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To find out role of serum ferritin levels as an early predictor of severity of dengue-an observational study

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***Abstract*---**Background: Ferritin is an acute-phase reactant and highly expressed by cells of the reticulo-endothelial system in response to infection and inflammation. Hyperferritinaemia is a hallmark of diseases characterized by extensive immune activation such as malignancy or viral infection, including dengue. Aims and Objectives: To find out if serum ferritin levels can predict the severity of dengue early. Material and Methods: Study design: This was a single centre, prospective observational study done on 50 patients with positive NS1Ag or dengue antibody serology Ig-M and are admitted in Paediatric ward or in Paediatric ICU during study period (December 2019 - May 2021) in tertiary care centre, Karad. Enrolled patients underwent serum ferritin level analysis at time of admission. Serum ferritin levels were later compared with the severity of the dengue disease (According to WHO dengue classification 2009). Statistical Analysis: Data was analyzed using computer software, SPSS-v21, DeLong et al (1988) and Binomial exact. Data was expressed in frequency and percentage as well as mean and standard deviation. A $p<0.05$ was taken as significant. Results: The mean serum ferritin levels were 298.59 ± 147.48 ng/ml in DF without warning signs, 370 ± 230.58 ng/ml in DF with warning signs, 1101.41 ± 426.64 ng/ml in severe dengue ($p<0.05$). Mean serum ferritin levels on day 1-3 was 299.50 ± 185.01 ng/ml in DF without warning signs, 258 ± 93.18 ng/ml in DF with warning signs, 1049 ± 500.17 ng/ml in severe dengue ($p<0.001$).Mean serum ferritin levels on day 4-5 was 304.33 ± 97.48 ng/ml in DF without warning signs, 379.71 ± 176.87 ng/ml in DF with warning signs, 1193 ± 434.16 ng/ml in severe dengue ($p<0.001$).Mean Serum ferritin levels on day 6-7 was 236 ± 0 ng/ml in DF without warning signs, 571.33 ± 429.48 ng/ml in DF

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with warning signs, 1133 ± 418.80 ng/ml in severe dengue (p>0.05). Conclusion: According to our study serum ferritin levels can be used as an early predictor of severity of dengue, especially with in first 5 days of onset of symptoms. A cut of > 423 ng/ml had a sensitivity of 100% and specificity of 81.58% to differentiate between severe and non-severe dengue fever from our study.

***Keywords*---**Dengue fever (DF), severity of dengue, serum ferritin.

Introduction

Dengue fever (DF) is a major public health problem with 50 million annual cases worldwide. It has been reported in more than 100 countries and continues to spread to previously unaffected regions.[1] It is an arboviral fever caused by the dengue virus. It is caused by 5 serotypes of Dengue virus namely DENV1, DENV2, DENV3, DENV4, DENV-5 and is transmitted to human beings by the bite of Aedes mosquitoes. [2] [3] Infection with any serotype usually results in asymptomatic infection or mild non-specific febrile illness, but in a subset of patients, severe disease develops, characterized by a transient capillary leakage syndrome (dengue haemorrhagic fever/ DHF).[4] Immunity of host plays an important role in dengue fever pathogenesis.[5] The exact mechanism of different clinical manifestations of dengue is not clearly understood till date. Most accepted theory is virus strains of secondary infection causing increased antibody and memory T-cells production resulting in cytokine storm.[6] Other theories considered are shift to Th 2 response in severe dengue from Th 1 response in mild dengue.[7] These finally result in haemorrhage and shock due to damage of endothelium, platelets and various organs leading to vasculopathy and coagulopathy.[8] This unpredictable disease course and the need to differentiate DF from other causes of fever to make an early and sensitive diagnosis of acute dengue virus infection is thus important. Other arboviral diseases present in India include Japanese Encephalitis, Chikungunya, and Kyasanur Forest Disease but dengue fever remains the most common cause of the arboviral epidemic outbreak.[9] The case fatality rate of dengue is 1% while the fatality rate is as high as 3-5% in certain sections of rural India.[10] The clinical features of dengue fever can be explained by endothelial damage and capillary leakage due to increased capillary permeability.[5] In addition to the current laboratory markers for dengue, serum ferritin levels were described to be associated with clinical disease severity in children. In many cases ferritin levels higher than 500 µg/L were detected, defined as hyperferritinaemia. Ferritin is an acute-phase reactant and highly expressed by cells of the reticulo-endothelial system in response to infection and inflammation.[11] Ferritin binds iron, limiting its availability in the circulation. Because many pathogenic microorganisms need iron for their proliferation, this mechanism is favourable for the host. Moreover, iron deficiency enhances the immunological performance of lymphocytes, neutrophils and macrophages. Hyperferritinaemia is a hallmark of diseases, characterized by extensive immune activation, including hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS).[11] HLH can be congenital or triggered by an external stimulus, such as malignancy or viral infection, including dengue. NK cells and CD8+ T lymphocytes are impaired in their cytotoxic function in patients with

HLH, which results in reduced clearance of infected and antigen-presenting cells from the circulation. This may lead to an exaggerated immune response with proliferation of dendritic cells, tissue macrophages and T-cells, contributing to a cytokine storm. [11]

Material and Methods

Aim and objectives: To find out if serum ferritin levels can predict the severity of dengue early. *Study design:* This was a single centre, prospective observational study done on 50 patients with positive NS1 antigen test or dengue antibody serology for Ig-M and are admitted in Paediatric ward or in Paediatric ICU during study period (December 2019 - May 2021) in tertiary care centre, Karad. The study was approved by institutional ethics committee before initiation (IEC protocol number: 195/2019-2020). *Inclusion criteria:* Children with positive NS1 antigen test or dengue antibody serology for Ig-M were included in the study. *Exclusion criteria:* Fever with any other causes who did not fit inclusion criteria and child with preexisting hematological disorders and iron load status were excluded from the study. *Method of collection of data:* Children admitted in Krishna Hospital with positive NS1 antigen test or dengue antibody serology for Ig-M between the age group of 2 months to 14 years and fulfilling inclusion and exclusion criteria were included in this study. Dengue serology was sent for all those who had come with complaints of fever and clinical features suggestive of dengue. Diagnosis of dengue was made on the basis of NS1 antigen positivity and/or detection of IgM antibodies using a commercially available one-step immunochromatographic assay. Demographic profile, day of illness at time of presentation and relevant information have been collected using a structured proforma by interviewing the parents of the participants. Clinical presentation on admission was noted and complete blood counts (CBC) and serum ferritin levels were sent on admission. This is followed by daily vitals monitoring and watching for bleeding manifestations if any and any other complications. CBC was done every alternative day from day of admission till day of discharge/death or CBC was done if any bleeding manifestations were noted or clinically indicated. Serum ferritin levels were sent only if patients were presented within 7days of onset of symptoms. Serum ferritin levels were done by *IMMULITE,* a solid phase enzyme labeled chemiluminescent immunometric assay. The solid phase (bead) is coated with monoclonal murine anti-ferritin antibody. The liquid phase consists of alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-ferritin antibody. The patient sample and the reagent are incubated together with the coated bead for 30 minutes. During this time, ferritin in the sample forms the antibody sandwich complex with monoclonal murine anti-ferritin antibody on the bead and the enzyme conjugated polyclonal goat anti-ferritin antibody in the reagent. Unbound patient sample and enzyme conjugate are then removed by centrifugal washes. Finally, chemiluminescent substrate is added to the test unit containing the bead and the signal is generated in proportion to the bound enzyme. Enrolled patients were classified into DF without warning signs, DF with warning signs and severe dengue based on clinical and lab parameters according to WHO dengue classification 2009.[12] Serum ferritin levels were later compared among non-severe dengue cases (DF without warning signs and DF with warning signs) and severe dengue cases. We also compared serum ferritin levels based on day of illness with respective to severity of the disease. *Statistical analysis:* Data

was analyzed using computer software, Statistical Package for Social Sciences (SPSS), DeLong et al (1988) and Binomial exact. Data was expressed in frequency and percentage as well as mean and standard deviation. To elucidate the associations and comparisons between different parameters, Chi square (x2) test was used. A p <0.05 was taken as significant.

Results

A total of 50 children with positive NS1 antigen test and or dengue antibody serology for Ig-M and who were admitted in our hospital during the study period from December 2019 to November 2021 were included in this study. In our prospective observational study, the mean serum ferritin levels were 298.59 ± 147.48 ng/ml in DF without warning signs, 370 ± 230.58 ng/ml in DF with warning signs, 1101.41 ± 426.64 ng/ml in severe dengue. This observation was statistically significant (p<0.05). [Table 1] [Figure 1]

Table 1
Mean serum ferritin levels

	DF without warning signs (n=22)		DF with Warning signs (n=16)		Severe dengue (n=12)		*p* value
	Mean	SD	Mean	SD	Mean	SD	
Serum ferritin	298.59	147.48	370	230.58	1101.41	426.64	<0.0001*

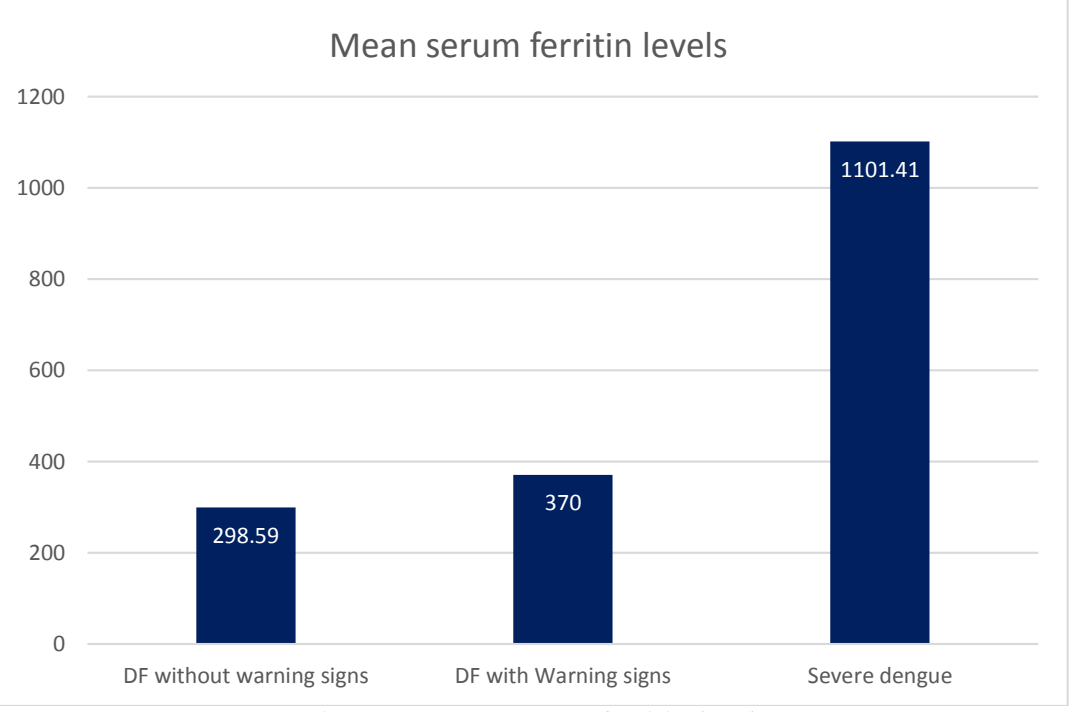

Figure 1. Mean serum ferritin levels

Mean serum ferritin levels on day 1-3 was 476.70 ± 432.65 ng/ml, on 4 – 5 days was 432.38 ± 323.24 ng/ml and on 6-7 days were 810.25 ± 508.89 ng/ml. [Table 2] [Figure 2]

Table 2
Day of illness and mean serum ferritin levels

Day of illness	Ferritin (Mean ± SD)
1 – 3 days (n=24)	476.70 ± 432.65
4 – 5 days (n=18)	432.38 ± 323.24
6 – 7 days (n=8)	810.25 ± 508.89

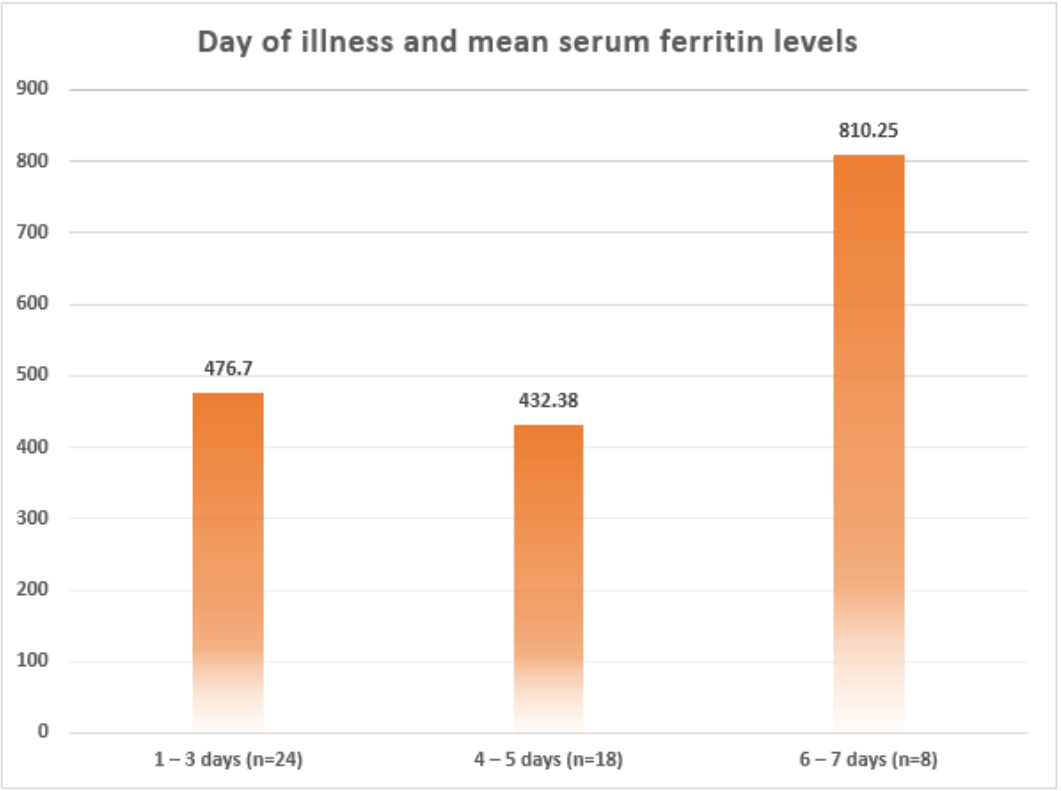

Figure 2. Day of illness and mean serum ferritin levels

Serum ferritin levels were significantly higher (p<0. 001) in severe dengue group when compared with non-severe dengue group in 1–3 days (1049 ± 500.17 ng/ml vs 299.50 ± 185.01 ng/ml and 258 ± 93.18 ng/ml) and 4-5 days of day of illness (1193 ± 434.16 ng/ml vs 304.33 ± 97.48 ng /ml and 379.71 ± 176.87 ng/ml). Even though serum ferritin levels were higher in severe dengue group after 5 days of illness (1133 ± 418.80 ng/ml in severe dengue vs 236 ± 0 ng/ml and 571.33 ± 429.48 ng/ml in non-severe group), this observation is not statistically significance with respective to severity of disease in our study (p=0.17). [Table 3] [Figure 3]

Table 3
Severity of Dengue and serum ferritin levels based on day of illness

Day of illness	DF without warning signs	DF with warning signs	Severe dengue	*p* value
1 – 3 days	299.50 ± 185.01	258 ± 93.18	1049 ± 500.17	<0.001*
4 – 5 days	304.33 ± 97.48	379.71 ± 176.87	1193 ± 434.16	<0.001*
6 – 7 days	236 ± 0	571.33 ± 429.48	1133 ± 418.80	0.17

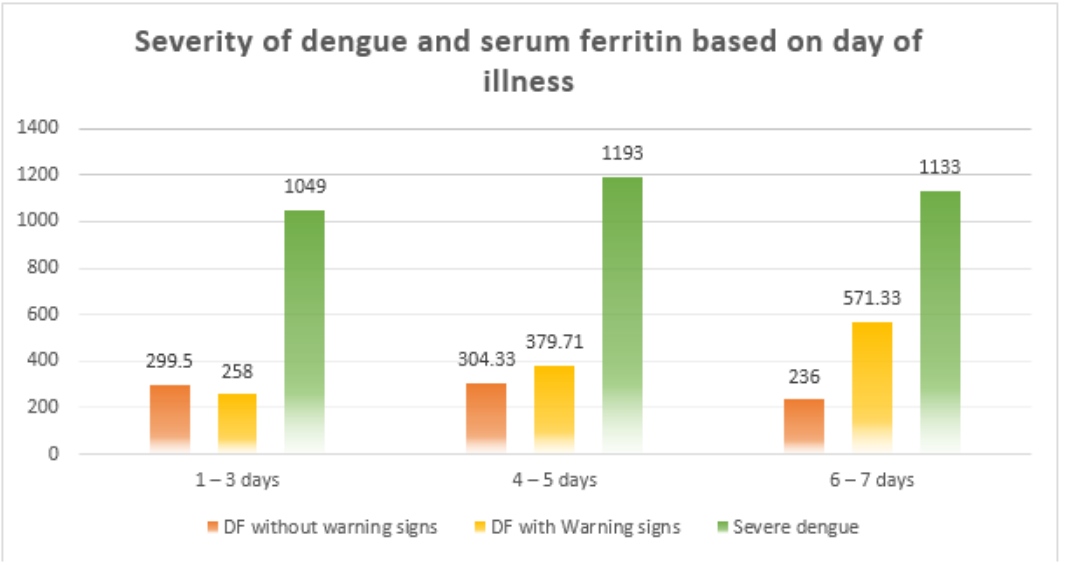

Figure 3. Severity of Dengue and serum ferritin levels based on day of illness

In our study by using ROC analysis curve, we got a cut of value of > 423 ng/ml that had a sensitivity of 100% and specificity of 81.58% to differentiate between severe and non-severe dengue fever. [Table 4] [Figure 4] [Table 5]

Table 4
A receiver-operator characteristic curve analysis of ferritin and severity of dengue

Area under the ROC curve (AUC)	0.969
Standard error [a]	0.0211
95% Confidence interval [b]	0.877 to 0.998
z statistic	22.293
Significance level *p* (Area=0.5)	<0.0001

[a] DeLong et al., 1988, [b] Binomial exact

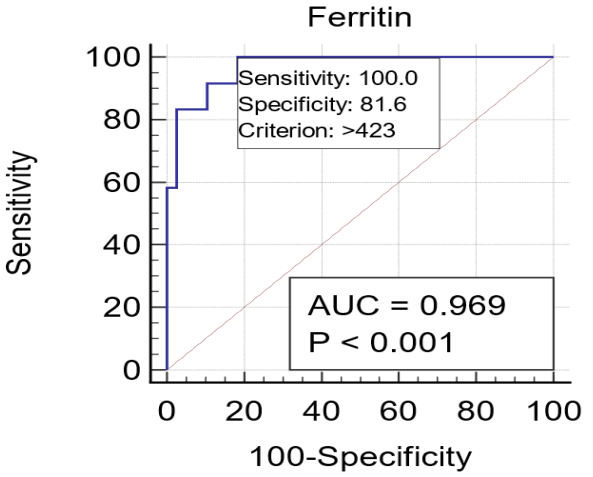

Figure 4. A receiver-operator characteristic curve analysis of ferritin and severity of dengue. Area under the ROC curve values of 0.969 (95% CI:0.877-0.998), Cut-off value 423 ng/mL, sensitivity 100% specificity 81.6%

Table 5
Youden index

Youden index J	0.8158
Associated criterion	>423
Sensitivity	100.00
Specificity	81.58

Discussion

In this study we evaluated patients with dengue fever presenting as inpatients in paediatric ward or PICU of a tertiary care centre in an urban setting to find out if serum ferritin levels can be used as an early marker for predicting severity of disease in dengue patients. The mean serum ferritin levels in our study were 298.59 ± 147.48 ng/ml in DF without warning signs, 370 ± 230.58 ng/ml in DF with warning signs, 1101.41 ± 426.64 ng/ml in severe dengue. This observation was statistically significant (p<0.05). Similarly, study done by *Chandrashekar M et al* reported the use of ferritin level in serum >1,200 ng/ml as a method for predicting progression to DHF starting on Day 4 of the disease.[13] Chaiyaratna et al conducted a study to evaluate the levels of ferritin, an acute-phase reactant, in predicting the risk of dengue haemorrhagic fever (DHF) in patients with dengue infection. Study included one hundred seventy-seven Thai children (100 males, 77 females), 4-16 years old (median age 11 years) with DF (n = 44) and DHF (n = 133). The results reveal that sensitivities on days 5, 6 and 7 of illness were 81.5, 84.4 and 89.9%, respectively, and the specificities were 42.4, 39.0 and 36.4%, respectively. High serum ferritin levels > or = 1,200 ng/ml may be a predictor of dengue haemorrhagic fever.[14] In 2017, *Roy Choudhary et al* conducted a study to evaluate ferritin as a surrogate marker for dengue infection where 30 cases of

clinically and serologically confirmed dengue fever were included. Ferritin level in dengue cohort was significantly higher than the infective or inflammatory etiology (OFI) group. The best cut-off for ferritin level to differentiate dengue from OFI was found to be 1291 ng/ml. The sensitivity at this cut-off is 82.6% and the specificity at this cut-off is 100%.[7] 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). 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 ± 568.31 ng/ml in non-severe cases vs. 1264.71 ± 492.59 ng/ml in severe dengue on day of admission; 418.19 ± 404.59 ng/ml vs. 1490.74 ± 359.40 ng/ml 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.[15] Similarly, our study also showed significant difference between non severe group and severe dengue group. Mean serum ferritin levels in our study on day 1-3 of illness was 476.70 ± 432.65 ng/dl, on 4 – 5 days of illness was 432.38 ± 323.24 ng/dl and on 6-7 days of illness was 810.25 ± 508.89 ng/dl. Mean serum ferritin levels of severe dengue group in first 5 days of illness (1049 ± 500.17 ng/ml and 1193 ± 434.16 ng/ml) were significantly higher than in non-severe dengue groups (299.50 ± 185.01 ng/ml, 258 ± 93.18 ng/ml, 304.33 ± 97.48 ng/ml and 379.71 ± 176.87 ng/ml) in our study and this is of statistically significance with $p<0.001$ suggesting that serum ferritin levels can be used as an early marker for predicting severity of dengue with in first 5 days from onset of illness. Even though mean serum ferritin levels were higher after 5 days of illness in severe dengue group (1133 ± 418.80 ng/ml) when compared to other groups (236 ng/ml and 571.33 ± 429.48 ng/ml) this observation is not statistically significance.

	Present study	Petchiappan V et al [16]
Day 1-3	1049 ± 500.17	-
Day 4–5	1193 ± 434.16	2000
Day 6-7	1133 ± 418.80	2000

In the present study, a cut off of serum ferritin >423 ng/ml had a sensitivity of 100% and specificity of 81.58% to differentiate between mild and severe dengue. *Chaiyaratna et al* study reported that high serum ferritin levels > or = 1,200 ng/ml may be a predictor of dengue haemorrhagic fever.[14] *Roy Choudhary et al* reported that serum ferritin of 1291 ng/ml is best to differentiate between dengue and OFI with a sensitivity of 82.6% and specificity of 100%.[7] The lower serum ferritin values for diagnosing severity of dengue disease according to our study is might be because of higher sensitivity value that we have considered in this study when compared to other studies. *Suresh et al* in their study reported that on D1, the serum ferritin level was a "good" predictor of severe dengue, with an area under the curve (AUC) of 0.863 with standard error (SE) = 0.043 and a 95% CI from 0.778 to 0.947 ($P < 0.05$). On D4, serum ferritin was an "excellent" predictor of severe dengue, with an AUC of 0.947 with SE = 0.021 and a 95% CI from 0.907 to 0.988 ($p<0.05$).[17]

Conclusion

According to our study serum ferritin levels can be used as an early predictor of severity of Dengue especially with in first 5 days of onset of symptoms. A cut of > 423 ng/ml had a sensitivity of 100% and specificity of 81.58% to differentiate between severe and non-severe Dengue fever from our study.

Bibliography

1. Fever DH. Diagnosis, Treatment. Prevention and Control. Geneva: World Health Organization (WHO) and Special Programme for Research and Training in Tropical Diseases (TDR). 2009;3.
2. Bancroft TL. On the aetiology of dengue fever. Austral. Med. Gaz. 1906;25:17-8.
3. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med J Armed Forces India. 2015 Jan;71(1):67-70.
4. Halstead SB. Pathogenesis of dengue: dawn of a new era. F1000Research. 2015;4.
5. Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. Clinical microbiology reviews. 2009 Oct;22(4):564-81.
6. Wilder-Smith A, Ooi EE, Horstick O, Wills B. Dengue. The Lancet. 2019 Jan 26;393(10169):350-63.
7. Roy Chaudhuri S, Bhattacharya S, Chakraborty M, Bhattacharjee K. Serum ferritin: a backstage weapon in diagnosis of dengue fever. Interdisciplinary perspectives on infectious diseases. 2017 Oct 2;2017.
8. Chaturvedi UC, Nagar R. Dengue and dengue haemorrhagic fever: Indian perspective. J Biosci. 2008 Nov;33(4):429-41.
9. Broor, Shobha & Devi, L.. (2015). Arboviral Infections in India. Indian Journal of Health Sciences and Care. 2. 192. 10.5958/2394-2800.2015.00035.8.
10. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva: World Health Organization; 2009. 1, EPIDEMIOLOGY, BURDEN OF DISEASE AND TRANSMISSION.
11. Valero N, Mosquera J, Torres M, et al. Increased serum ferritin and interleukin-18 levels in children with dengue. Brazilian Journal of Microbiology: [publication of the Brazilian Society for Microbiology]. 2019 Jul;50(3):649-656. DOI: 10.1007/s42770-019-00105-2. PMID: 31243722; PMCID: PMC6863311.
12. The revised WHO dengue case classification: does the system need to be modified? https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3381438.
13. Sekhar MC. Ferritin as a Marker of Severe Dengue in Children: Asian Journal of Clinical Pediatrics and Neonatology. 2020 Oct 10;8(3):7–11.
14. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit K. Serum ferritin levels in children with dengue infection. Southeast Asian J Trop Med Public Health. 2008 Sep 1;39(5):832-6.
15. Soundravally R, Agieshkumar B, Daisy M, Sherin J, Cleetus CC. Ferritin levels predict severe dengue. Infection. 2015 Feb 1;43(1):13-9.
16. Petchiappan V et al. Int J Res Med Sci. 2019 Mar;7(3):876-881.

17. Channapatna Suresh S, Hanumanthaiha R, Ramakrishna C, Sandeep R, Narasimhasetty PS, Ramakrihna V, Raju B. Serum Ferritin as a Prognostic Indicator in Adult Dengue Patients. The American Journal of Tropical Medicine and Hygiene. 2020 Dec 28: tpmd201111.