Original article:

Incidence of bleeding manifestations in dengue fever patients having thrombocytopenia: a randomised clinical study in a tertiary hospital setting

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ABSTRACT:

Dengue is a mosquito-borne viral disease that is a threat to global health. This was a randomized study of 50 patients from June 2016 to October 2016. Patients having thrombocytopenia at any point during admission were taken into consideration and their history of bleeding manifestations was compared to the level of platelet count. On history, only 16 patients complained of bleeding manifestations like Malena, bleeding per rectum, excessive menstrual bleed, gum bleeding, blood in sputum or vomitus however while examining each patient, only 17 patients (34%) had petechiae. Statistically, a platelet cut off on the first day of presentation was taken as 42,500 cells/mm3 and this when compared to incidence bleeding manifestations in the patients was found to be a significant level (p value= 0.002). Even when an average platelet cut off, taking into consideration all the platelet values ($> 46,500$ cells/mm^3) was compared to bleeding manifestations it was found to be significant (p value= 0.001). Patients were also found to have deranged of liver functions however this was not statistically significant in this study. In conclusion, this study shows that an average platelet cut off can be achieved which can help prognosticate the disease progression or tendency to develop bleeding manifestations in a hospital setting.

INTRODUCTION:

Dengue, caused by dengue virus, is one of the most prevalent mosquito-borne arboviral infection in India. Nearly 70% of the 96 million apparent infections occur in Asia, in which India is making up to one-third of the total.

Spread by *Aedes aegypti* mosquito, Dengue has 4 distinct serotypes (dengue 1, 2, 3, 4). Recovery from one serotype infections offers immunity against that particular serotype but subsequent infections by other serotypes increase the risk of developing severe dengue.

Symptoms of dengue fever include high-grade fever accompanied by severe headache, pain behind the eyes, joint pains, vomiting and rashes on the body(1). Rarely fatal, but however complications are part of severe manifestations of Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) which can be prevented with early detection and inappropriate interventions. However WHO revised its classification in 2009 and classifies dengue now as dengue fever without warning signs, dengue fever with warning signs and severe dengue. Warning signs include abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy, restlessness, liver enlargement >2 cm and increase in HCT concurrent with rapid decrease in platelet count(1,2).

Dengue hemorrhagic fever involves evidence of vascular damage, petechiae and local hemorrhaging. Shock, multifocal hemorrhage, ARDS and neurologic signs (e.g. Seizures or coma) predict a poor prognosis. The patient

599

tends to have a triad of symptoms like hemorrhagic manifestations, evidence of plasma leakage, platelet count of 1,00,000/microliters. The mortality rate in these patients is 10-20% but if Dengue shock syndrome develops mortality can reach 40%.

The outcome of these patients is usually predicted with hematocrit, liver enzymes, coagulation profile, and platelet count. Increased hematocrit, elevated liver enzymes, and altered coagulation profile and decreased platelet count predicts the severity of the disease(3). Thrombocytopenia is always considered as a predictive parameter for inpatient management of dengue fever as well as recovery parameter in DHF and DSS. Different mechanisms have been hypothesized to explain dengue-associated thrombocytopenia, including the suppression of bone marrow and the peripheral destruction of platelets(4). Thrombocytopenia may be associated with alterations in megakaryocytopoieses by the infection of human hematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction (platelet activation and aggregation), increased destruction or consumption (peripheral sequestration and consumption)(5). Hemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation(5).

A study by Gubler et al in 2002 states that activation of the immune process and direct marrow suppression by the viral particles is responsible for a drop in platelets. It has been proposed that platelets are sensitized by autoantibodies and then destroyed by the reticuloendothelial system of the body. These autoantibodies against glycoproteins of platelet are recognized in 80% of patients(6).

AIMS AND OBJECTIVES:

This study aims at assessing the incidence of thrombocytopenia in dengue hemorrhagic fever in a Tertiary Hospital setting in Mangalore. In this study, we also aimed to find a relationship between thrombocytopenia and bleeding manifestations. To achieve a cut off level below which bleeding manifestation could be predicted and hence the overall outcome of the patient can be assessed.

METHODOLOGY:

The study is a randomized, time-bound, hospital-based study that was conducted on patients admitted in Department of General Medicine in Justice K.S. Hegde Charitable Hospital, affiliated to K.S. Hedge Medical Academy, a unit of deemed to be NITTE University, Deralakatte, Mangalore. The period of study was from June 2016 to October 2016.50 random patients were taken. All those taken were above 18 years of age and diagnosed to have Dengue fever based on Dengue Card Test that checked for NS1 antigen and dengue IgM.

These patients were checked for their platelet counts and those with a falling trend in platelet count or those with less than 1, 50,000 cells/mm^3 were considered into the study. Patients with any bleeding disorders such as VWB disease or those suffering from ITP or any hematological malignancy were excluded from the study.

Evaluation of blood samples collected from all patients with NS 1 Antigen, IgM, IgG antibodies positivity experiencing febrile illness, clinically consistent with dengue infection was done. Patient history with regard to symptoms and bleeding manifestations was noted and clinical details and investigations were entered in the respective proforma. A whole blood sample was taken in an EDTA tube and was sent to the Hematology Laboratory in Justice K.S. Hegde Charitable Hospital. Repeated samples were sent during the course of hospital stay till platelets reached normal levels. Routine liver functions were also assessed.

RESULTS:

The average age seen in this study was 44.98 years with a maximum of 65 years and a minimum of 23 years. There were a majority of women in this study (29 women; 58%). All the patients came with a history of fever on initial evaluation followed by headache (60%), myalgia (52%)and vomiting (50%) being the most common symptoms. By history only 16 patients complained of bleeding manifestations like Malena, bleeding per rectum, excessive menstrual bleed, gum bleeding, blood in sputum or vomitus. On examination only 17 patients (34%) had petechiae. All patients were NS1 antigen positive by Card test.
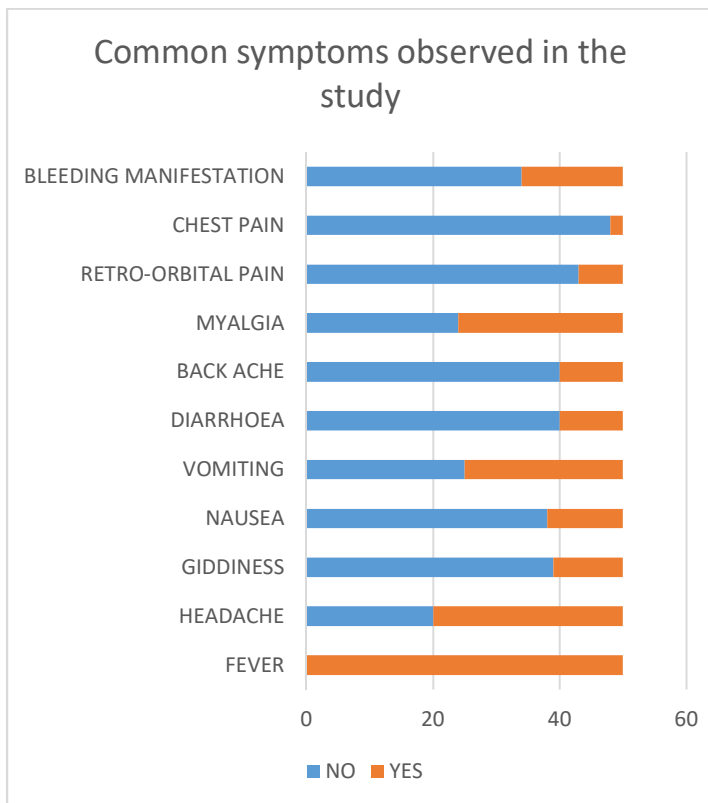

Fig 1: Distribution of patients according to symptoms.

Table No.1 shows the average distribution of platelet counts seen during the course of hospital stay. We see that only 11 patients had a longer hospital stay as per this table.

On statistical analysis, comparing of the test group least platelet count (cutoff was taken as 46500) with the Gold standard of bleeding manifestation, the test group has a sensitivity of 81.2 % and specificity of 70.6%. The test has a positive predictive value of 56.5% and Negative predictive value of 88.9%. The test and the gold standard agree on 37 out of 50 having a diagnostic accuracy of 74%. The Kappa value of 0.465 indicates good agreement with a p-value of 0.001.   (Table No.2 and Table No.3)

		LEAST PLATELET COUNT	PLATELETS DAY 1	PLATELETS DAY 2	PLATELETS DAY 3	PLATELETS DAY 4	PLATELETS DAY 5	PLATELETS DAY 6
N		50	50	50	41	35	22	11
Mean		66960	81000	85140	90804.88	108171.4	120227.3	171545.5
Std. Deviation		44025.48	56031.7	57222.09	55035.09	57043.06	46542.28	183030.8
Minimum		6000	6000	13000	11000	12000	30000	42000
Maximum		179000	237000	219000	218000	263000	218000	703000
Percentiles	25	29500	33000	36000	46000	65000	89750	72000
	median	56000	76500	61000	75000	106000	118000	115000
	75	101250	116250	136250	142000	148000	151000	187000

Table No.1: Platelet distribution during the course of hospital stay.

			BLEEDING MANIFESTATION		Total	P value
			ABSENT	PRESENT		
LEAST PLATELET COUNT (cutoff of 46500)	>46500	Count	24	3	27	
		% within LEAST PLATELET COUNT (cutoff of 46500)	88.9%	11.1%	100.0%	
		% within BLEEDING MANIFESTATION	70.6%	18.8%	54.0%	
	<=46500	Count	10	13	23	
		% within LEAST PLATELET COUNT (cutoff of 46500)	43.5%	56.5%	100.0%	
		% within BLEEDING MANIFESTATION	29.4%	81.2%	46.0%	
Total		Count	34	16	50	0.001
		% within LEAST PLATELET COUNT (cutoff of 46500)	68.0%	32.0%	100.0%	
		% within BLEEDING MANIFESTATION	100.0%	100.0%	100.0%	

Table No.2: Comparing the least platelet (cutoff 46500) with bleeding manifestations.

			BLEEDING MANIFESTATION		Total	P value
			ABSENT	PRESENT		
PLATELETS DAY 1 (cutoff of 42500)	>42500	Count	27	5	32	
		% within PLATELETS DAY 1 (cutoff of 42500)	84.4%	15.6%	100.0%	
		% within BLEEDING MANIFESTATION	79.4%	31.2%	64.0%	
	<=42500	Count	7	11	18	
		% within PLATELETS DAY 1 (cutoff of 42500)	38.9%	61.1%	100.0%	
		% within BLEEDING MANIFESTATION	20.6%	68.8%	36.0%	
Total		Count	34	16	50	0.002
		% within PLATELETS DAY 1 (cutoff of 42500)	68.0%	32.0%	100.0%	
		% within BLEEDING MANIFESTATION	100.0%	100.0%	100.0%	

Table No.3: Comparing least platelet on day 1 (cutoff 42500) with bleeding manifestations.

When individually comparing the platelets on day 1 (cutoff was taken as 42500) with the Gold standard of bleeding manifestation the test group has a sensitivity of 68.8 % and specificity of 79.4%. The test has a positive predictive value of 61.1% and Negative predictive value of 84.4%. The test and the gold standard agree on 38 out of 50 having a diagnostic accuracy of 76%. The Kappa value of 0.466 indicates Good agreement with a p-value of 0.002.

18 of the 50 patients received platelet concentrate transfusion depending on the severity of bleeding manifestation. However, all 50 patients did not have good outcomes. 6 patients did not survive (12%) and the rest recovered with minimal complications. 19 out of the 50 patients had deranged Liver function tests excluding 16 with mild alterations in liver enzymes. A comparison between the level of platelet count and deranged LFTs was checked for and was seen that when platelet cut off was taken as 42,500 cells/mm^3, there was a lesser number of patients with normal LFT when compared to those with platelet counts >42,500 cells/mm^3. Chi-square test on this showed a poor significance (p value=0.395). This explains that the first day of platelet count could not assess the prognosis and also

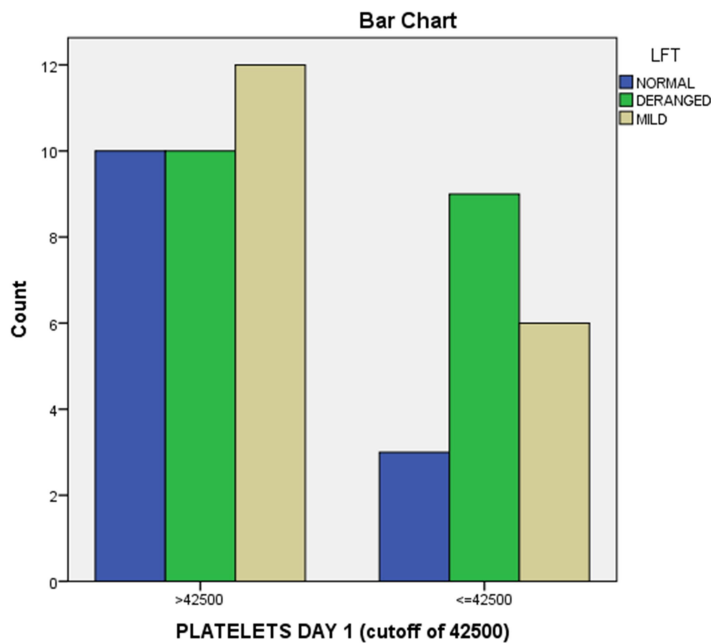shows that some patients probably had a deranged LFT irrespective of the platelet levels.

Fig.2: Comparing average platelet count on day 1 with deranged LFT

			LFT			Total	Chi-square
			NORMAL	DERANGED	MILD		P value
PLATELETS DAY 1 (cutoff of 42500)	>42500	Count	10	10	12	32	0.395
		% within LFT	76.9%	52.6%	66.7%	64.0%	
	<=42500	Count	3	9	6	18	
		% within LFT	23.1%	47.4%	33.3%	36.0%	
Total		Count	13	19	18	50	
		% within LFT	100.0%	100.0%	100.0%	100.0%	

Table No.4: Distribution of patients on day 1 of platelets.

DISCUSSION:

This study assesses the relationship between platelet level and expectation of bleeding manifestations in those patients suffering from Dengue fever. As per the study, we see that the majority were women and the average age of the study group was 44.98 years. All the patients had a fever as the main presenting complaint. Out of 50 patients gave a history of bleeding manifestations however 17 patients were found to have petechiae in the study. Statistically, a platelet cut off on the first day of presentation was taken as 42,500 cells/mm^3 and this, when

604

compared to the incidence of bleeding manifestations in the patients, was found to be a significant level. This means that those with platelets lower than or equal to 42,500 cells/mm^3 were bound to have bleeding manifestations as per this study. Similarly, if an average platelet count considering all the days of admission, average cut off was taken as 46,500 cells/mm^3 it also gave a significant result.

19 out of 50 study patients have severely deranged Liver function tests and 16 had mild derangement. When this was compared with the above-mentioned platelet cutoffs, a statistically significant result could not be achieved. However, we can infer that thrombocytopenia and liver dysfunction may have some relation. One study showed during hospitalization, leukocytosis, elevated AST and ALT levels, and a low platelet count was associated with increased mortality ≤7 days after the illness onset(7). In our study, we see that those with lower the platelet counts showed a derangement of AST, ALT levels however not significant relation was obtained. In another study by Suhendro Suwarto et al, tried to determine the associations of nonalcoholic fatty liver disease (NAFLD) with laboratory markers of dengue severity and length of hospital stay. In this, 267 dengue-infected patients were taken and showed serum AST levels immediately increased following dengue infection, and this change was followed by an increase in ALT levels within 24 to 48 hours. Also in the same study, patients in the leakage group with NAFLD had significantly more severe thrombocytopenia than did those without NAFLD. A previous study reported that the thrombocytopenia observed in DENV patients may be due to the increased adherence of platelets to sites of vascular endothelial injury(7)

In a study by Ing-Kit Lee et al in Taiwan, analyzed 1086 adults with dengue during a 12-year period. Three scoring models were established in the study. In the study, thrombocytopenia (platelet count < 50 × 109 cells/L) was an independent predictor of mortality ≤3 days of presentation and in the overall study population. Although thrombocytopenia is not directly responsible for the risk of bleeding per se in dengue, a significantly lower platelet count of <50 × 109 cells/L exacerbates the bleeding complication, which can lead to an unfavorable outcome as per the study(7).

Another study by Tewari et al assessed 443 adults and 57 children and found to have a fever as the major complaint followed by myalgia. 337 patients had thrombocytopenia in the study and only 36 of these had bleeding manifestations(8). Of these 36, 27 patients had petechiae. 10 patients had a platelet count <10,000/mm^3, 23 patients had platelet count was between 11–20,000/mm^3 while in 3 patients the platelet count was between 21–30,000/mm^3.In comparison to our study, the patients had a much lower cut off of platelets which showed bleeding manifestations.

A study by Shradha Kathri et al in Manipal, India saw 106 patients with dengue fever and assessed to see if any variation in platelet parameters of patients with dengue fever and thrombocytopenia(9). However, no relation was found. However, in the study group, the 20 patients had mild thrombocytopenia (1, 00,000 -1, 50,000 cells/mm^3). No bleeding manifestations were seen in any of the patients. Our study had more patients with lower levels of thrombocytopenia hence validating the level of thrombocytopenia and bleeding manifestations.

In another study done by Rajesh Kumar Khare and Pulak Raj, there is with a positive correlation between platelet count and complications(10). The lower the platelet count the higher the risk of complications. The lack of correlation between low platelet counts and bleeding manifestations was seen in this study.58 patients were taken

into consideration which is near similar to our study with an average platelet level taken as 77,000 cells/mm^3 which is higher when compared to our cut off of 42,500/46,500 cells/mm^3.

It has been suggested that thrombocytopenia arises from both decreased production of cells from bone marrow associated with increased peripheral destruction of platelets. Several mechanisms are involved in thrombocytopenia and platelet dysfunction in dengue, indicating the complexity of dengue immunopathogenesis(11). Patients with lower platelets have higher chances of having complicated dengue fever. In our study, there is a positive relation between thrombocytopenia and bleeding manifestations. If platelet counts on the day of presentation are less than 42,500 cells/mm^3 patients highly likely to develop bleeding manifestation if he hasn't already and hence prolonging the hospital stay and also increasing the risk of complications.

However, in this study, we did not assess the complications in each patient. Hence we could not analyze the level of thrombocytopenia and severity of complications. Also, this was a time-limited, Hospital bound study which led to small study size.

CONCLUSION:

Platelet count can be used to predict the complication rate in a patient admitted with dengue fever.

Our study shows a significant relationship between platelet count less than or equal to 42,500 cells/mm^3 on the first day of presentation or any platelet value less than 46,500 cells/mm^3 and bleeding manifestations. Therefore, close monitoring of thrombocytopenia is required to prevent patients from developing complications of dengue fever and hence better outcomes.

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