SEVERE THROMBOCYTOPENIA IN PATIENTS WITH SECONDARY DENGUE HEMORRHAGIC FEVER IN PAKISTAN

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Abstract:
Objective: To evaluate the frequency of severe thrombocytopenia in patients with dengue hemorrhagic fever in a tertiary care hospital in Pakistan
Methodology: A cross sectional study was conducted at Liaquat National Hospital, Hyderabad, Pakistan between September 2018 and January 2019. All patients presenting with consistent fever and tested positive for dengue fever were included in the study. Those with obvious signs and symptoms of other diseases and were negative for dengue IgM and/or IgG were excluded from the study. Demographic and clinical characteristics; age, gender, marital status, job status, number of children, laboratory reports, and serological data were recorded. Data was further stratified into two groups, Dengue Fever and Dengue Hemorrhagic Fever. SPSS v 24 was used to analyze data. A p-value of <0.05 was considered statistically significant.
Results: A total of 170 patients were included in the study. The mean age of patients was 29.5 + 12.41 years. Out of the 170 patients, 27 (9.8%) were diagnosed with secondary dengue fever. In the present study, it was found that in patients with primary dengue fever, 80 (55.9%) had mild, 44 (31.1%) had moderate, and 19 (13.0%) had severe thrombocytopenia, whereas; in patients with secondary dengue fever 7 (26.9%) had mild, 14 (53.8%) had moderate, and 5 (19.2%) had severe thrombocytopenia (p=0.0001).
Conclusion: The present study indicates that severe thrombocytopenia is a frequent clinical manifestation in patients with dengue hemorrhagic fever.
Keywords: dengue fever, dengue hemorrhagic shock, platelets, thrombocytopenia

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INTRODUCTION:

Approximately about 100 million patients are diagnosed with dengue fever every year, out of these 200,000 are reported from Asia [1]. Dengue fever is hyper endemic in Pakistan and neighboring countries and poses a serious threat to the health-care sector and public health. The viral disease is a major concern for rising mortality and fatality among endemic regions specifically in the South Asian countries [2]. In Pakistan, the first outbreak of dengue fever was in 1994, since then there have been many dengue virus outbreaks in different parts of the country. The number of dengue fever cases has been on the rise and the most common serotypes reported are DV-2 and DV-3. [3-5].

Incidence of thrombocytopenia is a serious clinical complication in dengue fever patients which may worsen the prognosis and clinical outcome of the disease. It has been found that patients who have secondary dengue infection are more at risk of developing severe thrombocytopenia. Secondary dengue infection is when a patient has a positive past history of dengue fever and is currently infected by a different serotype of the dengue fever which has been found to be severe. This is due to more severe immunological response in the body [6].

The decrease in production of platelets in the bone marrow as indicated by the rise in megakaryocytes - the immature platelet cells and the concomitant increase in the destruction of platelets from peripheral blood are two mechanisms involved in the development of thrombocytopenia in patients with dengue fever [7, 8]. Several studies suggest that the activation and dysfunction of platelets is implicated in the pro thrombotic complications in dengue hemorrhagic fever and dengue shock syndrome [9].

METHODOLOGY:

A cross sectional study conducted at Liaquat National Hospital, Hyderabad, Pakistan between April 2018 to December 2019 for a study duration of 20 months. After obtaining the ethical clearance from the institutional review board of Liaquat National Hospital, Hyderabad, patient recruitment was initiated. Informed written consent was taken from all patients prior to data collection. A non-probability convenience sampling technique was used to select patients. All patients presenting with continuous high-grade fever for the last 7 days or more, and other consistent signs and symptoms or a history of dengue fever were included in the study. Secondary dengue fever was diagnosed when presence of IgG antibodies against dengue virus were detected. The patients who presented with obvious signs and symptoms of other diseases including Salmonella infection causing typhoid fever, malaria, hemoglobinopathies, coagulopathy, and those who tested negative for dengue IgM and/or IgG were excluded from the study. A sample size of 170 was calculated using the previous study by Khan E, et al, where the prevalence of dengue fever was observed to be 26.3% in Pakistan [9].

Demographic profile and clinical information such as the age, gender, marital status, job status, number of children, laboratory reports, and serological data for each of the participants were recorded. All the patients were further stratified into two groups those with primary dengue fever with and secondary dengue fever. All patients were treated as per protocol and were hospitalized for a period of four weeks at minimum.

The data was entered and analyzed through the statistical package for software sciences (SPSS v. 24). The continuous data was presented as mean and standard deviation while the categorical data was presented as frequency and percentage. The association between the demographic characteristics and the severity of the illness was also determined using a Chi square test. All data was presented in tabular and graphical form. A p-value of less than 0.05 was considered as statistically significant.

RESULTS:

During the study period a total of 170 dengue fever patients were admitted through the emergency department of Liaquat National Hospital, Hyderabad. Out of these 126 (74.2%) were male while 44 (25.8%) were female patients. The mean age (standard deviation) of patients in our study was 29.5 ± 12.41 years. The patients presented with complaints of continuous high-grade fever, headache, nausea and vomiting, myalgias, characteristic skin rash, and lethargy. Out of the 170 patients, 27 (15.8%) were diagnosed with secondary dengue fever. See table 1 for details.

Demographic Characteristics	Group A Primary Dengue fever n = 143	Group B Secondary Dengue fever n = 27
Age group *Younger than 21 years* *21-35 years* *36-55 years* *56-65 years* *Older than 65 years*	131 (9.2%) 86 (60.1%) 22 (15.5%) 17 (11.8%) 5 (3.4%)	2 (7.4%) 17 (62.9%) 5 (18.5%) 2 (7.4%) 1 (3.7%)
Gender *Male* *Female*	108 (76%) 35 (24%)	15 (55.6%) 12 (44.4%)
Presenting symptoms *High grade fever* *Cough* *Headache* *Nausea and vomiting* *Muscle ache/Myalgias* *Rash* *Diarrhea*	143 (100%) 11 (8%) 35 (24.4%) 39 (27.7%) 36 (25.6%) 59 (21.8%) 2 (7.6%)	27 (100%) 3 (11.5%) 16 (57.7%) 9 (34.6%) 14 (50%) 8 (30.8%) 5 (17.6%)

The study evaluated the severity of thrombocytopenia in patients with dengue. It was found that in patients with primary DF, 80 (55.9%) had mild, 44 (31.1%) had moderate, and 19 (13.0%) had severe thrombocytopenia, whereas; in patients with secondary DF 7 (26.9%) had mild, 14 (53.8%) had moderate, and 5 (19.2%) had severe thrombocytopenia with a significant p-value of 0.0001. See figure 1 and table 2 for details.
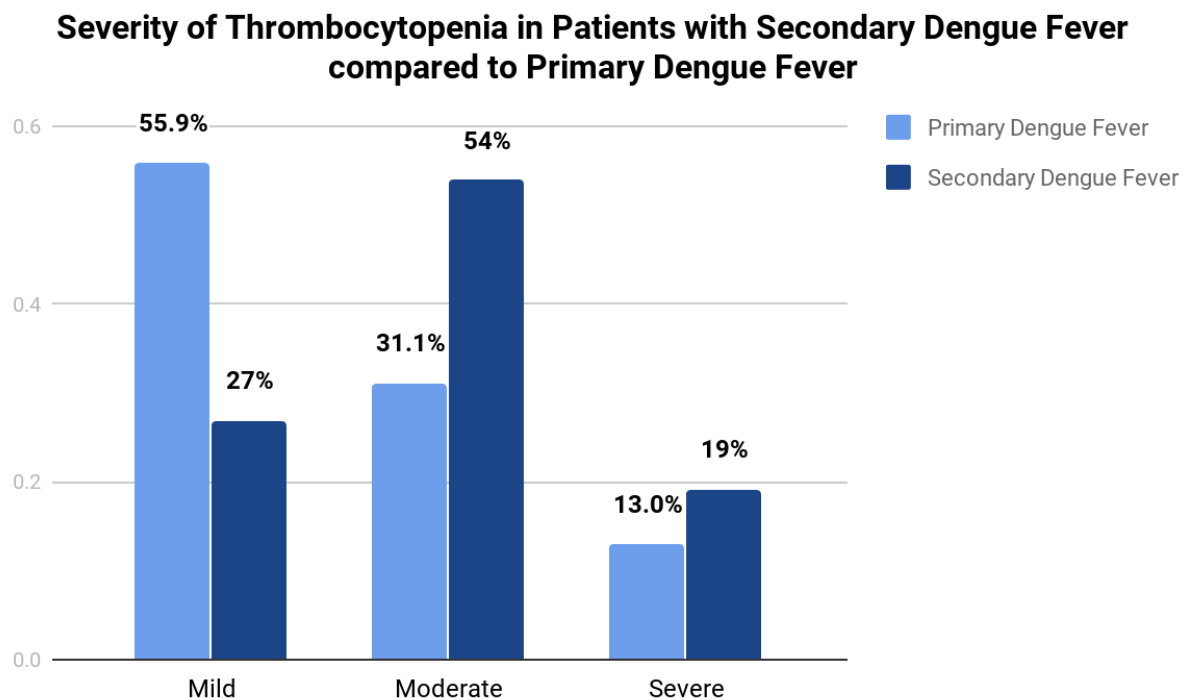
Figure 1: Severity of thrombocytopenia in patients with Secondary DF and Primary DF (n=264)

Table 2: Association of different hematological indices and abnormalities with the secondary DF and primary DF

Independent variable	Primary Dengue Fever (n=143)	Secondary Dengue Fever (n=27)	P-value
Hemoglobin levels Severe deficiency (< 8) Moderate deficiency (8-10) Mild deficiency (10-12) Normal (>12)	4 (2.9%) 31 (21.4%) 44 (31.1%) 64 (44.5%)	4 (15.4%) 6 (23.1%) 8 (30.8%) 8 (30.8%)	0.856
Thrombocytopenia Mild (100-150) Moderate (50-99) Severe (<50)	80 (55.9%) 44 (31.1%) 19 (13.0%)	7 (26.9%) 14 (53.8%) 5 (19.2%)	0.0001
Leukopenia Present Absent	66 (45.88%) 77 (54.28%)	8 (31%) 19 (69.2%)	0.089
Lymphocytosis Present Absent	21 (14.7%) 122 (85.3%)	3 (11.5%) 24 (88.5%)	0.589
Neutropenia Present Absent	35 (24.8%) 107 (75.2%)	11 (42.3%) 16 (57.7%)	0.006
Monocytosis Present Absent	34 (23.5%) 109 (76.5%)	19 (73.1%) 7 (26.9%)	0.001

DISCUSSION:

According to the World Health Organization, thrombocytopenia in dengue fever is an indicator of clinical severity and a prognostic marker for clinical outcome [10]. As per the WHO criteria and guidelines of 2009, thrombocytopenia is present if the platelet count has declined to less than 150,000 per mm^3 of blood [11].

In the present study we evaluated the presence and severity of thrombocytopenia among patients with secondary dengue fever. Patients with a secondary dengue infection have a recurrent infection i.e. they have a past history of dengue fever. The course of disease in patients with secondary dengue infection is more severe and associated with a worse prognosis [6].

In the present study it was found that the majority of the patients with secondary dengue fever had developed moderate to severe thrombocytopenia with a platelet level of 99,000 or below per mm^3 of blood. When compared with the patients diagnosed with dengue fever for the first time, it was further suggested that patients with secondary dengue fever had significantly more severe thrombocytopenia (p=0.0001). See figure 1 and table 2 for details.

Our findings are reinforced by a previous local study by Nasim et al, in 2013 which suggested that thrombocytopenia is a common manifestation in patients with dengue fever and is associated with the severity of the disease. They observed that 95% of their patients had thrombocytopenia at presentation [12]. Nevertheless, they did not stratify their study population according to the severity of the thrombocytopenia and their sample size was much smaller compared to the present study. The other demographic characteristics of the study were similar to the present study.

Thrombocytopenia is a severe clinical complication associated with dengue fever patients and poses a serious threat to the overall prognosis of the patient [13]. Earlier studies indicate that decline in platelet count can cause major bleeding in dengue fever patients and can be fatal if not treated promptly and aggressively [14-15]. The majority of the patients suffers from thrombocytopenia in the first week of the disease and is treated with platelet transfusion to prevent bleeding and subsequent death. In a small yet significant number of patients with dengue hemorrhagic fever and shock, the blood clotting abnormalities due to the severe thrombocytopenia associated with the secondary

effects of hypoxia and acidosis, may result in true disseminated intravascular coagulation (DIC) and major hemorrhage [16]. In some rare cases thrombocytopenia may even persist days after the patient has already recovered from dengue hemorrhagic fever [17].

In secondary dengue infections, the autoimmune reaction is much stronger; therefore, there is more severe disease course. In a study Saito et al, it was found that the patients with secondary dengue infection had a mean $\pm$ SD platelet count of $47\cdot9 \pm 34\cdot6 \times10^3/\mu l$ [18]. They also observed that patients with dengue hemorrhagic fever were associated with severe thrombocytopenia.

Some randomized controlled trials indicate that severe thrombocytopenia may aggressively be treated with anti D immunoglobulin. The study suggested that the patients who were treated with anti D immunoglobulin had an accelerated increase in platelet count; $91,500/mm^3$ compared to those who were administered a placebo; $69,333/mm^3$ at 2 days of start of treatment [19]. This indicates that anti D immunoglobulin is efficacious and relatively safe for the treatment of severe thrombocytopenia secondary to dengue hemorrhagic fever.

In another local study by Raza et al, it was observed that about 57 (95%) of their patients with dengue hemorrhagic fever in the study presented with thrombocytopenia at the time of admission [20]. The study further evaluated that severe thrombocytopenia of platelet count of less than $15,000$ cells/mm^3 of blood in dengue hemorrhagic fever patients was significantly associated with comorbidities (p=0.005). Other significant variation between the two groups (DF and DHF) were raised ALT levels which were 2.7 times higher than normal in DHF patients (p = 0.032) and AST levels which were raised up to 10 times than normal in all DHF patients. Further research can evaluate the significance of these liver enzymes in predicting the clinical severity of the disease.

CONCLUSION:
The present study indicates that severe thrombocytopenia is a frequent manifestation in patients with dengue hemorrhagic fever. Prompt treatment and management is crucial to prevent further deterioration of the patient prognosis and outcome.

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