

ASSOCIATION OF THROMBOCYTOPENIA AND PROLONGED BLEEDING TIME IN DENGUE WITH THE CLINICAL OUTCOME OF THE PATIENT

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ABSTRACT

Objective: To find out the frequency of thrombocytopenia and prolonged bleeding time in dengue fever and its effect on clinical outcome of patients.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was conducted, from August 2016 to February 2017 in the Haematology department, at Pakistan Naval Ship (PNS) Shifa, Karachi.

Methodology: The study was conducted on serologically proven positive dengue IgM cases fulfilling inclusion and exclusion criteria. Platelet count was done on Beckman Coulter and Sysmex analyzers and then rechecked manually by peripheral blood smear. Bleeding time measured by the Duke method. The clinical outcome was evaluated on the day of discharge (based on survival of the patient and their lab values) or the patient expired and their lab values.

Results: A total of 135 patients were evaluated in the study. The mean age of the patients was 29.12 ± 13 yrs (range: 2-63 years). The thrombocytopenia was observed in one hundred and eleven (82.2%) patients. Severe thrombocytopenia was observed in nine (6.7%) cases, moderate thrombocytopenia was found in thirty (22.2%) cases and mild thrombocytopenia was observed in seventy-two (53.3%) cases. Bleeding time more than nine minutes was observed only in one case. Out of 135 patients, two (1.48%) expired and one hundred and thirty-three (98.52%) survived and were discharged.

Conclusion: We observed that platelet count can be a predictor of disease and its progression, but the absence of either cannot be used to exclude the progression of the disease. Dengue continues to be a significant health problem in Pakistan.

Keywords: Bleeding time, Clinical outcome, Dengue, Thrombocytopenia.

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INTRODUCTION

In tropical and subtropical countries of the world, dengue is a major health problem affecting roughly 50 to 100 million people per year with increasing geographic extension every year¹. Generally, 2.5% of these affected people may expire². Dengue fever is an acute febrile disease caused by an arbovirus in Flavivirus genus and transmitted by the bite of *Aedes aegypti* mosquito. There are five serotypes DENV 1, DENV 2, DENV 3, DENV 4, and DENV 5³. These mosquitoes also play part in the transmission of other viral infections like Chikungunya, Zika, and Yellow fever⁴. There are certain environ-

mental conditions that favor collection and breeding of mosquitoes particularly rainy and post-rain season. *Aedes aegypti* mosquito proliferates in stagnant water, like buckets, flower vases, coolers, buckets, and unused tires.

In Pakistan, dengue has become a serious health issue. Sindh and Punjab are the provinces where outbreaks of dengue have been reported and now it is endemic here⁵. Dengue is a complex disease in terms of host-virus relation, virus-vector relationship and variability of clinical manifestations. It presents with a wide range of clinical spectrum ranging from mild to severe life-threatening form, including undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). DHF and DSS are the life-threatening forms of dengue. Progression to severe form is indicated

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by increased vascular permeability and plasma leakage⁶. If it remains un-noticed or untreated, it may cause circulatory collapse or even shock.

Clinical features depend on viral strain, age and immune status of patient⁶. Clinical features include high-grade fever, bone pain (break-bone fever or bone crusher disease), epigastric pain, vomiting, erythematous rash, and bleeding tendencies.

Diagnosis of dengue infection can be made in the laboratory by demonstration of specific antibodies by complement fixation, neutralization test or enzyme-linked immunosorbent assay (ELISA). Hybridization probe technique for nucleic acid and reverse transcriptase Polymerase chain reaction (PCR) are the newer tests for confirmation of diagnosis.

The haemostatic changes, which occur in dengue include:

- a) Vasculopathy manifests as petechiae.
- b) Platelet abnormalities include thrombocytopenia and dysfunctional platelet (impaired ADP-induced aggregation and ADP release). Generally, the severity of clinical presentation correlates with thrombocytopenia level.
- c) Coagulopathy manifests as mild to moderate prolongation of prothrombin time and partial thromboplastin time. Clotting factor assay shows mostly mild to moderate reduction.

In dengue, thrombocytopenia is mainly due to increased immune-mediated clearance of platelets and decreased production of platelets⁷. There is a decreased megakaryocyte number in marrow during the early febrile phase. Later on, megakaryocyte number becomes normal or increased. Initially, the platelet counts usually drop to below 100,000/ul for 1 to 2 days before defervescence and after 3 to 5 days in the majority of the cases, the counts then normalize during convalescence^{8,9}.

Pseudo thrombocytopenia might be a reason when there is no bleeding, although apparently there is severe thrombocytopenia. Severe thrombocytopenia causes chances of hemorrhage al-

though the platelet count may not directly correlate with the bleeding manifestation.

In dengue, there is only supportive or symptomatic treatment and no specific treatment. However, in routine practice, blood components, especially platelets are being transfused indiscriminately with an opinion to "prevent" occasionally life-threatening complications including bleeding.

This study was aimed to assess the trends in platelet count in patients with dengue fever and its correlation with prolonged bleeding time and outcome of the patient. The study will be helpful to clinicians for early detection of the severity of disease as these parameters may have a correlation with clinical outcome. These investigations are beneficial to reduce morbidity and mortality of the patients and these tests can be performed in the under-resourced laboratory.

Unnecessary use of blood component transfusion can be avoided with regular monitoring of these parameters. It will help to ensure the availability of blood components, especially platelets to the right patients, as well as to avoid unsafe exposure to transfusion transmissible diseases¹⁰.

This study will be helpful in the future development of guidelines for better management in dengue and hence minimize unnecessary exposure of the patient to platelets.

METHODOLOGY

This cross-sectional study was carried out in the department of Haematology, PNS Shifa Karachi, from August 2016 to February 2017. The sample size was calculated by using the WHO sample size calculator, considering 7% margin of error and a 95% confidence interval. The estimated sample size was n=135. The sampling technique was Non-probability purposive sampling. Permission from the institution's ethical board was taken. Prior to enrollment in the study, verbal informed consent was taken from all patients or their nearest relative. All the serologically proven positive dengue IgM cases that were reported in the pathology department and fulfilling inclusion and exclusion criteria were

included in the study. All cases of either gender having positive dengue Immunoglobulin M (IgM) serology dengue infection, diagnosed on the basis of enzyme-linked immunosorbent assay (ELISA) and immunochromatographic assay (ICT), and were willing to take part in the study, were included in the study. Patients with acute febrile illnesses and negative dengue IgM

was rechecked manually by peripheral blood smear. Bleeding time was measured by the Ivy method. The clinical outcome was evaluated on the day of discharge (based on survival of the patient and their lab values) or the patient expired and their lab values. Platelet count: $<1,50,000/\mu\text{l}$ was taken as thrombocytopenia while $<20,000/\mu\text{l}$ as severe thrombocytopenia,

Table-I: Frequency of thrombocytopenia and prolonged bleeding time in dengue patients (n=135).

Haemostatic Manifestations in Dengue		At the time of Admission, n (%)	On Discharge n (%)
Platelet Count	Thrombocytopenia		
	≥1,50,000 / ul	24 (17.8%)	32 (23.7%)
< 1,50,000 / ul	No	111 (82.2%)	103 (76.3%)
<20,000 / ul	Severe	09 (6.7%)	1 (0.7%)
	>20,000 to <50,000 / ul	30 (22.2%)	10 (7.4%)
	>50,000 to < 1,50,000 ul	72 (53.3%)	92 (68.1%)
Bleeding Time	Normal	134 (99.3%)	135 (100%)
	> 9 Minutes	1 (0.7%)	-

Table-II: Association of thrombocytopenia in dengue patients with outcome (n=135).

Haemostatic Manifestations in Dengue		n	On Discharge	
Platelet Count (ul)	Thrombocytopenia		Survive	Expired
≥1,50,000	No	32	30 (93.8%)	2 (6.3%)
< 1,50,000	Yes	103	103 (100%)	-
<20,000	Severe	1	1	-
>20,000 to <50,000	Moderate	10	10	-
>50,000 to < 1,50,000	Mild	92	92	-

p-value was 0.059* (level of significance at <0.05), Confidence level 95%, *Fisher's exact.

Table-III: Frequency of prolonged bleeding time in dengue patients with respect to outcome (n=135).

Bleeding Time	n	On Discharge	
		Survive	Expired
2 to 9 minutes	135	133 (98.5%)	2(1.5%)
> 9 Minutes	-	-	-

serology, known cases of bleeding disorders, chronic liver disease, malaria, and typhoid fever were excluded from the study.

For this study, blood samples (in EDTA containing tube) of patients were taken on the first day of admission and on the day of discharge (in survivors) and if the patient expired during a hospital stay, the latest reports were considered and Performa filled accordingly. Thrombocytopenia was done on Coulter and Sysmex analyzers (KX-21) and to avoid false thrombocytopenia it

$>20,000/\mu\text{l}$ to $<50,000/\mu\text{l}$ as moderate thrombocytopenia and $>50,000/\mu\text{l}$ to $<1, 50,000/\mu\text{l}$ mild thrombocytopenia respectively.

The obtained data were entered in SPSS-17 for statistical analysis. The mean and SD were calculated for age, and percentages and frequency were calculated for gender, thrombocytopenia, bleeding time and clinical outcome. In order to analyze the association between thrombocytopenia in dengue and clinical outcome, a

chi-square test was applied. The p -value ≤ 0.05 was assumed as significant.

RESULTS

A total of 135 serological proven dengue-infected cases were included in this study. The mean age of the patients was 29.12 ± 13.11 years (Range: 2-63 years). There were 111 (82.22%) males and 24 (17.78%) were females.

At the time of admission, thrombocytopenia was found in one hundred and eleven (82.2%) patients (platelet count $< 1,50,000$ /ul) as presented (table-I). Severe thrombocytopenia was observed in nine (6.7%) cases, moderate thrombocytopenia was in thirty (22.2%) cases and mild thrombocytopenia was observed in seventy-two (53.3%) cases.

While at the time of discharge, thrombocytopenia was observed in one hundred and three (76.3%) patients, in which severe thrombocytopenia was observed in one (0.7%) case, moderate thrombocytopenia was found in ten (7.4%) cases and mild thrombocytopenia was observed in ninety-two (68.1%) cases. Bleeding time was prolonged (above 9 minutes) in one case at the time of admission while this has become normal at the time of discharge (table-I). At the time of discharge, bleeding time was normal in all patients.

Out of 135 patients, two (1.48%) had expired and one hundred and thirty-three (98.52%) survived and discharged (table-II). There was no significant association of level of thrombocytopenia and with clinical outcome of the patient (p -value=0.059).

Two male patients expired and their age was above 30 years (40 years and 58 years).

DISCUSSION

The frequency of dengue fever has increased in Pakistan as we observed a number of outbreaks in the past few years. Although we encountered a number of patients with serologically confirmed dengue, the actual number is much larger. This includes all patients with false negative serology, patients with a mild flu-like

illness in whom dengue was not considered a possibility, also patients with mild disease and normal platelet count who did not seek treatment at the hospital.

Until now, most studies have enrolled moderately small numbers of cases and usually focusing on severe disease, in which complications because of secondary multiple organ system dysfunctions confound the results. To date, there have been only a few studies documenting serial changes in platelet count and bleeding time during the course of the illness, and their association with outcome. This study strongly emphasizes routine complete blood counts, especially platelet count, as the haemostatic manifestations evolve over a period.

In our study, cases were reported mostly in the rainy season, while in winter, cases were reported infrequently possibly due to cold and fewer mosquitoes.

As observed in the previous studies thrombocytopenia was the most common and most significant laboratory finding in dengue¹¹⁻¹³. An important finding of this study was 82.2% of patients had thrombocytopenia, most of them with moderate (22.2%) or mild (53.3%) thrombocytopenia. While at the time of discharge, thrombocytopenia observed in one hundred and three (76.3%) patients in which severe thrombocytopenia observed in only one case, moderate thrombocytopenia was in 10 cases and mild thrombocytopenia observed in ninety-two (68.1%) cases. It was also observed by Nagaram *et al.* According to them the thrombocytopenia was found in all cases of dengue, severe thrombocytopenia (platelet count $< 20,000$) was in thirty-four (19.5%) cases¹⁴. Although, thrombocytopenia is the most common finding in dengue we could not rule out the possibility of dengue only if the patient has a normal count. The association of thrombocytopenia in dengue fever has been proved as significant (p -value ≤ 0.05). In addition, there is a gradual normalization of platelet count during the course of disease in most of the patients.

Bleeding time prolonged in only one patient admitted with severe thrombocytopenia (Platelet count $8 \times 10^9/\text{ul}$) and with the passage of time, platelet count and bleeding time, gradually improved, as he discharged with platelet count $72 \times 10^9/\text{ul}$ on day 7 of hospital stay. In the majority of patients, bleeding time was within the normal range, but in a few patients, it was towards upper limits (7 or 8 minutes). Therefore, we can say that in these patients there would be compensatory increased production of platelet and early recovery. In our study, we noticed that with increasing severity of thrombocytopenia, there is increased bleeding time. Fortunately, a small number of patients had severe thrombocytopenia that was similar to the study by Banerjee *et al*¹⁵.

Our findings endorsed by Basher *et al*. According to them the patients with a platelet count of $>100,000/\text{ul}$ are usually asymptomatic and bleeding time remains normal, but platelet count $<50,000/\text{ul}$ causes mildly prolonged bleeding time⁸. Therefore, we can assume that the remaining platelets are functionally normal. However, few studies showed poor or no association between thrombocytopenia and bleeding diathesis^{14,16,17}.

In our study, mortality was around 1.48% (two cases), which is lower than observed in previous studies with higher mortality of 6.25%, 1.7% and 10% respectively^{11,14,16}. In these patients, the platelet count and bleeding time was towards the upper normal limit but was normal ($p\text{-value}=0.059$). One of the two patients, who expired, had secondary bacterial meningitis and expired due to cardiorespiratory arrest on the 4th day of hospital stay.

It was worth mentioning here that thrombocytopenia and raised bleeding time were not sole factors in causing complications in every case and there could be some other risk factors that contribute to it (deranged coagulation profile or raised fibrin degradation products due to disseminated intravascular coagulation (DIC) or as in our case patient is having co-infection)¹⁸.

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CONCLUSION

The thrombocytopenia could be a predictor of dengue and its progression, but the absence of either could not rule out the progression of the disease. As these investigations were easy to perform and are helpful to pick the patient with severe dengue infection.

In addition, thrombocytopenia should not be the only indicator to transfuse platelets "in all cases". The community and treating physicians should be educated regarding the judicious transfusion of platelets. Unnecessary platelet transfusion should be avoided, especially during an epidemic, when there is scarcity of its availability.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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