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Original Research Article

A PROSPECTIVE OBSERVATIONAL STUDY EVALUATING SERUM FERRITIN LEVELS WITH THE SEVERITY OF DENGUE FEVER IN CHILDREN

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ABSTRACT

Background: Dengue fever is the fastest spreading viral disease transmitted by mosquitoes and is regarded as a major public health concern in tropical and subtropical locations around the globe. The clinical spectrum may range from dengue fever without warning signs to dengue with warning signs to severe dengue. The progression to severe dengue remains difficult to predict. Acute inflammation causes a rise in serum ferritin, an acute phase reactant. Measuring serum ferritin levels during dengue fever can help identify illness development early on. This study was conducted to assess the relationship between serum ferritin levels and the severity of dengue fever in children **Materials and Methods:** Children under the age of 18 who were hospitalized to the pediatric ward and intensive care unit of a tertiary care hospital in southern India were the subjects of this prospective observational study. From June 2023 to January 2025, 92 children who tested positive for dengue with NS1 or IgM antibodies participated in the study. All children underwent a thorough clinical history and examination. Serum ferritin levels were checked on the third day of dengue infection. **Result:** The patients in this study had an average age of 9.53 years, with 54 males (59%) and 38 females (41%). The most prevalent symptoms reported in this study were fever (100%), followed by myalgia (73.9%), GI symptoms 61 (66.5%), headache 56 (60.9%). Complications included plasma leak in 52 (56.5%) and shock in 18 (19.6%) cases. 74 (80%) cases were non-severe dengue, with 18 (20%) cases being severe dengue. 15 children tested positive for IgM ELISA and 77 for the NS1 antigen. The average hematocrit was 38.63 ± 4.8%. The platelet count averaged 81086.9 ± 48102.6 cells/µL. The mean WBC count was 5068.15 ± 1839.2 cells per µL. The median AST was 197.68 + 163.501 units/L, and the mean ALT was 142.82 + 124.011 units/L. Gallbladder edema was seen in 56.5% of cases, ascites in 39.1%, and pleural effusion in 40.2%. The median blood ferritin levels in non-severe dengue cases were 181 (153-181) micrograms/L, whereas in severe dengue cases they were 731 (553-1553) micrograms/L (p value < 0.001).The average hospital stay for non-severe dengue cases was 4.86 days, while severe dengue cases required 6 days. **Conclusion:** Children with severe dengue frequently develop hyperferritinemia. On the third day of sickness, median blood ferritin levels > 730 micrograms/L indicate a higher risk of severe dengue infection.

INTRODUCTION

Dengue fever is the most common viral disease transmitted by mosquitoes to people. It is spread by Aedes aegypti mosquitoes and is caused by four dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of the genus Flavivirus. Infection provides lifelong immunity to the infecting virus serotype, but not for other serotypes.

Dengue infections are expected to reach 7.6 million by 2024, according to WHO. Nearly half the world's population resides in the countries where dengue is endemic. The World Health Organization (WHO) recognizes dengue as one among 17 neglected tropical diseases. Each year, thousands of severe dengue cases occur, resulting in approximately 20,000 deaths and the loss of 264 DALYs per million population annually. The case fatality rate for dengue

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fever varies across countries, with children and young adults most commonly affected by fatal outcomes. Globally, reported dengue cases have risen significantly, from 2.4 million in 2010 to 5.2 million in 2019. Among the thirty most highly endemic countries, five are in Asia: India, Indonesia, Myanmar, Sri Lanka, and Thailand. In the Southeast Asia Region (SEAR), the incidence of dengue has more than tripled over the last decade, with cases increasing from 0.19 million in 2011 to over 0.45 million in 2015 and 0.68 million in 2019. Similarly, deaths increased from 1,050 in 2011 to 1,684 in 2019. However, in the year 2020, the number of dengue case reports and deaths in 7 SEAR member states declined to 0.26 million and 928, respectively.[1]

In India, the incidence of dengue has shown a rising trend in recent years. In 2018, 1,24,493 instances were reported, and this number grew to 2,05,243 in 2019. Although there was a decline to 44,585 cases in 2020, the numbers surged again to 193,752 in 2021 and 233,251 in 2022. The case fatality rate (CFR—deaths per 100 cases) has significantly declined over the years, from 3.3% in 1996 to 0.3% in 2014. It further decreased to 0.2% between 2015 and 2018 and reached 0.1% in 2019, a level that has been sustained since then.[1] The 2009 WHO classifies dengue according to levels of severity: dengue without warning signs; dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets); and severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure).[2,3]

Severe dengue is caused by a complicated interaction between the virus and the immune system. The underlying pathophysiological mechanisms leading to progression of disease are antibody-dependent enhancement, cytokine storm, vasculopathy, and coagulopathy. Serum ferritin levels, a measure of the acute-phase response, correlate with illness severity and indicate a strong inflammatory response. Antibody-mediated mechanisms enhancing inflammatory reaction causing cytokine storm similar in nature to that observed in secondary hemophagocytic lymphohistiocytosis (HLH) has been implicated in the pathophysiology of severe dengue disease. Various biomarkers have been studied to assess complications in critical dengue illness, including serum ferritin (SF), serum ceruloplasmin, platelet count (thrombocytopenia), interleukin assays, and rising hematocrit levels.[4,5] Of all the severity biomarkers studied, serum ferritin levels have shown the greatest promise.[4,6] Measurement of serum ferritin is easy, cheap, and readily available in most intensive care units (ICUs).[7] A prognostic indicator could provide a proactive approach in managing severe dengue infection and anticipate potential complications. The study aimed to examine the relationship between serum ferritin levels and severity of disease in children with dengue fever. Secondary objectives were to address the dengue related complications like hepatomegaly, plasma leak, shock, bleeding manifestations, deranged liver enzymes, and renal impairment. There is a cytokine storm in dengue, leading to the release of inflammatory cytokines, which in turn elevates the ferritin levels.

MATERIALS AND METHODS

This prospective observational study was conducted over a period of 8 months (June 2023 to January 2024) in the pediatric department at a tertiary care hospital in southern India. Proper approval from the Institutional Ethical Committee was obtained before commencement. Written informed consent was taken from parents. The study included children aged 6 months to 18 years with laboratory-confirmed dengue fever by NS1 and/or dengue IgM ELISA and admitted to the pediatric ward/PICU within the first 72 hours of the onset of fever. Children with co-infections (like malaria, typhoid, or rickettsial fever), any other systemic illnesses, or any congenital anomaly, and patients/parents not willing to sign the informed consent forms were excluded.

Based on the National Guideline for Clinical Management of Dengue was published in collaboration with the World Health Organization (WHO) in 2018 for efficient management of dengue, cases classified into three major categories based on their severity: dengue fever (group A), dengue fever with warning signs (group B), and severe dengue (group C). Here the former two (groups A and B) are non-severe groups.

The diagnosis of dengue fever was confirmed by an NS1 antigen-based ELISA test (J. Mitra kit, India) or dengue serology for IgM for primary dengue infection (kit from the National Vector Borne Disease Control Program, Pondicherry, and the National Institute of Virology, Pune, India). Blood samples collected from children with a provisional diagnosis of dengue fever were screened by both NS1 Ag and MAC-ELISA.

A predesigned proforma was used to capture details about the clinical and laboratory profiles, as well as warning signs. Serum ferritin levels were determined using an immunoenzymatic colorimetric technique on day 3 of dengue infection and reported in micrograms/liter. Both severe and non-severe dengue cases had their levels analyzed.

Details of clinical and laboratory profile, warning signs were recorded in a predesigned proforma. Serum ferritin levels were measured using Immuno enzymatic colorimetric method on day 3 of illness of dengue and reported in micrograms/litre. Between cases of severe and non-severe dengue, the levels were compared.

Statistical analysis: Data were statistically analyzed using Statistical Package for Social Sciences version 1.0, Inc. Chicago, USA) for MS Windows. Qualitative data expressed in proportions. Quantitative data is expressed in means, medians, and standard deviations. The chi-square test/Fisher's

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exact probability test was used as a test of significance. P Value<0.05 was taken as significant.

RESULTS

We enrolled 92 cases of dengue; the mean age of patients was 9.53±3.01 years. 58.7% of the patients were males and 41.3% were females, indicating male predominance [Table 1]. 56.5 % of the patients were from urban areas, and 43.5% were from rural areas, indicating urbanization leads to more dengue virus transmission [Table 2]. 4.3% of the patients had a previous history of dengue. With respect to clinical presentation, 100% of the patients had fever, 73.9% had myalgia, 66.3% had vomiting/abdominal pain, 65.2% had dengue rash, 64.1% had petechiae/mucosal bleed/melena, 60.9% had headache, 72.8% had organomegaly,56.5% had signs of leak, and 19.6% had shock. On ultrasound examination, gallbladder edema was detected in 56.5% of patients, followed by pleural effusion was detected in 40.2% and ascites in 39.1% cases [Table 3]. 83.7% had NS1 antigen positive, and 15 (16.3%) had IgM+ELISA [Table 4]. Mean Hb% was 10.74 ± 2.4 g/dl. The mean hematocrit was 38.63±4.8 volume%. The mean platelet count was 81086.9±48102.6 cells/µL. The mean WBC count was 5068.15 ± 1839.2 cells/µL. The mean CRP was 16.15±15.5 mg/L (0.8-48.4). Mean aspartate transaminase (AST) was 197.68 + 163.501 U/L (range 30-978). The mean alanine transaminase (ALT) was 142.82+ 124.011 U/L (range 856–142.82) [Table 5]. 36.9% of patients were having dengue fever (DF), whereas 43.5% were having dengue with warning symptoms (DWS) and 19.6% were having severe dengue (SD) [Table 6]. Median ferritin was 181 (153-181) micrograms/L in non severe dengue, 731(553-1553) micrograms/L in severe dengue, with a p value of < 0.001, which was statistically significant [Table 7].

Table 1: Gender wise distribution of the study population

Gender	Number of cases	Percentage
Male	54	58.7%
Female	38	41.3%
Total	92	100%

Table 2: Distribution of cases based on Residence

Place of Residence	Number of cases	Percentage
Urban locality	52 (56.5%)	56.5%
Rural locality	40 (43.5%)	43.5%
Total cases	92	100%

Table 3: Symptoms and Signs among study population

Symptoms and Signs	Number of cases	Percentage
Fever	92	100%
Myalgias	68	73.9%
Vomiting/Abdominal pain	61	66.3%
Dengue rash	60	65.2%
Bleeding manifestations	59	64.1%
Headache	56	60.9%
Organomegaly	67	72.8%
Signs of plasma leak	52	56.5%
Shock	18	19.6%
Ultrasound findings:		
Gall bladder wall edema	53	56.5%
Pleural effusions	37	40.2%
Ascites	36	39.1%

Table 4: Depicting diagnosis of dengue cases based on NS1antigen/ IgM ELISA

NS1 antigen/IgM ELISA	Number of cases	Percentage
NS1 antigen positive	77	83.7%
IgM ELISA positive	15	16.3%
Total	92	100%

Table 5: Labaratory parametres among study population

Lab Parametre	Mean Value	Standard Deviation
Hb (g/dl)	10.74	±2.4
HCT volume %	38.63	±4.8
TLC cells/mm3	5068.15	± 1839.2
Platelets cells/mm3	81086.9	±48102.6
CRP mg/L	16.15	±15.5
AST U/L	197.68	+ 163.501
ALT U/L	142.82	+ 124.011

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Table 6: Distribution of patients based on Dengue Categories

Dengue Category	Number of cases	Percentage
DF	34	36.9%
DWS	40	43.5%
SD	18	19.6%
Total	92	100%

For the purpose of comparing laboratory parameters, we created two categories for this study: non-severe dengue (DF and DWS: 74 cases) and severe dengue (SD: 18 cases)

Table 7: Non severe dengue vs Severe dengue Lab Parameters

Lab Parameters	Non severe dengue (74)	Severe dengue (18)	P value
Age in years	9.51±3.07	9.61±2.8	0.903
Hb%	11.351±2.26	8.1±0.87	< 0.0001
TLC cells/mm3	5317.1±1865.8	2046.8±323.6	< 0.0001
HCT volume%	38.528±5.11	39.08±3.42	0.66
Total Proteins g/dl	6.2±1.1	5.3±0.624	0.001

Table 8: Median Lab Parameters of Non Severe dengue Vs Severe dengue

Lab Parameter	Non Severe dengue (N=74) Median (IQR)	Severe dengue (N=18) Median (IQR)	P value
Platelets cells/mm3	91000(45000-111250)	37500(27500-45000)	0.000
AST U/L	99.5(78.5-99.5)	350(325-450)	0.000
ALT U/L	88(55.5-145.50)	241(196-458)	0.000
Serum Creatinine mg/dl	0.5(0.4-0.7)	1.1(0.9-1.1)	0.000
Serum Ferritin microgram/L	181(153-181)	731(553-1553)	0.000
CRP mg/L	4.4(2.4-21.2)	31.5(22-31.5)	0.000

As few of the lab parameters were extremely distributed, a nonparametric test called the Mann-Whitney test was used to compare medians and interquartile range (IQR) of the above variables. P values for the above parameters were statistically significant.

DISCUSSION

In recent decades, its incidence has increased significantly, posing a major public health concern, especially in countries with limited healthcare resources. Children under the age of 18 are particularly vulnerable to severe manifestations of the disease, which may include dengue hemorrhagic fever and dengue shock syndrome—conditions that can lead to significant morbidity and mortality if not identified and managed promptly. Early identification of patients at risk of developing severe dengue is crucial for timely intervention and improved outcomes. Dengue fever contributes significantly to morbidity and mortality in Southeast Asian endemic regions. Clinical phenotypes of dengue in children and adults differ, with children experiencing higher incidences of shock compared with adults. Additionally, mortality due to SD is 15.9 times higher in children under 14 years of age. In our study, male cases (58.7%) were more reported than female cases (41.3%). In Prabhavathi R et al8 and Rizwan Ishtiaq et al,[9] studies showed a greater number of dengue cases in male children, 52% and 87.3%, respectively, whereas Shilpa Khanna Arora et al10 showed almost equal numbers of cases.

In the present study, 19.5% of children developed severe dengue, a proportion that is close to the findings of Shilpa Khanna Arora et al,[10] (20%). The study found that 43.4 percent of children had dengue with warning symptoms (DWS), which is lower than the number reported by Arora et al,[10] (62.35%) and Prabhavathi R et al8 (52%). In contrast, in Rizwan Ishtiaq et al study,[9] the classification used was Dengue Hemorrhagic Fever (DHF), which may correspond to DWS or SD depending on severity, but exact classification into DWS or SD was not specified.

In our study, 56.5% of dengue cases were from urban areas and 43.5% of cases were from rural areas, like in Vong et al and Chew et al studies.[11,12]

The findings in this study suggest that while fever is the most common symptom across all groups, the other symptoms, like abdominal pain, vomiting, myalgia, and bleeding manifestations (petechiae, mucosal bleed, melena), become more prevalent as the disease progresses. This aligns with the increasing systemic effects of dengue, including capillary leak and immune activation, which lead to greater symptom severity. Myalgia and bleeding manifestations, in particular, are often linked to the body's response to vascular damage and platelet dysfunction, which become more pronounced in severe dengue. The presence of these symptoms in higher frequencies in severe cases further highlights the clinical features that may be used to predict disease progression. However, symptoms like rash were still present in both severe and non-severe cases, indicating their commonality in dengue and limiting their ability to differentiate between the severity levels in all cases. The current research also discovered greater levels of AST compared to ALT.

In the present study, serum ferritin levels showed a progressive increase with the severity of dengue infection, with mean values of 162.5 ng/mL in the

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Dengue Fever (DF) group, 210 ng/mL in the Dengue with Warning Signs (DWS) group, and 731 ng/mL in the Severe Dengue (SD) group. This trend highlights the potential of ferritin as a biomarker reflecting the degree of systemic inflammation and immune activation associated with worsening disease severity. In the present study median serum ferritin levels (731 microgram/L) were high in severe dengue group, compared to non severe cases (181 microgram/L), which was statistically significant (p value 0.000).

Lakshmanan et al,[13] conducted a retrospective cohort study involving critically ill pediatric patients and observed a median ferritin level of 8,105 ng/mL (IQR: 2,350–15,765) in severe dengue cases, suggesting a strong association between hyperferritinemia and disease severity, including poor clinical outcomes.

Similarly, Sekhar M.C,[14] identified ferritin levels ≥1,200 ng/mL as a useful cutoff to predict severe dengue in children, emphasizing its diagnostic and prognostic significance. Though the ferritin levels in the present study were comparatively lower than those reported in these studies, the increasing trend across clinical categories (DF, DWS, and SD) remains consistent and supports the use of serum ferritin as an adjunctive marker for assessing disease severity.

Other useful markers in predicting severe dengue found in our study were anemia, leukopenia, low total proteins, severe thrombocytopenia, transaminitis (AST>ALT), serum creatinine, and CRP.

Our study showed an inverse relation between hemoglobin and the severity of dengue in children. 67% of children with severe anemia had a risk of severe dengue. This is in inline with the findings of Jakribettu et al,[15] where low Hb is identified as a key parameter in predicting severity. Similarly, Nandwani et al,[16] also highlighted that early low Hb is a predictor of severe dengue. The present study shows a clear declining trend of white blood cell (WBC) counts with increasing severity of dengue: DF cases had a mean WBC of 5860.8 cells/mm³, DWS had 4855 cells/mm³, and SD had the lowest at 2046.8 cells/mm³. This indicates that leukopenia is a sensitive indicator of dengue, especially in severe cases. Studies including Hemant Jain et al,[17] Saha et al,[18] and Dhrubajyoti et al,[19] consistently reported low WBC counts across all dengue types, supporting the role of leukopenia in early diagnosis of dengue. Thus, decreasing trends of leukocyte counts are an important guide for progression to severe dengue.

Hepatic dysfunction in the form of markedly elevated liver enzymes was higher in severe and complicated dengue in comparison to classical dengue fever. Dengue infection can cause mild to severe liver dysfunction in children, including elevated transaminases, jaundice, and liver cell loss. Severe and complicated dengue showed higher levels of increased liver enzymes compared to dengue fever The current research also discovered greater levels of AST compared to ALT. Rise in AST is more common, and levels are higher in severe dengue than in non-severe dengue (DF, DWS). Higher AST levels in DF may be due to muscle breakdown and hemolysis, as AST is also present in non-hepatic locations such as red blood cells and muscles. Supporting this observation, a study done by Kuo CH et al,[20] in 1992 evaluated approximately 270 patients with dengue. According to this study, over 90% of the patients showed abnormal AST levels when compared to ALT. Mean total serum protein levels were lower (5.3±0.624 g/dl) in severe dengue than in non severe dengue cases (6.2±1.1 g/dl), consistent with the study conducted by Arora et al.[10]

CONCLUSION

One of the earliest markers for severe dengue is serum ferritin. An increased risk of developing severe dengue is linked to higher ferritin levels, especially in the early stages of the disease. Anemia, notable leukopenia, marked transaminitis (AST), thrombocytopenia, and hypoproteinemia were additional useful test markers that can be used to predict severe dengue.

Limitation: Because the study only measured serum ferritin levels once at admission, it might not account for the dynamic fluctuations in serum ferritin levels over the duration of the illness. Lower rates of severe dengue in the study. Before a definitive conclusion could be made, more research with a larger sample size of severe cases would be necessary.

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