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Relevance of Serum Ferritin Levels with Severity of Dengue Fever and Thrombocytopenia; A Prospective Study

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ABSTRACT

Objective: To determine the relevance of increasing levels of serum ferritin with the severity of dengue fever and platelet count.

Study Design: Prospective longitudinal study.

Place and Duration of Study: Medicine Department, Combined Military Hospital, Rawalpindi Pakistan, from Jul to Nov 2023.

Methodology: 110 admitted cases of dengue were selected. Serum ferritin levels and platelet count were done on day one and five of the indoor stay. The levels of serum ferritin were then compared with the severity of dengue. According to the WHO, patients without warning signs of dengue were classified as grade A, those with warning signs as grade B, and patients with severe dengue were classified as grade C.

Results: The levels of Serum ferritin rose significantly on the day five. On day one, the median ferritin was 600.0 (812.5–350.0) ng/ml and median ferritin on day 5 was 1100.0 (2000.0–646.50) ng/ml. Our study results showed that serum ferritin level was increased at day 5 with a statistically significant *p*-value <0.001. Sixty-three (57.3%) had grade A, 31(28.2%) had grade B and 16(14.5%) had grade C dengue. Hyperferritinemia was observed in 47%, 27%, and 16% of grade A, B, and C patients, respectively. Correlation of Serum Ferritin with Platelet count at day one and day five showed that patients had thrombocytopenia with raised serum ferritin level at day five.

Conclusion: The severity of dengue fever is correlated with elevated blood ferritin levels, which is quite relevant.

Keywords: Dengue fever, Dengue with warning signs, Dengue without warning signs, Ferritin levels.

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INTRODUCTION

Dengue is caused by dengue viruses belonging to the family Flaviviridae and is transmitted by Aedes aegypti mosquito.[1] Lifestyle shifts, increased urbanization, and poor water storage practices all contribute to the spread of mosquito breeding grounds, which in turn causes dengue fever, which is on the rise. Dengue virus can be classified into five serotypes (DENV 1–5). Dengue ranges from a dynamic febrile illness with body aches to Dengue shock syndrome (DSS), Dengue hemorrhagic fever (DHF), and multi-organ failure. The major pathogenic mechanism in dengue involves hyper inflammation and vascular leakage, leading to multi-organ damage.[2] Ferritin, an acute-phase protein, is produced by reticulo-endothelial cells as a reaction to infection and inflammation. Raised serum ferritin levels are a clinical marker of coagulation disturbances, immune activation, and disease severity. It can be useful both for diagnostic and treatment purposes. Hyperferritinemia is defined as levels of serum ferritin of higher than 500 µg/L. Hyperferritinaemia is seen in immune-activated diseases including haemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS). These conditions are characterized by fever, hepatomegaly, splenomegaly, lymphadenopathy, cytopenia, bleeding, hypofibrinogenaemia, hypertriglyceridaemia, abnormal liver function tests, raised ferritin levels and coagulopathy. In most cases, the symptoms of Dengue are found to be mild and self-limiting. Symptoms appear three to five days after the disease around defervescence,[3] time. Since the treatment for dengue is without specification, it is important to identify biomarkers to predict DENV infection severity to timely utilize the opportunities to prevent the progression of DENV infection severity.[4] Severe plasma leakage is defined as DSS or respiratory distress due to plasma leakage.[5] The raised serum ferritin level is characterized by rising levels of liver enzymes, low platelet count, and activation of coagulation and fibrinolytic systems.[6] DENV are

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single-stranded RNA viruses with structural proteins (capsid-C, membrane-M, and envelope-E) and seven nonstructural proteins (NS 1-5). The E protein triggers a protective immune response in the host. The nonstructural proteins are responsible for viral translation, transcription, and replication. Management of dengue is mainly supportive. When starting out, crystalloids are the way to go. Transfusing platelets as a preventative measure is not advised.[7] Clinically, dengue fever is suspected when acute febrile illness of 2-7 days is accompanied by headache, retro orbital pain, myalgia, rash, and hemorrhagic manifestations. Typically, the first five days of fever are sufficient for the ELISA approach to detect dengue fever by looking for NS1 antigen reactivity. IgM usually appears within 5–7 days of fever.[8] The febrile phase is followed by the critical phase and is characterized by plasma leakage. During this phase hyperferritinemia may be observed. During recovery, the hyperferritinemia also trends downwards, emphasizing ferritin's role as a severity marker in a self-limited pro-inflammatory state.[9] For an early prediction of disease severity, serum ferritin serves as a prominent biomarker.[10] The rationale of this study is to identify the importance of increasing levels of serum ferritin with the dengue fever severity.

METHODOLOGY

A prospective longitudinal study carried out in the Medicine department of a Tertiary care Hospital in Rawalpindi, from July to November 2023, after obtaining ethical approval from the Hospital Ethical Review Board (reference number 479/2023). The study was formulated to observe the relevance of increasing levels of serum ferritin with the severity of dengue fever and thrombocytopenia.

Inclusion Criteria: Patients with dengue fever, as determined by a positive Dengue NS1 antigen or Dengue IgM serology, were eligible to participate if they were indoor cases, over the age of 12, and of either sex.

Exclusion Criteria: Patients with chronic conditions like connective tissue disorders, inflammatory disorders and malignancy, pregnancy, acute infections and qualitative or quantitative platelet disorders.

One hundred and ten patients with dengue fever were selected through consecutive sampling from July to September 2023 when the incidence of dengue was on the high. After taking in-depth history, relevant clinical examination was done. Hospital registration number and informed consent from all dengue patients were taken. According to WHO guidelines patients without warning signs of dengue were classified as grade A, those with warning signs as grade B, and patients with severe dengue were classified as grade C. Hyperferritinemia was labeled as serum ferritin levels greater than 500 µg/L and thrombocytopenia with platelet count of less than 150x109/L. Serum ferritin levels and platelet counts were performed on day 1 and day 5 of admission. 3ml of blood was taken and analyzed on SYSMEX CS1600 automated coagulation Analyzer. Platelet count was performed by fully automated hematology Analyzer SYSMEX XP 100.

Data is analyzed using the Statistical Package for social sciences (SPSS) Version 23.00 and MS Excel 2023. Mann-Whitney U test and Pearson Correlation analysis are performed for the inferential statistics. Frequency and percentages are recorded for categorical variables, while for continuous variables, median is reported. A *p*-value ≤0.05 is considered significant.

RESULTS

A total of 110 patients were included in the study. Seventy two (65.5%) were males and thirty-eight(34.5%) were females (Table-I). Mean age of the sampled patients (n=110) was 35.5±12.4 years with minimum 12.00 and maximum 86.00 years, Out of 110 dengue positive patients sixty three (57.3%) were classified as grade A, thirty one (28.2%) grade B and sixteen (14.5%) grade C shown in Table-I.

Table-I: Demographic Characteristics of the Study Participants (n=110)

Study Parameters	n (%)
Age (Mean±SD)	35.5±12.4 years (Range: 12-86 years)
Gender	
Male	72(65.5%)
Female	38(34.5%)
Dengue Severity Grade	
A	63(57.3%)
B	31(28.2)
C	16(14.5%)

The median ferritin on day1 was 600.0 (812.5 – 350.0) ng/ml, with minimum 107 and maximum 1030 ng/ml, median ferritin on day 5 was 1100.0 (2000.0–646.50) ng/ml with minimum 116 and maximum 9703 ng/ml. Our study results showed that serum ferritin level was increased at day 5 with statistically significant *p*-value<0.001 shown in Table–II. Raised serum ferritin levels were identified in 47(42.7%)

grade A cases, 27(24.5%) grade B cases and 16(14.5%) grade C and 20(18.3%) patients had ferritin less than 500 ng/ml (Figure-1). Correlation of Serum Ferritin (ng/ml) level with Platelet count at day one shown in Figure-2 and day five is shown in the Figure-3 which shows that patients has thrombocytopenia with raised serum ferritin level at day five.

Table-II: Comparison of Serum Ferritin (ng/ml) Level at Day 1 and Day 5 (n=110)

Parameters	Day 1	Day 5	*p*-value
	Median (IQR)	Median (IQR)	
Serum Ferritin (ng/ml)	600.0 (812.5 – 350.0)	1100.0 (2000.0 – 646.50)	< 0.001

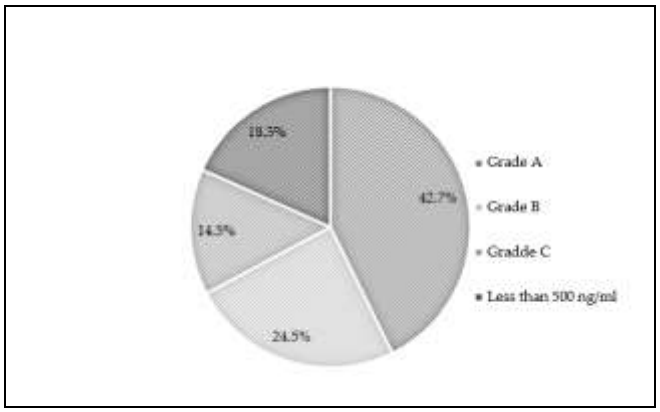

Figure-1: Hyperferritinemia of the Patients (n=110)

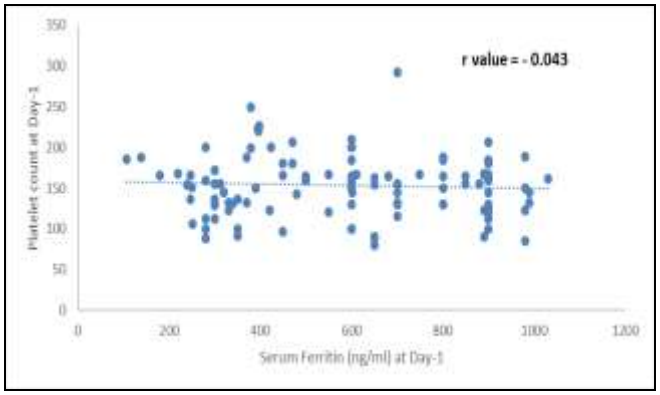

Figure-2: Correlation of Serum Ferritin (ng/ml) Level with Platelet Count at Day One

DISCUSSION

The arboviral disease most commonly transmitted by mosquitoes is dengue. According to the World Health Organisation, 2.5 billion individuals in the tropics and subtropics are in danger. The foundation of managing and preventing dengue-related mortality is early diagnosis of severe dengue[11].

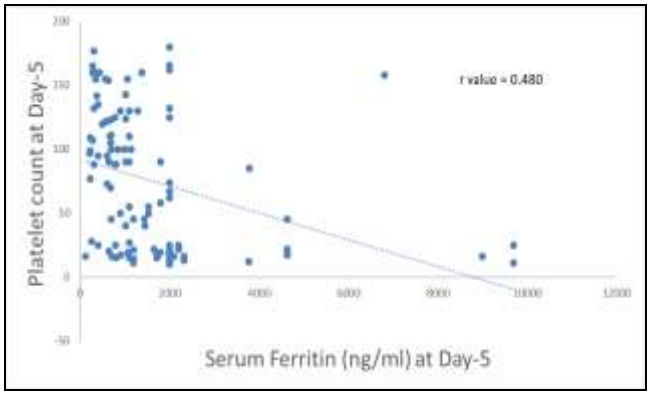

Figure-3: Correlation of Serum Ferritin (ng/ml) level with Platelet count at day Five

There are now three groups of dengue symptoms according to the new 2009 WHO classification: (1) severe dengue, (2) dengue with warning signals, and (3) dengue without warning signs. A rapid and severe fever is the most common symptom that patients experience. During febrile period patients has a rash, headache, bodyaches, and eye pain. Additionally, vomiting, nausea, and anorexia are prevalent. Dengue without warning signals is defined by these clinical characteristics. Continuous vomiting, fluid buildup (ascites, pleural effusion), abdominal pain or tenderness, bleeding from the mucosa (epistaxis), lack of energy, restlessness, enlarged liver (hepatomegaly >2 cm), and a sudden drop in platelet count are all red flags. In cases of severe dengue, symptoms such as altered consciousness, excessive bleeding, and/or organ damage (ALT > 1000 units/L) are present.[12] The severity of dengue fever is correlated with a higher serum ferritin level. Serum ferritin measurements noted on the fourth or fifth-day aid in evaluating dengue infection prediction.[8] Hyperactivation of the immune system and aberrant coagulation are hallmarks of dengue fever, which is characterised by elevated ferritin levels in infected individuals. Consequently, it is advised to closely observe patients whose ferritin levels are elevated. Ferritin levels taken on days 4 or 5 after dengue infection may be useful in predicting the severity of the disease. One hyperinflammatory disorder that can be deadly is secondary hemophagocytic lymphohistiocytosis, or HLD. Although dengue virus associated haemorrhage (HLH) can be deadly if not treated promptly, it is curable with the right medication. A group of serious symptoms known as "the hyperferritinemic syndrome" have been linked to elevated ferritin levels. One possible biomarker for early dengue severity prediction is elevated ferritin levels. Although cases of

dengue infection accompanied by HLH are uncommon, there have been reports of patients with hyperferritinemia and HLH in recent years. The dengue virus One serotype infection gives protection against that serotype for the rest of one's life. Nevertheless, a different serotype of reinfection is possible. Severe dengue can occur after a secondary infection with a different strain. There are three possible categories for patient therapy based on the clinical manifestations: Patients in Group A can be treated at home; those in Group B require hospitalisation; and those in Group C require emergency care. A significant rise in vascular permeability characterises DSS, the most severe form of DHF. The pulse pressure can determine the degree of plasma vascular leakage. Individuals with DSS typically have a pulse pressure below 20 mmHg. The primary goal of treating DSS is to quickly restore circulating plasma volume. Clinical parameters and serial hematocrit levels should be used to titrate the fluids. The preferred crystalloids for dengue fluid resuscitation16 are normal (0.9%) saline and Ringer's lactate.[13]

This investigation has significantly associated high serum ferritin levels with severe dengue fever. Kempegowda Institute of Medical Sciences Hospital in Bangalore was the site of a study by Suresh *et al*., One hundred dengue-positive patients had their ferritin levels checked on admission on days 1, during the fever phase, and day 4, after defervescence, in order to compare the levels with disease severity. With AUC (area under curve) =0.863, Standard Error (SE) =0.043, and a confidence interval (95%) between 0.778 and 0.947 (p<0.05), level of serum ferritin was found to be a reliable indicator of severe dengue on day one. On day four, serum ferritin demonstrated a high level of predictive power for severe dengue, with an AUC of 0.947, SE of 0.021, and 95% CI from 0.907 to 0.988 (p<0.05).[1] With 131 dengue cases studied, Moras *et al*., performed a descriptive research in the internal medicine departments of two separate tertiary referral hospitals. The majority of the dengue cases (87 out of 44) were classified as non-severe, while a small percentage (44 out of 87) were classified as severe. Ferritin levels were 4271 ug/l in the non-severe group and 9125.34 µg/l in the severe dengue patients. The p-value for this comparison was 0.003[6], indicating that it was likewise statistically significant. At University Hospital in Maracaibo, Venezuela, researchers Valero et al. studied 25 dengue patients. Ferritin and IL-18 levels were higher in dengue patients than healthy controls; however, severe cases showed the greatest levels of these molecules (p< 0.001).[14] In a database retrospective, Roy *et al*., looked at information from a corporate multispecialty hospital in Kolkata. Of the total number of febrile illnesses (OFI) studied, 30 were confirmed dengue fever cases, while 22 were not.[15] Ferritin levels were significantly greater in the dengue cohort compared to the OFI group (p-0.0001). The optimal ferritin level cut-off for distinguishing dengue from OFI was determined to be 1291. Here, 82.6% sensitivity and 100% specificity were achieved.[8] A retrospective investigation was carried out by Zhang *et al*., in Hangzhou 3A grade hospital on 171 dengue cases and 130 healthy individuals as a control group. The levels of serum ferritin were greater in the dengue fever group compared to the control group (z=6.930, p 0.001).[16] Liver damage, ALT, and AST levels were all higher in patients whose serum ferritin levels were high. The combination of dengue fever and liver injury was more likely in those with hyperferritinemia (≥500 ng/ml) (OR =8.120, p<0.001).[17]

On the other hand, some researches did not find a correlation between the two, i.e., ferritin levels and the severity of dengue. An Indian tertiary care teaching hospital was the setting for a prospective observational study by Williams *et al*., Dengue and bush typhus were the frequent infections among the 202 youngsters who were enrolled. At day one and day three of the hospital admission, the median ferritin levels were 798 (378, 3,205) µg/L and 429 (213,680) µg/L, respectively. The percentage change over 72 hours and admission ferritin values were not different between the children who had good outcomes and those who had bad outcomes. A teaching hospital's tertiary care unit was the site of the cross-sectional investigation by Mahabala *et al*., They inducted 80 people who had severe dengue fever. A ferritin level of 8613 ng/ml exhibited 67% sensitivity and 55% specificity for severe dengue progressing to HLH in contrast to a soluble IL-2 receptor level of 10,345 pg/ml, which had 100% sensitivity and specificity.[18]

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LIMITATIONS OF STUDY

Our study enrolled only admitted patients of dengue fever . During recent dengue outbreak a lot of patients were treated through OPD which were not included in our study. We conducted our study on a small number of samples.

However our data can be used for further studies on a large group of dengue patients.

CONCLUSION

In the course of dengue illness there is a window period when neither NS 1 nor Dengue IgM antibodies is detected. During this period physician can rely on levels of serum ferritin which markedly increase with the increasing dengue severity. Patients has thrombocytopenia with raised serum ferritin level at day five as compared to day one when platelet count was normal. Raised levels of serum ferritin serve as initial biomarkers of underlying dengue fever severity. As medical practitioners, we should realize the importance of early identification of raised levels of serum ferritin associated with dengue severity and its timely management. The results of the study need further analysis by large randomized controlled trials.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AR & NY: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

FAS & IK: Data acquisition, data analysis, approval of the final version to be published.

KAS & LY: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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