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Predictive correlation of adverse clinical outcomes with thrombocytopenia in dengue fever: A single center experience

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Abstract---Background: Dengue fever is the most widely distributed viral infection in the world with more than 100 million people affected. In this study we attempt to study the co-relation between the nadir of the plateletcount and the onset of warning signs (persistent pain abdomen and vomiting, mucocutaneous bleed or clinical fluid accumulations). A positive co-relation in between these two elements could help predict onset of shock and third spacing and facilitate prognostication and early intervention. Methods: We studied 82 patients with a diagnosis of dengue fever, the basis of which was clinical signs and symptoms with a positive NS1 antigen test. The nadir of thrombocytopenia and rise in transaminases were studied along with onset of various warning signs. Results: The nadir of thrombocytopenia correlates with onset of warning signs such as clinical fluid accumulation (two tailed P value <0.002) and mucosal bleed (two tailed P value <0.001). However, the rising value of

transaminases does not co-relate statistically with onset of warning signs in Dengue fever. Conclusions: The age of patients ranged from 18 to 60 with 23 out of 83 (27.7%) being in the 30-40 years age group. Clinical fluid accumulation was seen in 54 (64.7%) patients, mucosal bleed in 61 patients (74.3%) and liver was palpable more than 2 cm in 28 patients (33.7%). The importance of serial monitoring of platelets is important not only for the prediction of severe dengue, but also with the onset of other warning signs such as clinical fluid accumulation. This nuanced fact re-enforces the critical importance of platelet count predict other warning signs which contribute to morbidity and mortality in dengue fever.

Keywords---thrombocytopenia, dengue fever, mortality.

Introduction

Dengue, caused by a flavivirus, is a tropical disease spread by the vector *A.egypti* and *A. albopictus*.The etiological agent is a single stranded RNA virus 30 nm diameter (1). The virus has four distinct but closely related serotypes. In the Indian scenario, co-circulation of all four serotypes is prevalent according to studies from Pune(2), Hyderabad(3), Bhopal(4), Bangalore(3), Ernakulam(5), Dadar and Nagar Haveli (6)and Delhi(7). Dengue fever is the most widely distributed viral infection the world with more than 100 million people affected. It is endemic in tropical regions where conditions for viral replication and transmission are ideal. Although the disease occurs throughout the year, the outbreaks of this disease are more frequently encountered in the rainy season where multiple serotypes operate simultaneously, thereby increasing the incidence of severe dengue.

The cell mediated immunity is the basis for the so called original antigenic sin hypothesis (8), which describes a skewed immune response to subsequent infection with different serotypes, resulting in severe disease. This was based on Halstead's "antibody dependent immune enhancement theory" proposed in the 1970s. The abnormalities in homeostasis seen in severe dengue include vasculopathy(9), thrombopathy, coagulopathy(10) and bone marrow changes. These bone marrow changes range from mild and transient suppression to dengue induced HLH (11), dengue induced aplastic anemia (12) and dengue related myelofibrosis (13).

The fall of platelet count in dengue supposed to multi-factorial, including direct suppression of megakaryoctopoiesis, included platelet clearance by DENV induced apoptosis and anti-platelet antibodies. The disease is clinically characterized by fever, myalgia joint pain, mucosal bleed, clinical fluid accumulation and as already discussed, thrombocytopenia (14). Third spacing and bleeding manifestations may cause serious complications or death(14). The utility of monitoring the patient for warning signs has been corroborated by multiple studies and consensus statements (15). The rate of fall of platelets has been linked to various serious manifestations in Dengue such as (including but not limited to) ARDS, Dengue encephalitis and Dengue related acute liver failure.

According to existing literature onset of shock depends on presence or absence of enhancing and non-neutralizing antibodies, age (susceptibility to severe dengue drops considerably after 12 years of age), sex (females are more often affected than males), race (whites are more often affected than blacks), nutritional status (malnutrition is protective), and sequence of infections (e.g., dengue virus 1 infection followed by dengue virus 2 infection seems to be more dangerous than dengue virus 4 infection followed by dengue virus 2 infection). In 2016 an attempt was made to develop a risk score for severe dengue based on the above-mentioned parameters (16). In this study we attempt to study the co-relation between the nadir of the platelet count and the onset of warning signs (mucocutaneous bleed, clinical fluid accumulations and a palpable liver 2 cm below the right costal margin). A positive co-relation in between these two elements could help predict onset of shock and third spacing and facilitate prognostication and early intervention.

Methods

Study population

The Study design is depicted in Fig 1. We studied 82 consecutive admitted patients with a diagnosis of dengue fever, the basis of which was clinical signs and symptoms with a positive NS1 antigen test. This test when taken in conjunction with history and examination is now considered the gold standard for early diagnosis (17) and prompt management of dengue. The nadir of thrombocytopenia and rise in transaminases were studied along with onset of warning signs, namely, clinical fluid accumulation (ascites, pleural effusion or pedal edema, Grade 2 or higher) mucosal bleed (clinically detected wet or dry petechiae or purpura) and a palpable liver more than 2 cm below right costal margin. The pleural effusion so described above, was assessed either clinically or through a lateral decubitus chest radiograph. Similarly, ascites was assessed either through clinical examination or an abdominal ultrasound. We also attempted to study the rise in liver enzymes (SGOT and SGPT) as there is evidence in contemporary literature that a rise may predict severe dengue and associated conditions (including, but not limited to acute liver failure and other complications that may occur, secondary to dengue.)

Study Design

The study was a single center, observational study lasting 08 months. Every patient received treatment according existing standard of care. All patients with NS1 positive dengue were included, however, patients with dual infections (dengue and malaria) and the recently described triple infection (dengue, malaria and scrub typhus) were excluded as these are likely to cause an independent and confounding rise in transaminases. Patients with a previously diagnosed cause of low platelets (immune thrombocytopenia, hematological malignancies, aplastic anemia) were also excluded due to obvious confounding.

Study Outcomes

The profile of warning signs and associated clinical manifestations were studied. Statistical association of the nadir of thrombocytopenia and onset of other warning signs and its associated with rise in transaminases was studied.

Statistical Analysis

The t test for two independent samples, Levene's Test for Equality of Variances and t-test for Equality of Means was used to analyze the data. P value < 0.05 was taken as significant.

Results

Summary of results

The mean age of patients was 36 ± 10.1 , with a mean platelet nadir $92572/\text{mm}^3$. The AST and ALT peak was 4184 and 136 IU/L respectively. Clinical fluid accumulation was seen in 54 (65.03%, CI 95%, SD 1.9) patients, mucosal bleed in 61 patients (73.4%, CI 95%, SD 1.3) and liver was palpable more than 2 cm in 28 patients (33%, CI 95%, SD 2.1). Rare clinical manifestations such as dengue encephalitis (01 patient) and dengue ALF (01 patient) were also seen.

Based on the above score, 31.1% patients in our study had platelets less than 50,000 per microliter. However, on 16 (19.2%) of our patients had clinical hepatomegaly. Therefore, no more than one in five patients had a dengue severity score more of than 13, the accepted cut off having a predictive value for severe dengue.

Discussion

The mean age of patients was 36 ± 10.1 , with a mean platelet nadir $92572/\text{mm}^3$. The AST and ALT peak was 4184 and 136 IU/L respectively. Clinical fluid accumulation was seen in 54 (65.06%, CI 95%, SD 1.9) patients, mucosal bleed in 61 patients (73.4%, CI 95%, SD 1.3) and liver was palpable more than 2 cm in 28 patients (33.7%, CI 95%, SD 2.1).

The fall of platelets due to a multifactorial etiology has been discussed above and has been documented in several studies in the contemporary literature (18). Data regarding the complex pathophysiology of platelet fall in dengue continues to emerge with **Goldthorpe et al**, in 2017, proposing that the engagement of DENV EIII with thrombocyte progenitors could further contribute to thrombocytopenia (19). Also, **Michels et al** demonstrated the imbalance of angiopoietin 1 and 2 as contributing factors to thrombocytopenia in dengue (20). The role of CD61 as the punitive molecule causing thrombocytopenia was studied by **Noisakaran et al** (21).

In a study by **Bernard Filho et al**, they demonstrated a definite co-relation between thrombocytopenia and gingival bleeding (22). Our results are generally consistent with this finding. **Gamakaranage et al**, in 2012, showed a definite correlation between platelet fall and dengue hemorrhagic fever (23). **Strobol et al**

postulated that patients with previously diagnosed causes of platelets dysfunction have a deepened nadir of platelet count when they have dengue fever (24). Our study did not assess previously diagnosed platelet dysfunction and patients with other causes of thrombocytopenia were excluded. **Suwarto et al** showed an associated with pre-existing liver dysfunction and nadir of thrombocytopenia in dengue fever (25). From a treatment perspective, agents that have been tried with mixed but clinically insignificant results include corticosteroids, carica papaya extract, prophylactic platelet transfusion, oral calcium and immunoglobulin.

There have been attempts at exploring the utility of platelet fall to predict dengue hemorrhagic fever by **Kumar et al** (26), dengue encephalitis and early CNS involvement by **Alice et al** (27), post-partum hemorrhage by **Phi Hung et al** (28) and central retinal vein occlusion by **Velaitham et al** (29). Amongst Indian studies, **Ojha et al** in 2017 showed that platelet activation co-related with severity of dengue fever in their landmark article published in Nature magazine. A year later in 2018, **Sharma et al** explored the role of prophylactic platelet transfusion in Dengue. However, review of literature did not reveal any study that explores the correlation of thrombocytopenia with the occurrence of **other** warning signs, and in this regard, this study is unique. The linear relationship between thrombocytopenia and mucosal bleed remains, but this study shows the critical association between the occurrences of ascites, pleural effusion and pedal edema with thrombocytopenia. This opens up new avenues for research into the occurrence of complications and rare manifestations of dengue and their correlation with thrombocytopenia.

While we set out to explore and investigate the real time mathematical association (if any) between the nadir of thrombocytopenia and onset of other warning signs, we understand the obvious and direct association between a lower platelet count and increased incidence of mucosal bleed. While this may seem linear, the occurrence of clinical fluid accumulation and hepatomegaly cannot be corroborated through a similar linear relationship. Also, numerous infections (HBV, HIV, HCV, IgM HAV and IgM HEV) cause suppression of the bone marrow which may have contributed to the platelet in our patients. Some of these were tested in all patients who had deranged LFTs; however, we identify our inability to test for all these conditions and their confounding presence cannot be ruled out with scientific certainty.

Our study did not show a statistically significant association with transaminases rise and occurrence of warning signs. The utility of these parameters continues to remain critical in predicting dengue related acute liver failure and dengue related multi-organ dysfunction.

Observation Tables

Table 1: Clinical Characteristics of Patients Enrolled in The Study

S.no	Parameter	Mean(SD)(n=83)
1	Age	36±10.1
2	Sex(Male:Female)	5.1:1
3	Plateletnadirmean	92572±6958.8

4	AST(peak)		4184±214		
5	ALT(peak)		136(14.2)		
6	Clinicalfluidaccumulation		54±1.9		
7	Mucosalbleed		61±1.3		
8	rpalpablemorethan2cmbelowRCM		28±2.1		
CalculationTable1:ComparativeanalysisofPlateletcountandAST/ALTpe akwithmucosalbleed					
	Mucosalbleed	N	Mean	Std. Deviation	Pvalue
Platelet(lowest)	No	57	123824.56	62174.04	0.001
	Yes	26	24057.69	10960.08	
AST(initial)	No	57	131.30	126.56	0.674
	Yes	26	146.03	185.32	
ALT(initial)	No	57	101.40	95.93	0.626
	Yes	26	115.96	175.12	
CalculationTable:ComparativeanalysisofPlateletcountandAST/ALT peakwithclinicalfluidaccumulation					
	Fluidaccu mulation	N	Mean	Std. Deviation	P value
Platelet(lowest)	No	67	110692.5 4	65449.08	0.001
	Yes	16	16693.7 5	4924.15	
AST(initial)	No	67	131.99	123.79	0.620
	Yes	16	152.36	222.51	
ALT(initial)	No	67	101.63	95.37	0.522
	Yes	16	124.12	212.77	

For other factors AST(initial) & ALT(initial) the difference due to Fluid accumulation is not significant.

Dengue Severity score

S. no.	Clinical characteristics	Criteria	Assigned score
1	Age(years)	Morethan6	1
		Lessthan6	0
2	Hepatomegaly	Yes	8.5
		No	2
3	Systolicbloodpressure	Lessthan90	2
		More than90	0
4	Whitebloodcell (permicroliter)	Morethan5000	1
		Lessthan5000	0
5	Platelets	Lessthan50,000	4.5
		Morethan50,000	0

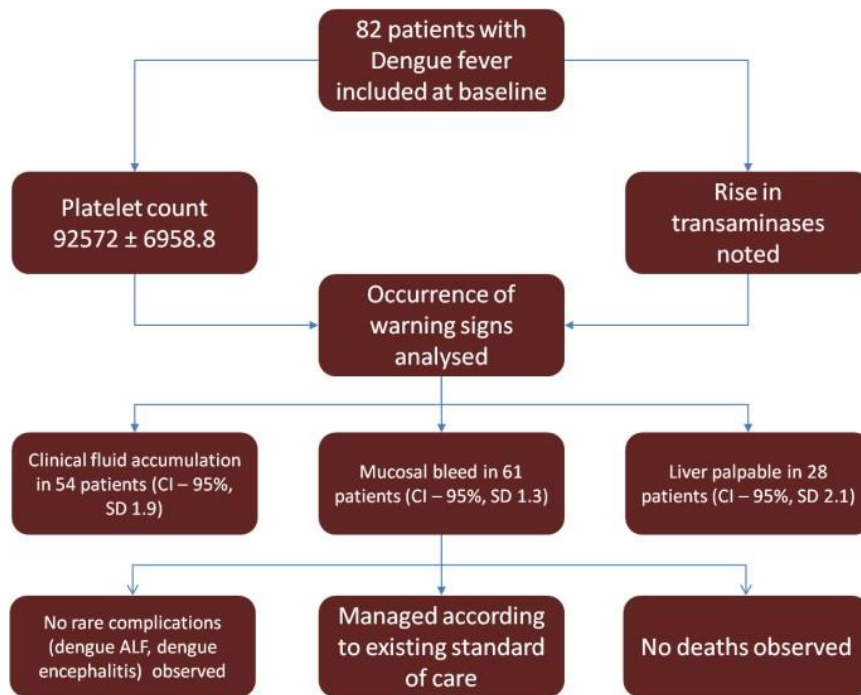


Fig2. Outcome measures in patients with Dengue Fever (n=83)

Conclusion

The importance of serial monitoring of platelets is important not only for the prediction of severe dengue, but also with the onset of other warning signs such as clinical fluid accumulation. This nuanced fact re-enforces the critical importance of platelet count predict other warning signs which contribute to morbidity and mortality in dengue fever.

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