Original Research Article

Clinico epidemiological features of dengue virus infection and correlation with serum ferritin levels

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Abstract

Problem Statement: Dengue is an acute systemic viral infection transmitted between humans by Aedes mosquitoes. Today the prevalence of Dengue infection to be almost 390 million per year, but only one fourth manifest clinically and are diagnosed. Our Determination of serum Ferritin level in Dengue patients and correlation of same, with severity and complications. **Methods:** The study was conducted in the Out-patient Department and in patient department of PMCH and newly diagnosed or suspected 200 adults and children were included during the period August 2017 to July 2018 in our study. **Results:** Therein lies the potential for serum ferritin to contribute. We performed the measurement of serum ferritin in 57 samples on the day of admission. These values are again grouped and searched for statistical significance. There was significant difference between the groups in terms of serum ferritin. The ROC analysis with each group concluded that a value of serum ferritin less than 1056 ng/ml is very likely to be non severe dengue and a value more than 1679ng/ml is probably going to be severe one. In between values, the prognosis couldn't be actualized but likelihood of non severe dengue with warning signs would be more. Serum ferritin measurement was also significantly low in DENV1 infection. **Conclusion:** Serum ferritin is a fairly good marker to prognosticate the severity. The values at the time of admission are significantly different in all the three groups of severity. It thus can be used as surrogate for predicting the course of illness.

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INTRODUCTION

Dengue is a viral fever transmitted by mosquitoes. The pathogenesis of the disease and also the magnitude of the problem include the characteristics of agent (Dengue Virus - DENV), vector (Aedes mosquito mainly), host (The humans) and also the environment. Dengue virus is small (50 nm in diameter) RNA virus included in the genus Flavivirus and family Flaviviridae. The genome contains single strand positive sense RNA of 11644 nucleotides that encodes for 3 structural (C-core, M-membrane and E- envelope) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A and NS4B) proteins[1]. Infection with a certain serotype can cross protect infection by other serotype for a certain period of time [few months[2]] to two years[3] and lifelong for the same serotype[4]. Mass global travel and transport results into hyper-endemicity of dengue virus in some places, leading to multiple serotypes spreading in same region at one point of time. Thus we get different serotypes in a single outbreak in a specific region[5]. DENV-5 another serotype is discovered recently in October 2013 in Sarawak state of Malaysia which is primarily having a sylvatic non human transmission cycle, causing milder form of disease.[6] Genetic re-assortment is thought to be temporal factor for the generation of the new type. Re-infection with a different serotype increases the risk of severity[7]. Dengue virus is spread by female Aedes mosquito taking a blood meal from an infected person (usually up to 5-12

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days from the onset of the symptoms i.e. the period of viremia) to a non immune person. Aedes aegypti is the main vector for dengue transmission and is prevalent throughout tropical and sub-tropical regions of the world[8]. Aedes aegypti is the principal vector in urban areas transmitting all four types of virus[9]. The mosquito lay eggs in damp surfaces just above the waterline and the adult emerges after 7 days. This may take several weeks if the temperature is low. The most important survival character of this egg of this mosquito is that it can be viable for more than one year in dry condition and can again re-emerge within 7 days when it comes in contact with water. The virus can remain dormant for the same period in the egg and become active when transformed to larvae. Aedes albopictus is a temperate climate mosquito, increasingly implicated in outbreaks of many states and places[10].It is secondary vector in Asia and transported to Europe and North America through used tyres and other natural goods. These mosquitoes have an excellent adaptive capability that made them viable in very low temperature and freezing points[11]. Aedes aegypti and Aedes albopictus has an average life span of 30 days and 8 weeks respectively though the life span may increase during the rains. Aedes aegypti usually resides in domestic environment and in household waters whereas Aedes albopictus prefers natural habitats like tree holes, rubber containers etc. All Aedes mosquitoes are day time feeder (peak biting is early in the morning and in the evening before dusk) with voracious appetite biting 5-7 persons at a time and also can fly up to a limited distance of 400 meters[12]. Vectorial competency (the combination of susceptibility to infecting virus, replicability and ability to transmit the virus in another host) of Aedes mosquitoes to Dengue is very high. Dengue viruses, having evolved from mosquitoes, adapted to non-human primates and later to humans. All of them can act as carries and also reservoir of dengue virus. The viraemia among humans builds up high titres of the virus two days before the onset of the fever (non-febrile) and lasts 5–7 days after the onset of the fever (febrile). It is only during these two periods that the vector species gets infected. Thereafter, the humans become dead-end host for transmission. The susceptibility of the human depends upon the immune status and genetic predisposition[13]. Both monkeys and humans are amplifying hosts and the virus is maintained by the mosquitos' transovarially via eggs.

METHODS

The study was conducted in the Out-patient Department and in patient Department of PMCH and newly diagnosed or suspected 200 adults and children were included during the period August 2017 to July 2018 in our study.

Inclusion criteria:

- Confirmed Dengue patients (NS 1 or Ig-M reactive by ELISA method from Dept of Microbiology PMCH .
- Those who are willing to participate in the study (consent being given by the patient or a close relative or guardian).

Exclusion Criteria

- Those who were unwilling to participate in the study.
- Patients with concurrent bacterial/viral/parasitic infection.

Dengue suspect patient attending to the OPD of PMCH and admitted in the IPD was screened for inclusion and exclusion criteria and was included as a case. Thorough history was taken and clinical features were recorded. Confirmation of Dengue infection was done by NS1 (within 5 days of fever) or Ig-M reactivity (after 5 days of fever) by ELISA method from Dept of Microbiology PMCH. Blood samples on the first day of observation was taken for routine complete haemogram, liver function test, and lipid profile, renal function tests which was done from Department of Biochemistry and Laboratory Medicine, STM. According the need for the patient's management repeated samples were also sent for estimation of different parameters like complete haemogram or liver function test or renal function test. All the data were recorded properly. Serum ferritin estimation (by ELISA method).

RESULTS

Epidemiological Parameters:

From the period of July 2016 to June 2017, we evaluated 310 admitted dengue patients in PMCH,patna.Those patients were clinically suspected to have Dengue Virus Infection and were diagnosed confirmedly by means of either NS1 reactivity or reactivity to Ig-M as per the day of fever as per recommendation in guidelines. Out of the investigated patients (57%) patients were males and the rest (43%) were females.

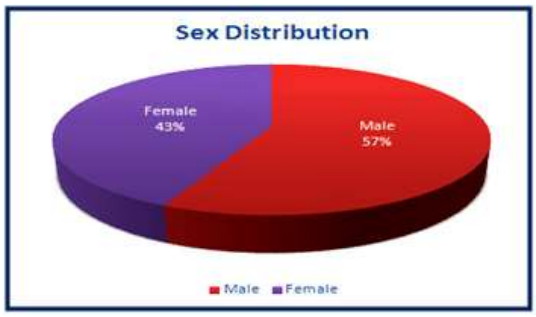

Figure: 1Sex Distribution

The mean age of the population was 30.13±14.15 years. The median age was 26 and IQR was 20 – 38.5 years. The age was similar in both sexes. The average age in male group was 28.45±12.33 years and the female group was 32.43±16.01years. The minimum age of the population was 10 years and the maximum age was 81 years. Incidentally both of the extreme ages were from the female group. Age group 20-29 years forms 31% of the total population. 10-19 years group comprises 24 % and 30-39 years forms 21%. Almost three fourth (76%) of the patients were aged less than 40 years. In lower age group males outnumber the females, but in higher age group, females were more.

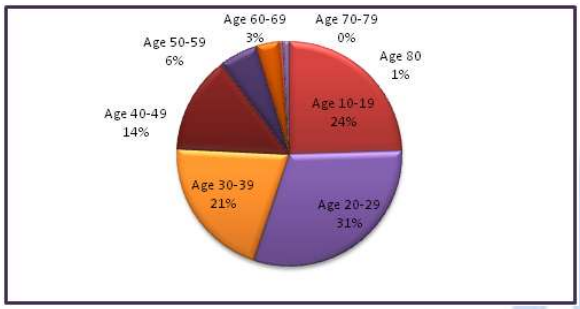

Figure 2: Age wise Distribution of patients

Hypertension was the most common co-morbidity found in patient population (39 patients) and diabetes was the second found in 14 patients. Other co morbidities include hypothyroidism, HIV reactivity, COPD. The patients with those co morbidities were depicted in figure 5. Among hypertensive's (39), most of the patients were on calcium channel blocker Amlodipine (58%). Among diabetics 11 persons were on OHA and the rest three were taking insulin. There is no apparent impact of the co morbidities on the outcomes as most of the patients recover from the illness with treatment. Only 6 patients died in the course of our study. Out of them, 3 had co morbidities which had no influence on outcomes. One patient had hypertension and diabetes, one had only hypothyroidism and another patient had immune-compromised status. In total population only 75 patients were found to have some type of addiction. Smoking and tobacco chewing head the list followed by alcohol intake. While observing the seasonal trend it was found that most of the cases started occurring from the first week of August and peaked at fourth week of September. There was a dip during the first week of October and then again a straight rise till last week of October. Gradually declining the outbreak almost stopped in last week of November

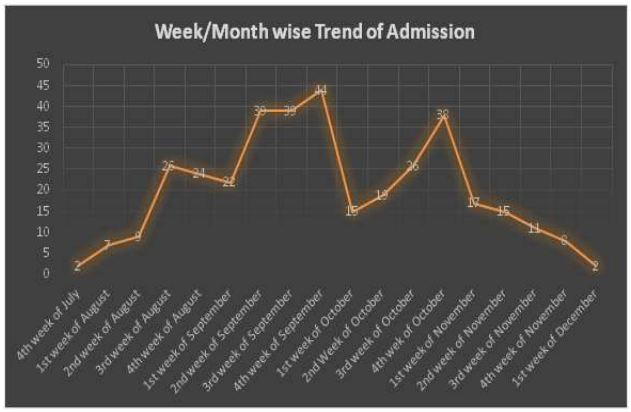

Figure 3: Seasonal Trend of Patient's Admission

Clinical Features

History of fever was present in all cases. Most of the patients were admitted with fever. 126 patients were admitted in an afebrile state for 24 hours due to some other reason, and another 62 patients did not have fever since the day of admission. The average day of fever in which patients got admitted was 4.8±1.9 days and the average day of illness in which the fever subsided was 5.09±1.65. So the fever in our dengue patients usually persists for 3.5 to 6.5 days. Average duration of hospital stay was 5.8±2.4 days. Average hospital stay for the severe cases was 7.13±3.45 days which was significantly higher than the number of hospital days in Non severe cases (vs 5.44±2 $p<0.001$) and also in cases Dengue with warning signs (vs 6.01±1.74 $p=0.0351$).

Table 1: The serum ferritin estimated in different serotype infection

	DENV 1	DENV 2	DENV 3	DENV 4
DENV 1	X	**0.0070**	0.1455	0.1810
DENV 2	**0.0070**	X	1.0	0.1978
DENV 3	0.1455	1.0	X	0.4286
DENV 4	0.1810	0.1978	0.4286	X

Table 2: Significance study of Ferritin values in different Serotype Infection

Serotype	Ferritin
DENV 1	694±472
DENV 2	1487±721
DENV 3	1465±408
DENV 4	1203±863

The significance or p values were calculated by Mann Whitney's Test.

The difference in average value of ferritin is present significantly between DENV 1 and 2. Though numerically higher there is no significant difference between serum ferritin of DENV 1 and other type of infection.

Serum Ferritin in DVI

We estimated the value of serum ferritin in 102 patients. The sample was sent at the day of admission. The average day of fever in which the sample was sent was 5.29±1.25 day (mostly 5th day). The minimum value of serum ferritin observed was 52.8 and maximum value was 4050. Out of 102 patients 79 patients had serum ferritin level > 500 ng/ml. This distribution of ferritin data is depicted in Histogram.

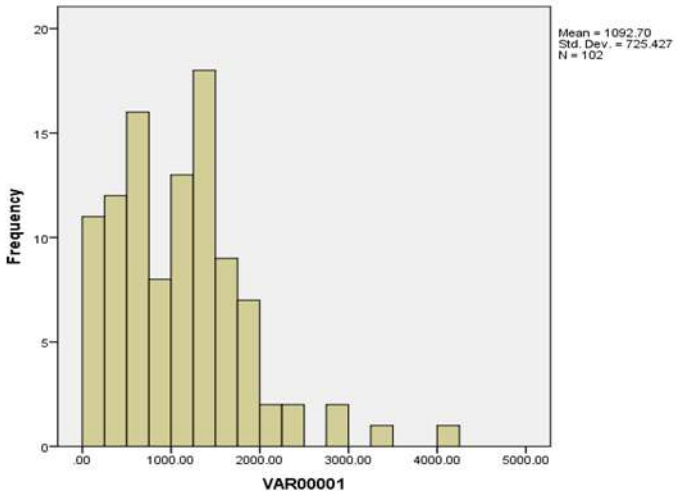

Figure 4: Histogram of values of serum Ferritin

Out of 102 patients, 51 patients were categorized as non-severe. Among the rest 28 were non severe dengue with warning signs and the other 23 were severe dengue. The detailed value of Serum ferritin is depicted in

Table 3: Detailed values of Serum ferritin indifferent groups

	Severe Dengue	Non severe Dengue with Warning signs	Non Severe Dengue
Sample Size	23	28	51
Lowest value	678.1	335	52.8
Highest value	4050	2158	1696.1
Arithmetic Mean	1842.36	1261.82	661.76
95% CI of mean	1491.59-2193.13	1089.94-1433.70	534.91-788.64
Median	1684	1305.25	583
IQR	1293.15-2321.75	1031.05-1633.75	303.77-1015.47
Standard deviation	811.15	446.26	451.07
Standard Error of mean	169.14	83.77	63.16
Coefficient of Skewness	1.1265 (p=0.0243)	-0.2908 (p=0.4874)	0.6476 (p=0.0544)
Coefficient of Kurtosis	1.1750 (p=0.1984)	-0.2212 (p=9467)	-0.7041 (p=0.1787)
D'Agostino Pearson test for Normal Distribution	Reject Normality (p=0.0346)	Accept Normality (p=0.7840)	Accept Normality (p=0.0637)
Comparision with Severe Dengue	X	p=0.0092 (calculated by Mann Whitney's U Test)	p<0.0001 (calculated by Mann Whitney's U Test)
Comparision with Dengue with Warning signs	p=0.0092 (calculated by Mann Whitney's U Test)	X	p<0.0001 (calculated by t test)
Comparision with Non severe Dengue	p<0.0001 (calculated by Mann Whitney's U Test)	p<0.0001 (calculated by t test)	X

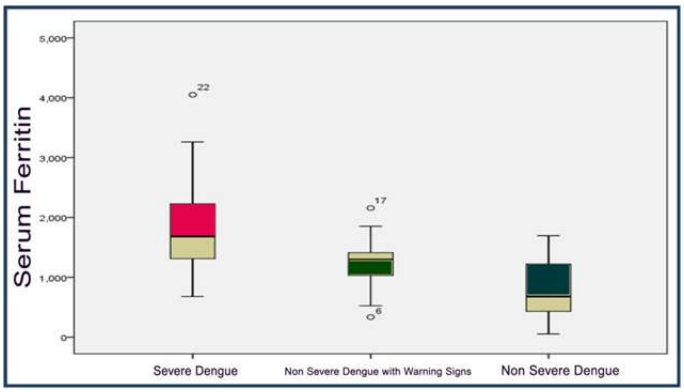

Figure 5: Box plot of the values of serum Ferritin in different severity groups

The level of serum ferritin is different in all three groups that reach the level of significance statistically. The value was significantly high in severe dengue and in Non severe dengue with warning signs than non severe dengue. More importantly the higher value significantly demarcates severe dengue from non severe dengue with warning signs. Out of proportion high value (4050 ng/ml) was noted in a patient with severe dengue developed hypotension and oliguria consistent with DSS had co existent E-B thalassemia. All the patients having pancreatitis had high value >500 ng/ml of serum ferritin. ROC analysis was done to calculate the trend of value in different group and also the cut-off value to demarcate between the groups with maximum sensitivity and specificity.

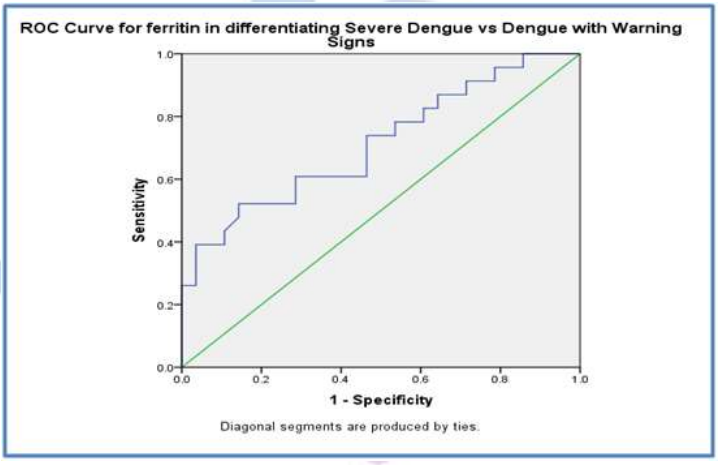

Table 4: Area Under the Curve (Severe dengue vs Dengue with WS)

Test Result Variable(s): Ferritin						
Area	Std. Error[a]	p.[b]	Asymptotic 95% Confidence Interval		Best Cut-off	Sensitivity at best cut/off	Specificity at best cut/off
			Lower Bound	Upper Bound			
					1679	52.2	85.7

The area under the curve as obtained by ROC analysis ferritin in predicting the accurate diagnosis of dengue with WS versus Severe was 0.714 with 95% confidence interval (.570, 0.857). The AUC between Severe and Non-Severe was 0.923 (0.0864-0.981) and between dengue with WS versus Non-severe was 0.811 (0.719-0.903). Also, the area under the curve is significantly different from 0.5 since p-value is 0.001 meaning that the ROC ferritin differentiates dengue with WS versus Non-severe significantly better than by chance. The accuracy of the test depends on how well the test separates the group being tested into those with and without the disease in question. Accuracy is measured by the area under the ROC curve. An area of 1 represents a perfect test; an area of .5 represents a worthless test.

A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system:

- .90-1 = excellent (A)
- .80-.90 = good (B)
- .70-.80 = fair (C)

- .60-.70 = poor (D)
- .50-.60 = fail (F)

The AUCs were 0.714, 0.811 and 0.923. Thus we can say that ferritin levels were found as a just fair predictor in differentiating dengue with WS versus Severe, fair to good predictor in differentiating dengue with WS versus Non severe and excellent predictor in differentiating Severe dengue over Non-severe Dengue. The best cut-offs and the sensitivity and specificity at this cut-off are mentioned in each chart.

Table 5: The Cut-offs is shown in this Table

	Non-severe vs WS	Severe vs Non severe	WS vs Severe
Cut-off	1056.5	1085	1679
Sensitivity	75	91.3	52.2
Specificity	78.4	98.4	85.7

It was evident that the differentiation between severe and non severe dengue is clear. But the separation lines between the other two groups are a bit blurred. Serum ferritin with other laboratory and clinical parameters can clearly identify the prognosis of the disease. The patients were managed conservatively according to WHO or NVPDCP guideline. Most of patients recover with conservative treatment satisfactorily. According to policies platelets were transfused only at platelet count below 10000/cmm or with significant bleeding. 31 patients had to transfuse with RDP or SDP. Out of them, 13 had platelet count less than 10000/cmm without any obvious bleeding and the rest 18 had significant bleeding with or without low platelet. 10 patients needed vasopressor support due hypotension in DSS not responding to fluid therapy. 4 had associated myocarditis in them. Non significant bleeding was treated with tranexamic acid. Significant bleeding was managed accordingly and with platelet transfusion as required. The per-vaginal bleeding was the most common form bleeding overall. Normal menstruation with normal amount and flow was not intervened. Abnormal bleeding was addressed either by tranexamic acid or projesterone or both. Platelet transfusion was given in requirement. Out of 29 abnormal PV bleeding cases 11 required platelet transfusion. All of them received tranexamic acid, additional projesterone was given in 21 patients. Among 359 patients in our study sample we lost 6 patients. Among them only two had the serum ferritin level at the time of admission. Their values are 3261 and 2834 ng/ml. These data are insufficient to comment on correlation with outcome. Analyzing the parameters of the expired patients the mean TLC was 3400 and 1500 being the lowest. 50000/cmm was the average platelet count and the lowest count was 10000/cmm. The patient having the platelet count of 10000/cmm died because of acute hemorrhage along with DSS. Mean SGOT and SGPT was 1135 and 413 IU/L respectively and the highest values observed were 3962 and 1222 IU/L. Acute hemorrhage with DSS was most common immediate cause leading to death in 3 patients. Myocarditis caused the demise in two and another patient died of encephalitis.

DISCUSSION

This study observed the outbreak of dengue fever in patna in 2017-18.Most of the patients admitted in our hospital were young adults. According to NVBDCP[7] there was a shift of age from pediatric population to young adult as was also observed in our study. This observation is also supported by a study from Lucknow[14] as well as the study conducted in STM Kolkata by Hati *et al*.[15]. In our study 55% of patients were from 10-29 years and a total of 76% patients were below 40 years of age. However, ours being a hospital primarily for adults, we have missed the paediatric population. Conversely Sarkar et.al found in a study in the year 2011 that the most common age group involved is 0-10 years[16]. In most of the studies male are slightly and non-significantly more. In our study also males comprised 57% of the population supporting the observation in the study from STM in 2005. Remarkably one study from ICMR by Sarkar *et al*.[16] and one study from Kolkata observed the female predominance of the patients[17]. Dengue fever was reported from almost all districts of Bihar.Patna headed the list with maximum number of cases.Since ours is a hospital in patna this finding is expected.There is no important recent study highlighting the co morbidities and addiction and their impact on course of the diseases and the outcome. We also do not find any relation with the co morbidity and the outcome including occurrence of severity. But the management of a dengue patient with co morbidities was challenging. Serum ferritin was also raised in all forms of dengue fever. We calculated the serum ferritin value in 102 dengue fever patient in all three groups at the time of admission only. This study did not have a control arm. We found that serum ferritin was significantly raised in severe dengue fever compared to non-severe dengue and also non-severe dengue with warning signs (<0.0001 and 0.0092). This observation was supported in studies from Aruba by Weg *et al*.They showed a similar association. In addition they also found that hyperferritinaemia was associated with increased marker of coagulation, low fibrinogen, increased viremia and most importantly was a surrogate for the group of dengue fever with warning signs. Another research, by Soundravally et.al.[18] performed the assay of serum ferritin in the day of admission as well as on the day of defervescence. They also found that the value was significantly raised in both

occasions between severe and non-severe dengue as they categorized the illness into two groups. They concluded that ferritin value more than 866.5 ng/ml was substantially sensitive and specific (86.9%, 83.3%) to determine the severity. In our study we classified the illness into three categories as specified by WHO, Severe dengue, Dengue with warning signs and non-severe dengue. We found that the ferritin level can substantially differentiate between all three classes of dengue on the day of admission. The elevated liver enzymes could only differentiate between severe and non-severe dengue but not for the other two comparators of dengue with warning signs with severe dengue and non-severe dengue. Low platelet in our study couldn't separate the possibility of severe dengue over dengue with warning signs. We also found the best cut off value of serum ferritin to be1679 ng/ml to differentiate severe dengue from the dengue with warning signs with a fair sensitivity (53%) and good specificity (86.6%). The cut off was higher in our study than the study in JIPMER which was 866.5 ng/ml. But another study from Thailand by Chaiyaratana *et al*.[19] found that ferritin value could significantly predict the severity of dengue fever from day5 to day 7 with a best cut off of 1200 ng/ml which was closer to our observation.

CONCLUSION

Dengue virus infection can present in any form and with any symptom related to any system in our body. This concept would definitely be helpful in making suspicion as early diagnosis and management with hydration would be necessary to prevent any complication. The fever in dengue usually persists for 5-6 days but may persist for 8 days. This needs a good judgment before going to an alternate diagnosis. Serum ferritin is a fairly good marker to prognosticate the severity. The values at the time of admission are significantly different in all the three groups of severity. It thus can be used as surrogate for predicting the course of illness. As a limitation we can comment that if the estimation of serum ferritin could be done serially it would be a better and stout option for a statistical superiority.

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