengue is an epidemic throughout the world and with its new upswings; it has caused an increased number of fatalities. In critical cases, increased serum ferritin has been observed to play a pathogenic role by enhancing the activity of immune cell producing a cytokine storm. **Material and Methods:** The study was conducted on 80 dengue patients admitted in Prasad Institute of Medical sciences, Banthra, Lucknow, U.P. All fever cases which are dengue NS1 antigen positive or which show classical signs and symptoms of dengue. In this study we were included Children from 1 month to 12 years of age. **Results:** The result of this study revealed that In severe dengue 14 cases out of 17 cases had serum ferritin levels of more than 1200 which is statistically significant compared to non severe cases where only 4 had high ferritin levels. **Conclusion:** It can be conclude that the serum ferritin level is a suitable marker for early diagnosis and prognosis of Dengue.
**Keywords:** Dengue, NS1 antigen.

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## Introduction

Dengue is an epidemic throughout the world and with its new upswings; it has caused an increased number of fatalities. It is a mosquito borne viral infection caused by flavivirus. It starts with a simple fever and the most of the cases recover. A few number of cases may lead to life threatening complications like dengue shock syndrome and dengue hemorrhagic syndrome and these patients require careful monitoring and assessment throughout the course of the disease. Even with the advancement of medical science, there is no proper treatment for dengue and patients, suffering with it, are only provided the supportive treatment.

Recently, in the patients of severe Dengue, a serious complication called as Macrophage Activation Syndrome (MAS) or Hemo-phagocytic Syndrome (HS) has been reported. This syndrome has been described as a dysregulated hyperactivated immune response caused by the disproportionate activation and proliferation of T cells and well differentiated macrophages.[1-3] This condition is an overwhelming systemic inflammation resulting in non-remitting hyper-pyrexia, hepato- & spleeno-megaly, hemorrhage, lymphadenopathy, and central nervous system (CNS) dysfunction. MAS/ HS is being increasingly reported in children suffering from severe dengue.[4-7] Because of the threatening nature, prompt diagnosis and treatment for these complications become extremely important. For the early diagnosis, a specific parameter needs to be identified. And it has been observed that hyperferritinemia (levels above 10000ug/L) is that needed parameter for MAS. Serum ferritin is an acute phase reactant. It is manufactured by reticuloendothelial cells in response to

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infection and inflammation. Serum ferritin level is selectively increased in dengue patients rather than in patients suffering from other febrile disorders.
Abnormal liver function tests, cytopenia, coagulopathy, hemophagocytosis, hypertriglyceridemia, hypoalbuminemia, and increased levels of serum sCD25 and sCD16 are also the markers of MAS.
This Hyperferritinemia has been observed to play two opposite type of roles in dengue patients. Increased serum ferritin level has been found to show a protective effect at the site of inflammation by its chelating property of the toxic free iron radicals.[8] On the other hand in critical cases, increased serum ferritin has been observed to play a pathogenic role by enhancing the activity of immune cell producing a cytokine storm.
It was aimed in the present study to predict the severity of the disease in its early phase by using the levels of serum Ferritin.[9]

**Methodology**
**Study Population:** The study was conducted on 80 dengue patients admitted in Prasad Institute of Medical sciences, Banthra, Lucknow, U.P

**Inclusion criteria:**
All fever cases which are dengue NS1 antigen positive or which show classical signs and symptoms of dengue.
Children from 1 month to 12 years of age

**Exclusion criteria:**
Patients who do not have serological evidence of NS1 positivity or IgM antibody.
Patient with anemia (Hb <11g/dl) and transfusion dependent chronic disease.
**Data collection:**
A previously designed proforma was used to collect the demographic and clinical details of the patients. All the patients underwent detailed clinical evaluation and appropriate investigations are noted.
**Study Duration:** The duration of this study was over a period of 2 years.
**Methodology:** History was taken in detail and duration of fever and other associated signs and symptoms noted. Necessary investigations were done. Child was then followed throughout the clinical course of the disease.
**Laboratory Investigations:** Total count, Platelet count, serum ferritin, USG abdomen, LFT, RFT, neuroimaging and other investigations if necessary.
**Serum Ferritin Estimation:** Ferritin is a globular protein found mainly in the liver, which can store about 2250 iron (Fe3+) ions. The ferritin molecule consists of a protein shell (Apoferritin) composed of heavy and light subunits, which surrounds a crystalline core containing iron oxide and phosphate. Ferritin is synthesized in the liver, spleen and numerous other body tissues, with major concentrations found in the liver, spleen, bone marrow, and intestinal mucosa. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which would be excreted in the stool. If ferritin is low there is a risk for lack in iron, which sooner or later could lead to anaemia. In the setting of anaemia, serum ferritin is the most sensitive lab test for iron deficiency anaemia. In contrast, serum ferritin levels are normal or increased in anemia associated with chronic disease. Elevated serum ferritin levels have been observed in acute and chronic liver disease and lymphoid malignancy (leukemia and Hodgkin lymphoma).
Ferritin is an acute-phase reactant, it is often elevated in the course of disease. In dengue serum ferritin is elevated because of macrophage activation.

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**Method and principle of serum ferritin estimation:** Immuno enzymatic colorimetric method for quantitative determination of Ferritin concentration in human serum or plasma. Ferritin ELISA test is based on simultaneous binding of human Ferritin to two monoclonal antibodies, one immobilized on microwell plates and the other conjugated with horseradish peroxidase (HRP). After incubation the bound/free separation is performed by a simple solid-phase washing. Then the enzyme HRP in the bound-fraction reacts with the Substrate (H2O2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H2SO4) is added. The colour intensity is proportional to the Ferritin concentration in the sample. The Ferritin concentration in the sample is calculated based on a standard curve.

## RESULTS

Out of 80 cases 23 were less than 5 years and 57 cases were more than 5 years of age. Among 80 cases, 43 were male and 37 cases were female. Out of 80 cases, 63 cases were non severe dengue and 17 were severe dengue. Out of 17 severe dengue cases, 1 case had fluid accumulation with respiratory distress, 11 cases had shock, 3 cases had bleeding, 2 cases had organ involvement (1 case had liver involvement with elevated liver enzymes and 1 other case had CNS involvement in the form of encephalitis). Out of 80 cases, 5 cases had serum ferritin < 300, 54 cases had 301-600 cases, 8 cases had serum ferritin 601 - 800 and 13 cases had serum ferritin levels >800. 47 cases had serum ferritin levels 301-600, 13 cases had levels between 601-800 and 2 cases had levels between 801-1200. 18 cases had serum ferritin levels of more than 1200. In severe dengue 12 cases out of 17 cases had serum ferritin levels of more than 800 which is statistically significant compared to non-severe cases where only 1 had high ferritin levels. In severe dengue 14 cases out of 17 cases had serum ferritin levels of more than 1200 which is statistically significant compared to non-severe cases where only 4 had high ferritin levels.

**Table 1: Distribution of Age**

| Age in years | No. of cases | Percentage |
|---|---|---|
| < 5 (1 - 5) | 23 | 28.75 |
| > 5 (5 - 12) | 57 | 71.25 |
| Total | 80 | 100 |

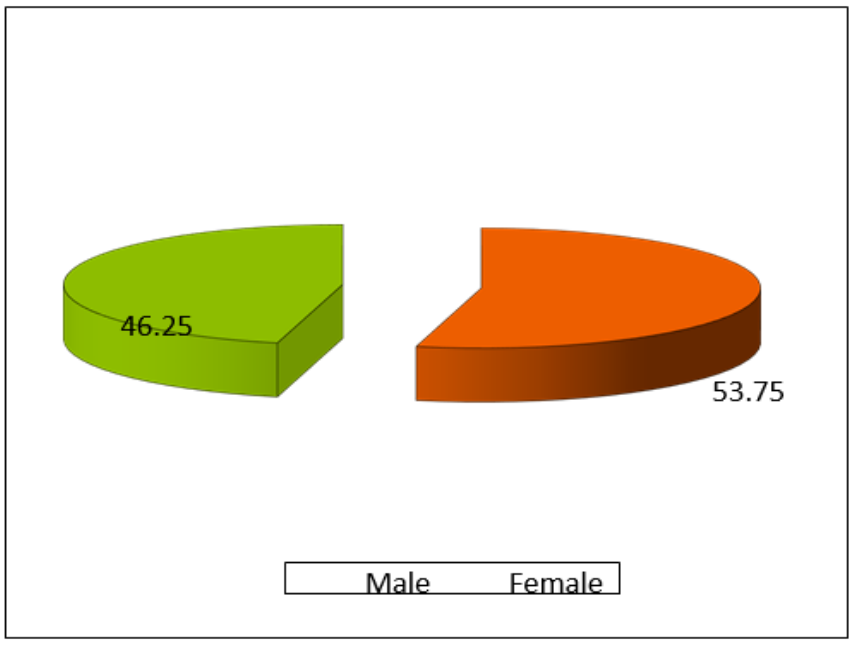

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**Figure 1: Gender Distribution**

**Table 2: Distribution of cases according to Dengue cases**

| Dengue | No. of cases | Percentage |
|---|---|---|
| Non severe | 63 | 78.75 |
| Severe | 17 | 21.25 |
| Total | 80 | 100 |

**Table 3: Distribution of cases according to Dengue cases complications**

| Complications | No. of cases | Percentage |
|---|---|---|
| Fluid Accumulation with Respiratory Distress | 1 | 1.25 |
| Shock | 11 | 13.75 |
| Bleeding | 3 | 3.75 |
| Organ Involvement | 2 | 2.5 |

**Table 4: Serum ferritin on day 3 & 4 of illness**

| Serum Ferritin (ng/ml) | Sr Ferritin Day 3 | Sr Ferritin Day 4 |
|---|---|---|
| < 300 | 5 | 0 |
| 301 – 600 | 54 | 47 |
| 601 – 800 | 8 | 13 |
| 801 – 1200 | 13 | 2 |
| > 1200 | 0 | 18 |
| Total | 80 | 80 |

**Table 5: Clinical diagnosis vs serum ferritin day 3**

| Day 3 | Ferritin > 800 (13) (ng/ml) | Ferritin < 800 (67) (ng/ml) |
|---|---|---|
| Severe dengue | 12 | 5 |
| Non severe dengue | 1 | 62 |
| p value | < 0.001 | Significant |

**Table 6: Clinical diagnosis vs serum ferritin day 3**

| | Ferritin > 1200 (18) (ng/ml) | Ferritin < 1200 (62) (ng/ml) |
|---|---|---|
| Severe Dengue | 14 | 3 |
| Non severe Dengue | 4 | 59 |
| p value | < 0.001 | Significant |

## DISCUSSION

In various studies involving adult patients and, in few studies, involving pediatric patients, it has been that Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) may finally lead to increased mortality if not diagnosed and treated early.

At present, there is no specific treatment or preventive modality against dengue infection and its management is mainly supportive and symptomatic.[10] It has been observed that most of the patients of dengue infection become asymptomatic within five to seven days and only 5% develop serious complications.[11] These complications may be life threatening. Therefore, it is extremely important to predict this condition well in advance to safe guard the life of patients. The elevated levels of acute phase reactants has been observed in severe dengue infections which plays an important role in diagnosis and prognosis of dengue.[12] Serum ferritin is one such acute phase reactant for dengue and it is excessively increased in dengue patients.[13]

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The present study was done to find out the role of elevation of serum ferritin in dengue patients during febrile phase with its severity. Outcome of the dengue and its mortality can be decreased by early prediction of the changes in levels of serum ferritn.

In the present study, duration of fever was noted and the mean febrile period was 4.78 days. Male preponderance was also observed in case of infection. This result of the present study was supported by one study 15, in which 57.14% of males suffered from the disease. Serum ferritin level was unaffected by the gender variation. Out of 80 pediatric patients studied in the present study, 23 were less than 5 years. While, 57 fall in the age group of 5-12 years.[14-17]

In one study15, serum ferritin level was measured only once while in others,[12,13,18] it wss measured at multiple times during the disease. In the present study, serum ferritin was measured more than once.

The mean value of serum ferritin on third day was 404.43 ± 102.82 and 830.006 ± 160.064, respectively in non-severe and severe dengue. This mean value increased respectively to 585.905 ± 193.082 and 1259.824 ± 192.461 on fourth day.

The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of serum ferritin were calculated for predicting the significance of severity of dengue for specific cut off values. It was 800 and 1200 ng/ml, respectively on day 3 and day 4.

| | **Value** | |
|---|---|---|
| | **Day 3** | **Day 4** |
| Sensitivity | 92.31% | 77.78% |
| Specificity | 92.54% | 95.16% |
| Positive predictive value | 70.59% | 82.35% |
| Negative predictive value | 98.41% | 93.65% |
| Accuracy | 92.50% | 91.25% |

The p value was statistically significant for both day 3 ($p<0.001$) and day 4 ($p<0.001$) of serum ferritin with disease severity.

## CONCLUSION

Dengue is a serious infection with life threatening complications like DSS/DHF. With the present results, it can be suggested that the serum ferritin level is a suitable marker for early diagnosis and prognosis of Dengue. This way, prompt diagnosis and treatment may help the treating physicians to save multiple lives.

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