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Original Research Article

A study to correlate level of thrombocytopenia with dengue seropositive patients and frequency of bleeding pattern

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Abstract: Thrombocytopenia is the major determinant of severity of dengue infection but bleeding may present with any number of platelet count among thrombocytopenic patients. The main objective in this study to correlate level of thrombocytopenia with dengue seropositive patients and frequency of bleeding pattern. The materials and methods in this prospective study, 263 patients were enrolled as dengue fevers were analyzed further with platelet count and various bleeding manifestations. In results the Petechiae (27.9%) was the most common bleeding manifestations followed by GI bleeding and gum bleed seen in 12.9% patients equally. Most of the patients (86.31%) showed thrombocytopenia but all of them were not presented with bleeding tendencies. 100 out of 135 i.e. 67% patients of dengue fever, 118 (120) Dengue hemorrhagic fever and 7 (8) dengue shock syndrome patients were thrombocytopenic (platelet count <1, 50,000 per cubic mm).Majority of patients with bleeding manifestations had moderate thrombocytopenia (20,000 – 50,000 mm^3). Thrombocytopenia increase with clinical severity of dengue infection and even moderate thrombocytopenia can also be seen among classical dengue fever. Frequency of bleeding pattern was not correlated with severity of thrombocytopenia as majority of cases were in moderate range thrombocytopenia i.e. 20,000 – 50,000 mm^3.
Keywords: DEN, DHF, DSS, NS1, SD, ELISA, PT.

INTRODUCTION

Dengue fever is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) also referred to as an arbovirus (arthropod-borne viruses). It is a disease with a wide clinical spectrum and a wide variety of presentations, ranging from asymptomatic to an undifferentiated fever (viral syndrome) to the more severe forms such as severe dengue (SD) or Dengue hemorrhagic fever (DHF) [1].

According to estimates of the World Health Organization (WHO), about 50 million cases of dengue fever occur annually worldwide and 2.5 billion people live in risk areas [1]. The frequency of dengue and its more severe complications dengue hemorrhagic fever and dengue shock syndrome has been dramatically increased since 1980. Dengue virus is the most common cause of arbovirus disease in the world causing an estimated 100 million cases of dengue fever, 250,000 cases of dengue hemorrhagic fever and 25,000 deaths per year [1,2].

The diagnosis of dengue fever is carried out based on clinical, epidemiological and laboratory data. Among laboratory tests, both non-specific [blood count, platelet count, tourniquet test, prothrombin time (PT), liver function tests and serum albumin concentration] and specific tests (viral isolation tests and serology for antibody examination) are used [1].

Leukopenia is the most prominent hematological change, sometimes with counts of < 2000/µL. However, there are reports of mild leukocytosis at the onset of the disease, with neutrophilia. Lymphocytosis is a common finding, with the presence of atypical lymphocytes. The hematocrit concentration should be monitored according to the days of illness, remembering that, with the progression to DHF, there will be a 20% increase in hematocrit from the patient's baseline [1,2].

Thrombocytopenia is a persistent finding in dengue fever and it can be regarded as strongest indicator of dengue fever, however absence of

thrombocytopenia should not rule out the possibility of dengue infection [2]. The normal range of platelet count in blood of healthy adults is 150,000 to 450,000/mm3 and counts less than 150,000/mm3 are referred to as thrombocytopenia [4]. It has been proposed that platelets are sensitized by auto antibodies, and then are destroyed by the reticulo-endothelial system of the body. These auto antibodies against glycoproteins of the platelet membrane can be identified in 80% of the patients [5].

Dengue viruses may directly interact with platelets and activate them. Dengue virus exposure could lead to inhibition of collagen-induced aggregation properties of platelets implicates the possible role of collagen receptors in virus-platelet binding or appears to occur because of complement activation and also because of peripheral sequestration [6,7]. Because dengue virus has been shown to suppress marrow production of platelets, both decreased production and increased utilization of platelets may contribute to bleeding early in infection. The relevance of these observations in the pathophysiology of dengue associated thrombocytopenia would center around platelet activation by virus during early viremic phases or in cases with prolonged viremia and subsequent removal of the active platelets by the liver and spleen. Moreover, if concurrent suppression of hematopoesis, the cascading effect would result in severe thrombocytopenia. Bone marrow studies in patients with DHF have shown marked depression of all marrow elements and down regulation of hematopoiesis [8]. Hemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation. Severe dengue infection, probably driven by a high early viral burden, leads to dysfunction of vascular endothelial cells, derangement of the hemocoagulation system then to plasma leakage, shock and bleeding [9].

It was observed that shortened platelet survival time and a defect in platelet adenosine diphosphate release in patients with DHF and Hemorrhage in Bangkok[10]. During dengue infection, a major fraction of circulating platelets has been activated; in other words, they are either removed from circulation or have lost the ability to promote clot formation [7, 8]. There is suppression of erythroid, myeloid and thromboid cells in 4-5 days. Hence the association with peripheral cytopenias, especially thrombocytopenia. Petechiae occur following lymphocytic dermal vasculitis [11].

Among biochemical variables, the most frequent changes occur in liver function tests such as in serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase and alkaline phosphatase levels, and serum albumin concentrations.

Dengue hemorrhagic fever is characterized by thrombocytopenia, spontaneous hemorrhages, and gradual plasma leakage that can lead to shock [2,12] Despite its clinical variability, the acute phase of dengue begins with fever that is indistinguishable from the initial phase of other acute febrile infectious diseases [2,12,13] Thus, acute dengue infection is often unrecognized until the appearance of the more severe forms of the disease. This observation leads to underestimation of the actual incidence, as well as inadequate or late treatment of a disabling and potentially lethal medical condition.

MATERIALS & METHODS

Study method

This prospective cohort study was conducted in department of medicine, GSVM medical college, Kanpur. Total 263 confirmed cases [based on the World Health Organization (WHO) criteria] of DF were included in this study, who have been admitted in our hospital between August 2011 and October 2014. DF was diagnosed on the basis of the positive serum immunoglobulin M (IgM) antibody and nonstructural protein 1 (NS1) antigen to DF. The serum IgM antibody was analyzed by the enzyme-linked immuno-or bent assay (ELISA) method using an IgM ELISA kit. It was a qualitative analysis and the titers were not measured. A detailed history and clinical evaluation was done. Past history of any bleeding disorder, chronic anaemia, thrombocytopenic disorder has been ruled out. Investigations like Hb, TLC, DLC, Platelet count, Haematocrit, LFT, RFT, Creatine kinase, PT/INR, aPTT, & electrolytes, USG abdomen, ECG & chest X-ray were done. HIV, malaria, and typhoid fever, chronic liver disease, abnormal PT/INR were ruled out.

RESULTS:

Out of 263 patients 135 (51.33%) patients were diagnosed to have DF, 120 (45.6%) patients were diagnosed as DHF and 8 (3.16%) patients were diagnosed as dengue shock syndrome based on WHO criteria. Most common symptom was Fever (100%), headache and myalgia presented equally in 58.94% cases, followed by retro-orbital pain 143(54.37%), abdominal pain 111(42.20%), mucosal bleeding (including GI Bleed, gum bleed, epistaxis, hematuria) 84(31.93%), and petechiae 72 (27.4%) were seen in patients. Tourniquet test was seen in 79 (30.0%) patients.

Over all petechiae (27.4%) was the most common bleeding manifestation. In DHF Petechiae (46.67%) was the most common bleeding manifestation. Most common orifice bleeding was gum bleeding (27.3%) followed by GI bleed (26.4%). In DSS most common orifice bleeding was GI bleed

(25.0%). Various manifestations of bleeding in dengue seropositive patients is shown in Table 1.

Table1: Distribution of patients according to bleeding sites

Sites	TOTAL (n=263)		DF (n=135)		DHF (n=120)		DSS (n=8)	
	NO.	%.	NO.	%.	NO.	%	NO.	%
Epistaxis	5	1.90	1	0.75	4	3.33	0	0.00
Gum-bleeding	34	12.93	0	0.0	33	27.27	1	12.50
GI Bleed	34	12.93	0	0.0	32	26.44	2	25.00
Hematuria	10	3.80	0	0.0	10	8.33	0	0.00
I.C.bleed	1	0.38	0	0.0	1	0.82	0	0.00
Petechiae	72	27.37	21	15.50	56	46.67	3	37.50

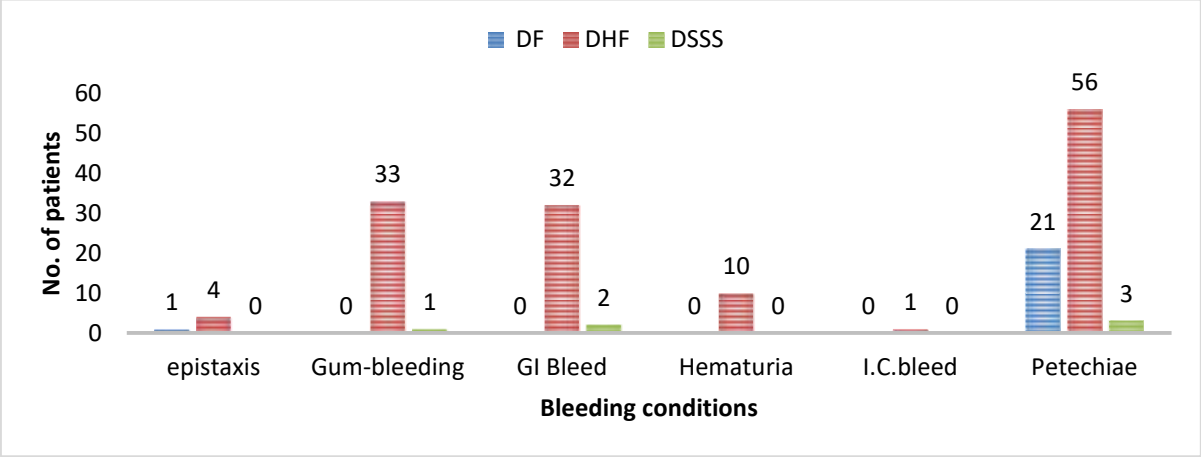

Fig-1: Distribution of patients according to bleeding sites

Most of the patients (86.31%) showed thrombocytopenia but all of them were not presented with bleeding tendencies. 100 out of 135 i.e. 67% patients of dengue fever, 118 (120) Dengue hemorrhagic fever and 7 (8) dengue shock syndrome patients were thrombocytopenic (platelet count <1, 50,000 per cubic mm) Table2.

Table 2: distribution of patients according to severity of platelet counts

Diagnosis	Platelet count (in cubic mm)			
	< 20,000	20,000 – 50,000	50,000–1,50,000	>1,50,000
DF(n = 135)	4	54	42	35
DHF(n = 120)	22	67	29	2
DSS(n = 8)	1	6	0	1

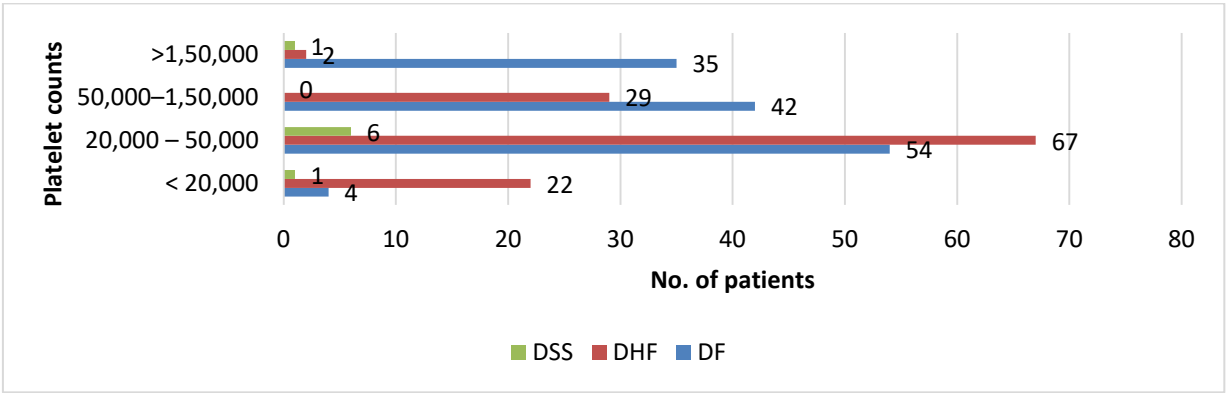

Fig-2: Distribution of patients according to severity of platelet counts

Among the patients of dengue hemorrhagic fever, Petechiae was the major bleeding manifestations, which was seen among 46.67% of patients, among mucosal bleeding gum bleeding was the most common followed by GI bleeding, seen in 27.5% and 26.67% patients respectively. Hematuria is seen in 8.3% of cases and 4(3.33%) patients had epistaxis. Association of various bleeding manifestations and level of platelet count was shown in table 3. It was seen that majority of patients (57%) with petechiae had platelet count in between 20,000 – 50,000 (per mm^3). 21(33) patients of DHF with gum bleeding had platelet count in between 20,000 – 50,000 mm^3. Most of the GI bleed 14 out of 32 had platelet count < 20,000 mm^3. 50% & 40% of hematuria patients had platelet count in between 20,000 – 50,000 and <20,000 mm^3 respectively. 7 patients of GI bleed out of 32 patients had platelet count > 50,000 mm^3.

Table-3: Distribution of patients according to severity of platelet count and various bleeding conditions

Bleeding manifestations in DHF (n = 120)	Platelet count (in cubic mm)			
	< 20,000	20,000 – 50,000	50,000–1,50,000	>1,50,000
Petechiae (n = 56)	12	32	12	0
Epistaxis(n = 4)	1	3	0	0
GI Bleed (n = 32)	14	11	7	0
Gum bleed (n = 33)	9	21	2	0
Hematuria (n = 10)	4	5	1	0

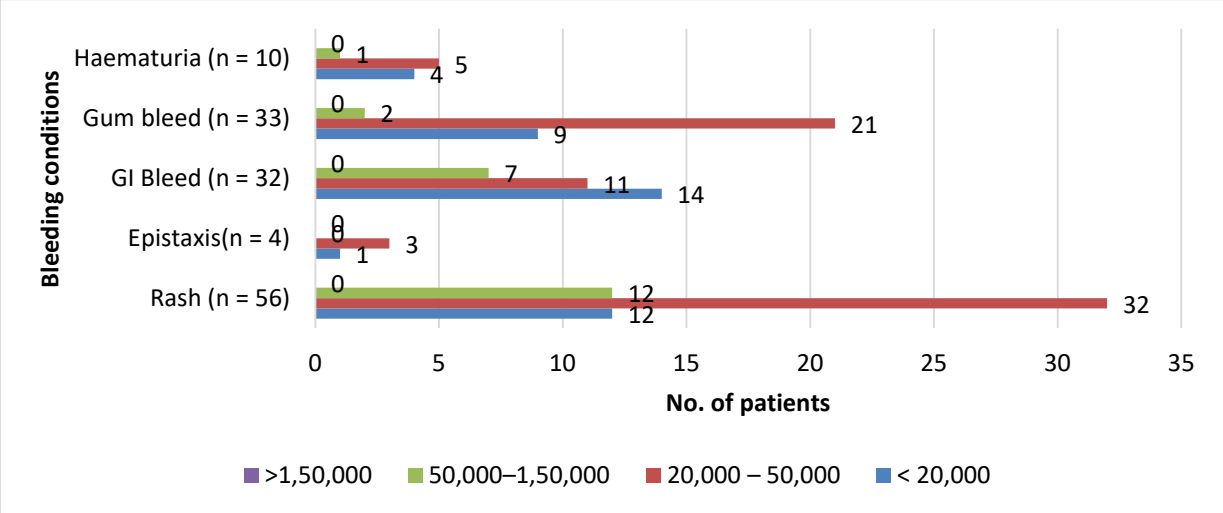

Fig-3: Distribution of patients according to severity of platelet count and various bleeding conditions

DISCUSSION:

We conducted a prospective study to correlate the level of thrombocytopenia with severity of dengue seropositive patients and variability in bleeding pattern. In the present study, various bleeding manifestations were observed in association with dengue infection. In this study, we found thrombocytopenia was seen in 86.31% of patients which was comparable to the study of Cherian T *et al.;* [16] i.e., 94.7%, Ageep *et al.;* [17], i.e., 81.4% and Butt N *et al.;* [18] i.e., 100%. It was seen because dengue viruses may directly interact with platelets and activate them. Dengue virus exposure could lead to inhibition of collagen-induced aggregation properties of platelets implicates the possible role of collagen receptors in virus-platelet binding or appears to occur because of complement activation. Low platelet count has been the hallmark for dengue virus infection and could vary widely in each case. Lower platelet value at admission had an association with more severe dengue spectrum [14]. Platelet count <50.000/mm^3 had a risk of DSS two times higher [15].

100 out of 135 i.e. 67% patients of dengue fever, 118 (120) Dengue hemorrhagic fever and 7 (8) dengue shock syndrome patients were thrombocytopenic (platelet count < 1, 50,000 per mm^3). In DHF & DSS, 55.8% & 75% patients had platelet counts in between 20,000 – 50,000 mm^3 respectively. 18.33% of DHF had < 20,000 mm^3. Among DF patients majority of patients i.e. 40% had platelet count in between 20,000 – 50,000 mm^3 and 35% had between 50,000 – 1,50,000 mm^3. It indicates that tendency of thrombocytopenia increases with clinical severity of

dengue fever but moderate thrombocytopenia may also be seen in non severe dengue infection.

Bleeding manifestation was also investigated in dengue patients to evaluate its association with the severity of thrombocytopenia. Over all petechiae (27.4%) was the most common bleeding manifestation. In DHF Petechiae (46.67%) was the most common bleeding manifestation which is similar to the study by Singh NP *et al*[19], and Khan E *et al.;* [3]. In DHF Most common orifice bleeding was gum bleeding (27.3%) followed by GI bleed (26.4%). In DSS most common orifice bleeding was GI bleed (25.0%). In a study conducted by Singh NP et al [19], gum bleeding, hematemesis and melena were observed in 40%, 22% and 14% cases, respectively. It was seen that majority of patients (57%) with petechiae had platelet count in between 20,000 – 50,000 (per mm3). 21(33) patients of DHF with gum bleeding had platelet count in between 20,000 – 50,000 mm3. Most of the GI bleed 14 out of 32 had platelet count < 20,000 mm3. Hematuria is seen mostly when platelet count is < 50,000. It was seen that major number of patients with mucosal bleeding had platelet count in moderate range than severe thrombocytopenia.

CONCLUSION:

We found that the tendency of thrombocytopenia increase with clinical severity of dengue infection and even moderate thrombocytopenia (20,000 – 50,000 mm^3) can also be seen among classical dengue fever. Frequency of bleeding pattern was not correlated with severity of thrombocytopenia as majority of cases were in moderate range thrombocytopenia.

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