Hyperferritinemia and Gallbladder Wall Oedema as Early Markers of a Severe Dengue Infection in a Developing Nation.

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Research article

Keywords: dengue, ferritin, gall bladder wall oedema, markers

Posted Date: June 25th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-36751/v1

Abstract

Background: Several chemokines, cytokine and biomarkers have previously been depicted in the pathogenesis of severe dengue fever. Despite the presence of clinical parameters, there have been no proven markers, during the period of defervescence to predict the progression of non-severe dengue cases to the severe dengue phase in the adult population. This study was undertaken to determine the likelihood of serum ferritin levels and gallbladder wall thickness measured within four days of febrile illness, as potential early predictors of severe dengue illness.

Methods: A prospective study was conducted among people visiting tertiary care hospitals in Mangalore, India. 131 participants who presented within 4 days of fever and who had Dengue NS1 antigen card test positive and confirmed Dengue IgM by ELISA were recruited in the study. Blood was tested for serum ferrritin levels and gallbladder wall thickness was measured using ultrasonography.

Results: 33.6% (44) of the cases had severe dengue and had a hospital stay of 6-8 days. Serositis, shock, hepatitis and renal failure were some of the significant complications associated with severe dengue cases. Rising transaminases, serum ferritin, H score and thrombocytopenia correlated well with the dengue severity.66 cases had evidence of gallbladder oedema and 89% of these cases developed severe dengue. Ferritin levels greater than 3825 ng/dl was established as a good cut-off for the severity of dengue with a sensitivity of 72.7%.

Conclusion: In poor socioeconomic and developing nation like India there exists the need for low priced laboratory and radiological tests to predict the severity of dengue cases so that they can be optimally treated to have a good outcome. We demonstrated the use of serum ferritin levels and gallbladder wall thickness >3mm detected early, during the third or fourth day of febrile illness, as potential markers of progression of dengue to its severity

Background

Dengue is a viral illness which is transmitted by the female mosquito of the species Aedes aegypti and less commonly Aedes albopictus that is rampant throughout the world especially in the tropics. It causes a wide spectrum of disease severity ranging from subclinical to severe flu-like symptoms in those infected [1]. The incidence of Dengue has increased considerably around the world in the last few decades. According to an estimate made by the World Health Organization [WHO], dengue incidence nearly amounts to about 390 million cases per year, of which 96 million manifests clinically [2]. Estimates of around 500,000 people with severe dengue require hospitalization each year and about 2.5% of those affected die. In India during the last few decades, there have been increased number of outbreaks and most of the states in India are currently almost endemic to dengue fever. Though the predominant serotype of the virus keeps changing each year, almost all the four serotypes are seen circulating in the environment [1].

Dengue has been classified by the WHO into 2 major categories: Non-severe dengue [with/without warning signs] and severe dengue. Non-severe dengue involves a high fever[104^0F/40^0C] accompanied by any 2 of the following symptoms during the febrile phase –nausea, vomiting, rash, aches and pains, positive tourniquet test, leucopenia. Three to seven days after the onset of the illness, the fever drops to below 100^0 F [38^0C] and the warning signs such as abdominal pain or tenderness, persistent vomiting, fluid accumulation, mucosal bleed, lethargy, restlessness, hepatomegaly and rising hematocrit with thrombocytopenia start to appear requiring strict medical intervention. Inevitable cases progress towards severe dengue evidenced by severe plasma leakage, fluid accumulation with respiratory distress, shock, severe bleeding and organ impairment [3].

Previous studies have shown the role of chemokines, cytokines and several other types of biomarkers that could be involved in the symptom severity of dengue fever [4–5]. Over the years the World Health Organization has attempted to find potential markers which could indicate the progression of dengue to its severe form. In view of this, few studies and reviews have been proposed to indicate potential markers of severity. Noteworthy among them is a meta-analysis conducted by Kuan-Meng Soo et al which proposed the role of IL-7, IL-8, IL-10, TGF-β and VEGFR2 as early indicators of severe dengue infection [6]. Although there have been studies reporting raised levels of cytokines, TNF-α, IL-2, IL-6, IL-10, IL-12 and IFN-Y involved in the pathogenesis in severe dengue cases, these markers have not proven to be beneficial in differentiating the early stages of dengue from the severe form [7–11]. Hence the diagnosis based on these markers and clinical symptoms help to distinguish severe dengue from non-severe only after the person has presented to the hospital with symptom severity. Hence there exists the need for markers to identify dengue during the early febrile phase before it progresses to the severe form so as to effectively manage the disease, reduce the rate of complications and death due to dengue. In a developing nation like India, mitigating the healthcare related costs due to outbreak of dengue is detrimental.

In addition to the biomarkers mentioned above, a study among the paediatric population showed that ferritin [an acute phase reactant] expressed by the reticuloendothelial cells as a response to inflammation and infection is allied with the severity of the disease [12]. A previous study by Ho T et al has deduced that ferritin is a discriminatory marker to differentiate dengue associated febrile illness from other causes for the same [13]. Serum ferritin in dengue fever shows marked levels in contrast to any other viral or bacterial illness and these high levels correspond to an exaggerated risk of developing complications. Some studies have shown a very strong relationship between the severity of dengue infection and rising ferritin levels [12]. During the course of dengue fever, there exists a period during which the Immunoglobulin M [IgM] antibody for dengue, the NS1 antigen and several other biochemical and blood parameters ruling out other febrile illnesses may be absent. During this phase the physicians are in a dilemma regarding the acceptable line of management. Since the progression of the dengue into its severe form can be prevented by optimal fluid correction, thereby preventing dehydration, there is a need to look for adjunct biomarkers which help us predict the progression of the disease during the early

stages [14]. The present study tried to look for ferritin levels done on the third or fourth day of the febrile phase, as an early predictor of severity of the disease

Hypovolemic shock due to the leakage of plasma into serosal cavities is prominent feature of severe dengue. Clinically it is difficult to determine leakage of plasma and quite often serum hematocrit is used as an adjunct to detect significant plasma leak [15]. Gallbladder wall thickening due to significant plasma leakage is one of the commonest findings in dengue fever [16]. A few studies indicate that ultrasonography plays an important role in detecting patients who progress to a critical phase by measuring the thickness of the gallbladder wall [17–19]. Very few studies have been undertaken regarding the validity of gallbladder wall thickening determined on the third or fourth day of febrile illness as a prognostic indicator of severity.

Hence, we undertook this study to determine if the serum ferritin levels and gallbladder wall thickness detected on the third or fourth day of the febrile phase could predict the severity of illness. Early identification of such patients could improve overall case outcome and facilitate more efficacious use of hospital reserves.

Materials And Methods

It is a descriptive study performed in the Infectious Diseases unit of the Department of Internal Medicine at two tertiary referral hospitals in Mangalore, India. This study got the approval of the institutional ethics committee and an informed written consent was taken from all patients who participated in the study after explaining the purpose of the study in a language known and understood by them. The sample size was determined based on sensitivity of ferritin for predicting severe dengue which was 77% with precision of 5% [20]. Hence from the available information the sample size was found to be 131 with confidence interval of 95% using Buderer's formula [21].

Sample size [n] was calculated based on sensitivity using the formula = $\frac{Z_\alpha^2 x S[1-S_n]}{L^2 x Prevalence}$

Where n – required sample size

S_n = anticipated sensitivity

A = size of the critical region [1-α is the confidence interval]

Z_α = standard normal deviate corresponding to 95% confidence interval and

L = absolute precision desired on either side [half width of the confidence interval] of sensitivity

Precision – 5%

Prevalence – 14%

All patients between 18–80 years of age who presented with acute febrile illness within 4 days of fever were screened for dengue by Dengue NS1 antigen card test. These were then confirmed with the help of serological test Dengue IgM by ELISA and those found positive were recruited for the study. On the 3rd or 4th day of fever, serum ferritin levels were done in the laboratory and quantified using electro-chemiluminescence immunoassay [ECLIA] and ultrasound abdomen was used to measure gallbladder wall thickness. All ultrasound abdomens were done by the same radiologist to avoid observer bias. Serum ferritin levels more than 500 ng/ml and gallbladder wall thickness more than 3 mm was considered significant. Blood samples were also obtained for complete blood analysis, renal function tests, liver function tests, serum ferritin as well as serum electrolytes. All admitted patients had their vitals monitored and were looked for complications like hypotension, bleeding, narrow pulse pressure, evidence of capillary leakage in the form of effusion, ascites or oedema and relevant investigations were done serially on case to case basis. H score was calculated which is a predictor for hemophagocytic lymphohistiocytosis disease [22].

Statistical analysis:

All of the quantitative variables were described in the form of mean, median and standard deviation [SD] and the qualitative [categorical] data was described in the form of frequency and proportion. Collected data was entered and analysed by using IBM SPSS Statistics for Windows, version 25.0 [IBM Corp., Armonk, N.Y., USA]. Results for quantitative variables was expressed and difference between the two groups was analysed using Mann–Whitney U test. To establish an optimal cut-of concentration for ferritin, we used the receiver operating characteristic [ROC] curve analysis. P value < 0.05 was considered to be significant.

Results

All patients between 18 to 80 years of age who presented with acute febrile illness within 4 days of onset of fever were screened and 131 of these patients who were confirmed to have dengue by serological test Dengue IgM antibody by ELISA were taken into consideration. The baseline characteristics of dengue patients are listed in Table 1. Out of the 131 laboratory confirmed cases 44 [33.6%] were classified as severe dengue according to the WHO 2009 definitions and the remaining 87[66.4%] were classified as non-severe dengue. Nearly 82/131 patients [62.6%] were in the age group of 31–60 years, thus affecting the working population and among them, 26 cases had severe dengue. While analysing the sex ratio, It was observed that majority of the victims were males [n = 131, 63.4%] and consequently more males [56.8%] were found to have severe dengue. Most of the victims were discharged from the hospital within 5 days of admission [n = 131, 56.5%] and the majority of the severe dengue patients had a hospital stay of 6–8 days. The number of patients who presented within 3 days of onset of fever were 68[51.9%] and the remaining 63 [48.1%] presented within 4 days of onset of the febrile phase. Table 2 enlists some of the clinical manifestations of the cases at the time of admission and the corresponding correlation of those complications with the severity of dengue. Some of the observed complications in our study were-serositis seen in 32 cases, 4 cases of bleeding manifestations, 4 cases of acute respiratory distress

syndrome, 6 patients with renal failure, 8 cases of shock, 5 subjects with hepatitis and one each case had encephalitis and myocarditis respectively. The number of patients who developed more than one of the above listed complications was 13 and this value was statistically significant when correlated with the severity of the disease. There was a significant association between the severity of dengue and complications such as renal failure [P < 0005], shock [P < 0005], hepatitis [P < 0001] and serositis [P < 0.005] when evaluated using the Chi square / Fischer's exact t-test. About 59.5% [78/131] of the cases had evidence of hepatomegaly and splenomegaly was seen in about 56.4% [74/131] of the cases. Table 3 outlines certain laboratory parameters studied among the 2 groups and their correlation with the disease severity. Serum levels of aspartate transaminase [AST], Ferritin, Platelet count, H score, Total count and Haemoglobin were estimated in all cases and expressed in terms of mean, median and standard deviation. The laboratory profile of most of the severe dengue patients was consistent with a mean AST level of 407.55 ± 679 [median 203], followed by mean ferritin level of 9125.34 ± 12453 [median = 568] and a mean platelet count of 26182 ± 24865 [median = 19000]. It is clear from the Table 3 that thrombocytopenia, rise in AST levels, H score and Ferritin levels have a significant positive correlation with the severity of dengue with the P value < 0.005. Table 4 shows the distribution of gallbladder oedema among cases of severe and non-severe dengue. Among the dengue victims who were evaluated, gallbladder oedema was present in nearly 66 cases. 39 patients [89%] among the ones who had severe dengue [N = 44] had a significant gallbladder wall thickening and this correlation between the severity of dengue with gallbladder oedema was found to be statistically significant [P < 0.000]. Table 5 shows a comparison of the laboratory parameters with respect to gallbladder wall oedema. Among the patients who had a significant gallbladder wall oedema, we also observed a higher trend of transaminases [mean AST = 379.6 ± 641], lower platelet count [mean = 23,378 ± 19207], higher ferritin levels [mean = 9972 ± 1043], a higher H score [mean = 169 ± 35] and a lower total count [mean = 4021 ± 1993]. The optimal cut-off concentrations for ferritin in severe dengue cases was receiver operating characteristic [ROC] curve analysis. The value which has a good Youden's Index was chosen as the best cut-off for predicting the severity in dengue [23]. The area under the curve as calculated from the graph is 0.788 [Figure]. The best cut-off value for serum ferritin was > 3825 ng/dl on the day of admission, with a sensitivity of 72.7% and a specificity of 62.1%.

Table 1
Baseline characteristics of Dengue patients [N = 131]

Baseline Charecteristics	Number [N = 131]	Percentage	Severe Dengue [N = 44]
Age group [Years]			
< 30	42	32.1	13
31–60	82	62.6	26
> 60	07	05.3	05
Sex			
Male	83	63.4	25
Female	48	36.6	19
Duration of hospital stay [days]			
3–5	74	56.5	18
6–8	49	37.4	23
> 8	08	06.1	03
Number of patients who presented within			
3 days of fever	68	51.9	22
4 days of fever	63	48.1	22

Table 2
Clinical manifestation of dengue cases at the time of presentation to the hospital

Complications	All dengue cases [N = 131]	Severe Dengue * [N = 44]		
		N	%	P value
Serositis	32	32	72.70	< 0.005
Bleeding	04	04	09	0.004
ARDS	04	04	09	0.004
Renal failure	06	06	13.60	< 0.005
Encephalitis	01	01	02	0.158
Shock	08	08	18.10	< 0.005
Hepatitis	05	05	11.30	0.001
Myocarditis	01	01	02	0.158
More than 1 complication	13	13	43.10	< 0.005
Hepatomegaly	78	33	75.00	0.010
Splenomegaly	74	26	59.09	0.669
*multiple responses				

Table 3
Comparison of laboratory parameters on the day of admission between Severe dengue and Non-severe dengue patients

Variables	Severe Dengue[44]			Non-severe [87]			P Value*
	Mean	Median	SD	Mean	Median	SD	
AST	407.55	203.50	679.00	180.87	113.50	312	0.000
Ferritin	9125.34	568.10	12453	4271.47	2152.00	4913	0.003
Platelet	26182	19000	24865	51736	32000	47922	0.001
H score	155.41	160.50	41.00	134.23	130.00	43	0.006
Total count	4270.45	390.00	1573.00	4077.47	3500	2221	0.143
Results were expressed in terms of mean, median and standard deviation [SD]							
*P value was calculated using Mann–Whitney U test. P < 0.05 was considered as statistically significant							

Table 4
Distribution of Gallbladder among severe dengue and Non-severe dengue cases

Parameters	GB Oedema		Total	Pearson Chi square	P value*
	Present [%]	**Absent [%]**			
Non- severe cases	27	60	87[66.4]	38.785	0.000
Severe cases	39	5	44[33.6]		
Total cases	66	65	131[100]		
*correlation of dengue severity in those with gallbladder Oedema					

Table 5
Comparison of laboratory parameters with respect to gallbladder oedema

Variables	**Gallbladder oedema [Present]**			**Gallbladder oedema [Absent]**			**P value***
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	
AST	379.62	193	641.58	131.75	93.50	119	0.000
Ferritin	9972.30	7740	1043	1768.60	1307	1561	0.000
Platelet	23379	17000	19207	63231	49000	51110	0.000
H score	169	165	35	113	115	31	0.000
Total count	4021.80	3800	1993.60	4264.60	3800	2060	0.655
Results were expressed in terms of mean, median and standard deviation [SD]							
*P value was calculated using Mann–Whitney U test. P < 0.05 was considered as statistically significant							

Figure: ROC curve of logistic regression model to predict the sensitivity and specificity of ferritin in determining the severity of dengue

The receiver operating characteristic [ROC] curve analysis was done to establish an optimal cut-of concentration for ferritin in determining the severity of dengue. With a sensitivity of 72.7% and 95% confidence interval, the area under the curve was found to be 0.788. This value was statistically significant [P=0.003]

Parameters	Sensitivity	Specificity	Area under the curve	Std. error	Asymptomatic 95%Confidence interval		P value
					Lower Bound	**Upper Bound**	0.003
Ferritin	72.7	62.1	0.788	0.44	0.702	0.873	

Discussion

Many studies have previously been done on the several cytokines, chemokines and clinical parameters to differentiate between severe dengue and non-severe dengue cases [24–25]. The validity of these biomarkers appearing in plasma before the onset of severe dengue phase may help to develop suitable measures for early prognosis of the disease. In our present study we tried to assess the ability of serum ferritin levels and gallbladder wall thickness as early predictors of severity. In our study dengue infection was more prevalent among patients aged 30–60 years than those less than 30 years of age. Although earlier dengue infection was considered to be an illness of the paediatric population it has shifted its distribution towards the adults and the elderly. Similar observations were made in a study conducted by Tauqeer Hussain Mallhi et al at a tertiary hospital in Malaysia [26]. Increase in the movement of the adult population, easier accessible health care facilities and the simplicity of reporting the causal factors of the disease to health-care workers might be the reason for this high occurrence of dengue fever among adults. Among the adults aged 30–60 years, a higher trend of severe dengue was found with 59% of the cases belonging to this category [Table 1]. This is in concordance with the study of Tauqeer Hussain Mallhi et al where dengue haemorrhagic fever [DHF] was seen fourfold times in patients aged > 40 [26]. It would most probably be attributed to the presence of secondary infections or the likelihood of past exposure to dengue infection leading to increased severity among this population [27]. The male population had a slightly higher rate of infection than the female population in our setting. These findings are in line with the outcome obtained by Anker & Arima study but in contrast to a study done by Murugananthan et al [28–29]. Although fever in dengue cases lasts for about 2–7 days, in our study we only included patients who presented with less than 4 days of febrile illness, to look for early predictors of severity. Majority of the patients [57%] had a hospital stay duration of 3–5 days which is comparable to previous studies done internationally [26, 30–32]. Prolonged stay in the hospital for about > 5 days was consistent with the severity of the disease amounting to 59% of the severe dengue cases. This is of particular significance in a developing nations like ours where resources are limited especially if dengue is endemic to that region. It also imposes an extensive burden on the health care facility.

There has been rising trend of organ involvement [liver, kidney, heart and brain] being reported increasingly in severe dengue cases [1]. Liver involvement was concurrently present in almost all patients with deranged liver enzymes and organ enlargement. Mean levels of transaminases were drastically higher among severe dengue cases in our study [Table 3] and is more predictive of severe bleeding outcomes according to another study done by Fariz-Safhan et al [33]. Several other studies by Kuo C H et

al, Nguyen T L et al have also reported similar elevations in both AST and ALT as predictors of severity [34–35]. Wahid et al demonstrated that a rising trend of liver damage could be expected to be seen by observing the patient for spontaneous bleeding manifestations. This was found amongst 9% of the severe dengue patients in our study similar to the results obtained by Wahid et al [36]. In addition, hepatomegaly accounted for 75% of the severe dengue cases [Table 2]. The spectrum of dengue induced renal injury can range from mild proteinuria to severe Acute Kidney Injury[AKI] and can be explained by the direct viral mediated damage or the formation of immune complexes in the glomeruli [37]. Renal failure was found to be present in 13.6% of the cases with severe dengue [Table 2] which is very similar to a study conducted by Khalil MA et al [38]. Multiple organ dysfunction [MODs] is the concomitant or sequential existence of derangement in the function of two or more systems along with an underlying critical disease state. MODs can cause various organ bleeds and is linked with an elevated mortality rate among severe dengue patients [39]. In our present study the various proportion of complications present were serositis [24%], bleeding manifestations [3.1%], acute respiratory distress syndrome [3.1], renal failure [4.6%], encephalitis [0.8%], shock [6.1%], hepatitis [3.8%] and myocarditis [0.8%]. Haematological profile of dengue patients were taken into consideration and compared to differentiate the severe dengue from the non-severe dengue cases. Thrombocytopenia was more common among the severe dengue patients [mean value = 26,182 ± 24,000] and this finding is consistent with previous reports by Fariz-safhan et al and Humayoun MA et al [33, 40]. This is predominantly due to increased destruction of platelets as a result of compliment activation, peripheral sequestration and marrow dysfunction and of late due to oxidative stress [20]. Platelets are also shown to adhere to the endothelial cells due to stimulation by the dengue virus [41].

In our study we analysed the various patterns of serum ferritin presenting in patients. Mean levels of ferritin computed among the severe dengue group was found to be 9125.34 ± 12453 whereas in the non-severe group, it was 4271 ± 4913. ROC analysis was plotted for assessing the sensitivity and specificity of ferritin to aid in the diagnosis of severe dengue. An area under the curve of 0.788 with a standard error of 0.44 and statistically significant P value of 0.003 [95% confidence interval 0.702–0.87] was obtained, which shows us that ferritin is a good marker of severe dengue. With a sensitivity of 73% and a specificity of 62% our study found the best cut-off level for ferritin to be > 3825 ng/dl. These finding are similar to the work done by Soundravally et al which showed higher levels of ferritin among severe dengue cases [mean = 12647 ± 492.59] than those with non-severe dengue [20]. Thus the raised levels of ferritin in severe dengue cases can be attributed not merely as an inflammatory product but rather have a pathologic role in activating mediators of cytokine storm. Since dengue predominantly activates the macrophages, the early severe rise in ferritin is believed to arise from such cells than merely the hepatocytes. Little different observations were made by Chaudhuri et al who compared the ferritin level differences in dengue fever and other febrile illness [OFI]. He found a higher sensitivity of 82.6% and a specificity of 100% for ferritin as an early marker. This distinction can be attributed to the fact that our study included a prospective analysis of confirmed dengue cases who presented on the third or fourth day of febrile phase rather than OFI group studied by Chaudhuri et al [14]. Moreover, hyperferritinemia in dengue was strongly associated with low platelet counts and rising levels of liver enzyme in a study

published by C.A.M van de berg et al [12]. These findings are in accordance with our study results [Table 4]. The findings were also similar to a cohort obtained for the period of 2010 DENV outbreak in Brazil in which raised levels of ferritin were linked to the severity of the disease and cytokine profile.

Of late, a phenomenon known as macrophage activation syndrome [MAS] also known as hemophagocytic syndrome [HS] is seen in cases with severe dengue. It is a systemic inflammatory condition occurring in response to disproportionate activation and proliferation of well differentiated macrophage and T cells that causes the immune system to have a hyper activated response [14]. A ferritin level above 10,000 µg/L is a definitive sign of MAS [42]. Since HS may be hard to tell apart from severe sepsis the availability of an easy score known as the H score allows clinicians to predict it in an individual patient and make appropriate treatment judgments at the earliest. The H score is a very well validated score to aid in the determination of reactive hemophagocytic syndrome which comprises of 9 variables equally weighted among clinical, cytological and biological aspects. The variables included in calculating the H score are – known underlying immunosuppression, temperature, organomegaly, cytopenias, ferritin, triglyceride, fibrinogen, serum glutamic oxaloacetic transaminase and hemophagocytosis features on bone marrow aspirate [22]. In our study the calculated mean H score among severe dengue patients was 155 ± 41 which was statistically significant which proved the probability of developing HLH in dengue would be between 25–40% whereas in non-severe dengue the score was 134 ± 43 with a 9–16% probability of developing HLH. H score has been proved to have a good performance with regards to validation data sets which ensures its credibility to be calibrated precisely to the spectrum of diseases linked with HS.

The presence of gallbladder wall oedema has been proved to have prognostic significance in various conditions like hypoalbuminemia, cholecystitis, acute hepatitis and liver cirrhosis [43–46]. Therefore, diffuse gallbladder wall thickening is seen in various conditions and its exact cause is usually determined by correlation of associated clinical findings and imaging features. The significance of gallbladder oedema in dengue was proven in previous studies and it was shown that it has prognostic significance [17]. Gallbladder wall thickness was hopeful as a test for severe dengue on day 3 or 4 of the febrile illness [before the onset of the critical phase] in determining the prognosis among the paediatric population. On the 5th or 6th day of the illness, a good sensitivity and specificity was obtained. A study done by James Colbert et al showed a high negative predictive value for detecting Dengue haemorrhagic fever/Dengue Shock Syndrome using ultrasound dependably in the hospital settings to rule out severe dengue in cases presumed to have suspected dengue as well as to determine the patients who needed to be monitored closely as well as which ones to hospitalize [47]. Significant gallbladder thickening [> 3 mm] was present in 89% [39/44] of the severe dengue cases whereas it was present in only 31% [27/87] of the non-severe dengue cases. These findings were in accordance with a similar study done by Michels M et al in which severe dengue patients had a gallbladder wall thickening of 3–7 mm and the non-severe cases had a mean value of 3.6 mm [15]. Jitendra et al had a similar study with mean GB wall thickness of 3.32–4.95 mm in non-severe dengue cases and a mean value 8.8 mm among severe dengue cases [16]. Our findings were consistent with the clinical deterioration of patients with non-severe dengue on the 3rd – 4th day of fever to severe dengue as the days progressed.

Conclusions

The current study also looked at Gallbladder wall oedema and its differences between with respect to laboratory parameters such as ferritin, AST, platelet count and H score. We found that thrombocytopenia, rising ferritin, AST and H score had a positive correlation with the presence of gallbladder wall oedema and this result was found to be statistically significant [Table 4]

All in all the prognosis of dengue in India is still worse compared to Western countries despite all the efforts taken for early diagnosis of the disease. In a poor socio-economic developing nation such as ours there exists the need for a low-priced and accurate measuring tool to predict the progression of the disease into severity during its defervescence. Based on the high sensitivity and a good specificity obtained in our study in predicting such cases, we can say that serum ferritin levels along with gallbladder wall thickness > 3 mm detected by ultrasonography on the third or fourth day of febrile illness can be used to accurately predict those patients who tend to progress towards severity.

Limitations

Patients who were mildly symptomatic and who refused admission or those that were treated on an outpatient basis were not included in the study. A control group with participants who tested negative for dengue was not taken into consideration for the study. RT-PCR method to determine viral copy number/ viraemia was not done due to financial constraints. The presence of gall bladder wall thickness > 3 mm was only used as a qualitative variable.

List Of Abbreviations

RT-PCR - real-time polymerase chain reaction

AST – Aspartate transaminase

MAS - macrophage activation syndrome

ECLIA - electro-chemiluminescence immunoassay

ELISA – Enzyme linked immunoassay

WHO – World Health Organization

Declarations

Ethical Approval and Consent to participate

Ethical approval was obtained from the institutional ethics committee at Kasturba Medical College Mangalore.

Informed written consent was obtained from participating subjects in the study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during the present study is included in this published article.

Competing interests

The authors declare that they have no competing interests

Funding

No funding was received for this study

Author’s contributions

ECM contributed substantially to the analysis and interpretation of the data and in preparing the final manuscript. NR contributed substantially to the conception and design of the study as well as the acquisition of data. BA provided critical revision of the article and final approval of the version to publish. RH performed the data analysis and contributed in formulating tables and figures. DM and SM provided critical approval of the version to publish

Acknowledgments

We would like to thank the medical superintendents from the two tertiary referral hospitals [KMC Hospital, Mangalore, India] for allowing us to conduct our study at the centre.

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Figures

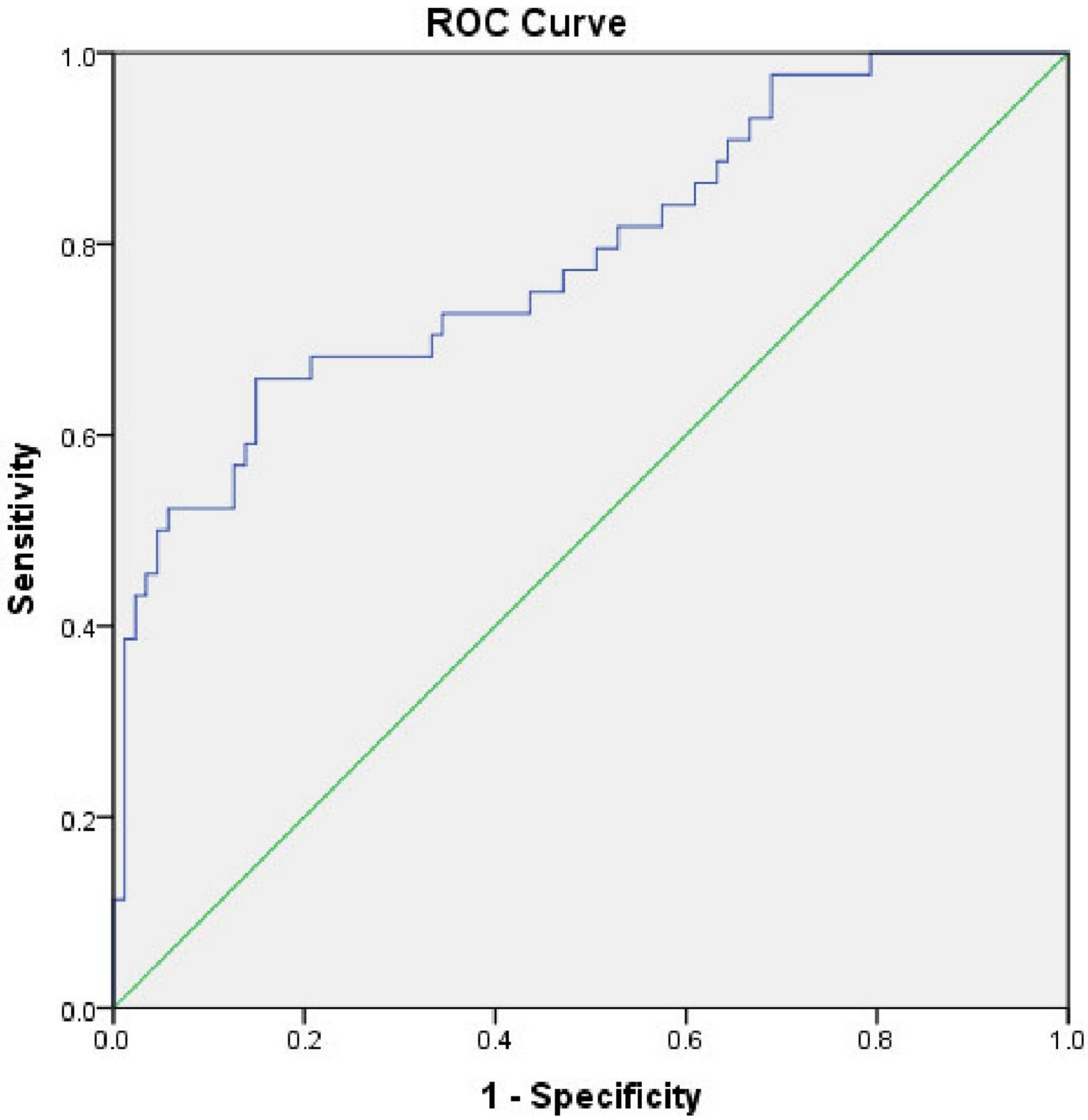

Figure 1

ROC curve of logistic regression model to predict the sensitivity and specificity of ferritin in determining the severity of dengue The receiver operating characteristic [ROC] curve analysis was done to establish an optimal cut-of concentration for ferritin in determining the severity of dengue. With a sensitivity of 72.7% and 95% confidence interval, the area under the curve was found to be 0.788. This value was statistically significant [P=0.003]