Original Article

Leucopenia as An Early Predictor of Thrombocytopenia and Hemorrhagic Manifestations in Dengue Fever: An Observational Study

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Abstract

Background: *Dengue fever is an endemic disease in Bangladesh that has shown increasing incidence eachpassing year. Dengue has a wide range of clinical presentations and laboratory findings. Thrombocytopeniais a common finding in dengue-infected patients along with other findings like leukopenia and hemorrhage in several forms. Thrombocytopenia is an indicator of disease severity andcan lead to severe bleeding manifestations. This study aims to find the association of leukopenia with thrombocytopenia and hemorrhagic manifestations.*

Materials and methods: *This prospective observational study was conducted in a tertiary care hospital in Chattogram, Bangladesh for a period of six months. A total of 100 patients with confirmed dengue infection who fulfilled the inclusion criteria were followed from the day of admission to recovery. Clinical and laboratory findings, Total Leucocyte Count (TLC) and platelet counts were noted. Pearson's Correlation Test was performed for correlation identification between leukopenia with thrombocytopenia and hemorrhagic manifestations in Dengue Fever.*

Results: *Around 60.72% of the patients with bleeding manifestations had a low Platelet count (<150000/cmm) and among them 91.2% had leucopenia (<4000/cmm). A significant correlation was observed among low TLC, thrombocytopenia and hemorrhagic manifestation (p value <0.001).*

Conclusion: *Leucopenia has the potential to serve as an additional laboratory marker to predict thrombocytopenia and subsequent hemorrhagic manifestation in patients with dengue fever.*

Key words: Dengue; Hemorrhage; Leucopenia; Outcome; Platelet.

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INTRODUCTION

Dengue Fever (DF) is an arboviral disease caused by one of the four serotypes of Dengue virus (DEN 1-4) belonging to the family Flaviviridae. The primary vector for the disease is Aedes aegypti mosquito, which is widely spread in the tropical and subtropical regions. It is an imperative public health challenge globally with more than 2.5 billion people at risk of infection in the tropics and subtropics.[1] Annually, an estimated 390 million (284-528 million) dengue infection occurs in around 129 countries, of which 70% of the burden contributor is in Asia.[1] The disease ranges from a mild subclinical infection to acute febrile illness to full blown shock.

In 2009, the WHO revised the classification of the disease from 1997 dengue case guidelines (Dengue fever, dengue hemorrhagic fever and dengue shock syndrome). The current scheme is based on the levels of severity, i.e. dengue without warning signs, dengue with warning signs (Abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy, restlessness, hepatomegaly and increased haematocrit concurrent with a rapid decrease in platelet count) and severe dengue.[1]

The modification was done as dengue affects different age groups and a wide geographical area. The course of DF depends on numerous underlying factors such as age, comorbid conditions, infecting serotype and secondary infection with other serotypes/organisms. Of all the clinical features, the disease severity and mortality appear to depend maximum on the occurrence of bleeding manifestations, which can vary from minor petechiae and gum bleeding to life-threatening internal hemorrhage and such manifestations are assumed as a result of underlying thrombocytopenia.[3] The exact mechanisms of thrombocytopenia in DF remain unclear, although many theories have been postulated for it.[4,5]

Dengue infection may be asymptomatic or can clinically manifest with symptoms ranging from non-specific viral infection to hemorrhagic fever and shock. Platelet counts are notably reduced in infected patients making them susceptible to bleeding. Such thrombocytopenia has been hypothesized to be due to factors like bone marrow depression, infection of megakaryocytes, the release of anti-platelet antibodies destroying platelets, altered vascular permeability, platelet consumption due to disseminated intravascular coagulation, platelet phagocytosis by macrophages.[6,7,8]

Alteration of various other hematological parameters are also noted in dengue infection. These include leukopenia, lymphocytosis with atypical lymphocytes on the peripheral blood smear, changes in hematocrit and alteration in liver enzymes.[9] This study aimed to evaluate the relationship between leucopenia with thrombocytopenia and subsequent hemorrhagic manifestations.

MATERIALS AND METHODS

This study was a hospital based observational study. The patients who presented with dengue fever in the Department of Medicine, Marine City Medical College & Hospital, Chattogram, Bangladesh from August 2023 to January 2024 were taken in study. The informed consent was obtained from each patient prior to commencement of this study. The detailed history and examination was carried out and each patient was investigated as per designed proforma.

Inclusion criteria
- Patients with a positive dengue serology (Non-Structural protein 1 antigen-NS1or IgM).
- Platelet count <150thousand/cumm.

Exclusion criteria
- IgG positive cases.
- Inherited Disorders of thrombocytopenia.
- Patients who received platelets transfusion during the study.
- Patients on antiplatelet medications.

Cases with confirmed dengue infection were followed from the day of admission till they either recovered clinically and were discharged or suffered from complications. Demographic details, clinical features and warning signs were noted.

Laboratory findings including TLC and platelet parameters were recorded using Mythic-22AL Automated Hematology Analyzer on venous samples collected in Ethylene-Diamine Tetra-Acetic Acid (EDTA) vials. The samples were analyzed within one hour of sample collection.

The patients were divided into 2 categories i.e. with hemorrhage and without hemorrhage. The indices were compared between these groups to look for any association between leucopenia with thrombocytopenia and subsequent hemorrhagic manifestations.

Data was collected and entered into Microsoft Office Excel Sheet 2019. Statistical analysis was performed using the latest SPSS version 23 software, including descriptive analysis. Pearson Correlation Test was performed to study correlation among leucopenia, thrombocytopenia and hemorrhagic manifestations. Any p value of <0.05 was considered significant.

RESULTS

Table I Gender of Respondents (n=100)

	Frequency	Percent
Female	42	42.0
Male	58	58.0
Total	100	100.0

Table I showing gender distributions where male was 58(58%) and female was 42(42%)

Table II Age group (n=100)

	Frequency	Percent
<20 years	44	44.0
21-30 years	18	18.0
31-40	20	20.0
41-50 years	6	6.0
>51 years	12	12.0
Total	100	100.0

Table II showing age group distributions where younger age groups were affected more than the older ones.

Table III Clinical Data (n=100)

Variables	Number	Percent (%)
Fever	100	100
Aches and pain	98	98
Eye pain	8	8
Headache	22	22
Vomiting	68	68
Diarrhoea	10	10
Rash	20	20
Bleeding	56	56
Elevated liver enzymes	26	26

Table III showing different symptoms where all patients had fever, 98(98%) patients suffering from aches and pain, 22(22%) had headaches and 68(68%) had vomiting. Other symptoms were rash, diarrhea and eye pain.

Table IV Serological test (n=100)

Tests	Number	Percent (%)
NS1	61	61
IgM	37	37
NS1 & IgM	2	2

Table IV showing 61(61%) patients were positive for dengue NS-1 antigen, 37(37%) patients were positive for dengue IgM by ELISA and 2(2%) patients were positive for both NS1 and IgM.
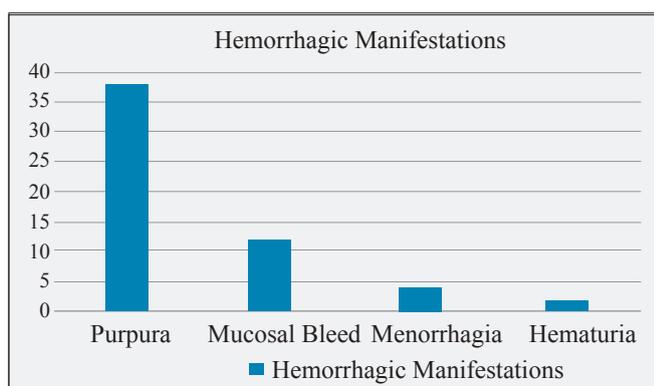
Figure 1 Hemorrhagic manifestations

Figure 1 showing 38 (38%) patients had purpura, 12 (12%) patients had mucosal bleeding, 4 (4%) patients had menorrhagia and 2 (2%) had hematuria.

Table V Comparison of hemorrhagic manifestation with thrombocytopenia

Thrombocytopenia (<150000/cmm)	Hemorrhagic Manifestation		Pearson Correlation (p Value)
	Yes (n=34)	No (n=22)	
	32000	100500	<0.001

Table V showing hemorrhagic manifestations in dengue fever were seen in those patients with thrombocytoepnia, reflecting the general population trend.

Table VI Comparison of hemorrhagic manifestation with MPV

Leucopenia (<4000/cmm)	Thrombocytopenia		Pearson Correlation (p Value)
	Yes (n=31)	No (n=3)	
	3820	5841	<0.001

Table VI showing thrombocytopenia in patients with dengue fever were seen in those patients who had leucopenia, reflecting the general population trend.

DISCUSSION

Dengue infection is associated with an increase in the vascular permeability and thrombocytopenia. The latter is also recognized by the WHO as a marker of clinical severity. The exact cause of thrombocytopenia in DF has not been elucidated. Possible pathogenesis includes direct suppression of the bone marrow by the virus, anti-dengue antibody mediated damage, peripheral consumption of platelets due to high levels of platelet-activating factor, isolated viral replication in the platelet or abnormal pooling of blood.[4] Studies have shown that DF is characterized by increased platelet activation (As evidenced by increased expression of P- selectin on the platelet surface), increased apoptosis (As shown by increased phosphatidylserine levels) and complement mediated destruction; all of which account for thrombocytopenia.[5]

It has been well established that a low platelet count is associated with increased risk of developing bleeding and other complications.[10] This is also in accordance with our observations. However, having a combination of markers for relation is more efficient than relying on a single parameter alone.

The role of immunological factors in the pathogenesis of Dengue Hemorrhagic Fever (DHF), particularly Dengue fever Shock Syndrome (DSS) was also studied. The complement activity, circulating Immune Complex (IC) histamine level, platelets and leucocytes were determined in the blood of 30 patients and 43 healthy persons. Besides other parameters, the leukocyte count was significantly reduced (p < 0.001). Furthermore, a marked correlation was found between the changes of the above parameters and the clinical stages of the disease.[10] In the DENV-1 study among the clinically apparent dengue fever patients the mean leukocyte count was 3000/cumm ± SD 780/cumm. Although minimum leukocyte counts (p = 0.28) and the duration of leukopenia (p = 0.55) did not reveal significant correlation.[11]

Leukopenia was frequently found and may be accompanied by varying degrees of thrombocytopenia.[12] Tzong-Shiann et al. suggested that leukopenia along with thrombocytopenia was useful for early diagnosis of dengue fever infection. The most notable laboratory findings in confirmed dengue fever case were included leukopenia (2966 ± 1896/cumm), thrombocytopenia (102 ± 45 × 103/cumm). The Positive Predictive Value (PPV) was high for combination of leukopenia with thrombocytopenia.[13] In the study conducted by Francisca Raimunda F et al leukopenia was the most prominent hematological change, sometimes with counts of less than 2000/cumm, as shown in our study also in which there were 51% cases were of leukopenia with 15 patients having leukocytes less than 2000/cumm.[10]

We had also tried the find out whether there was any correlation between the degrees of thrombocytopenia with the

degree of leukopenia. The correlation analysis between degrees of thrombocytopenia with severity of leukopenia was carried out in 100 positive cases. The correlation coefficient was +0.33. Which was considered as significant at the level of $p<0.01$. So it was concluded that there was high correlation between severities of thrombocytopenia with the degree of leukopenia.

CONCLUSION

Around 60.72% of the patients with bleeding manifestations had a low Platelet count (<150000/cmm) and among them 91.2% had leucopenia (<4000/cmm). A significant correlation was observed among low TLC, thrombocytopenia and hemorrhagic manifestation (p value <0.001).

DISCLOSURE

All the authors declared no competing interest.

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