

Full Length Research Paper

Incidence of thrombocytopenia in seropositive dengue patients

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Dengue has become a major health concern globally in recent decades. Dengue infected patients manifest a spectrum of symptoms and in severe cases the fate is mortality. A hallmark of dengue infection is thrombocytopenia which causes concern for the patients and treating doctors. This study aimed to evaluate the incidence of thrombocytopenia in seropositive dengue patients. Bleeding manifestation was also investigated in dengue patients to evaluate its association with the severity of thrombocytopenia. In this study, 750 individuals were screened for dengue infection by detecting immunoglobulin M (IgM) against dengue virus in their serum. Enzyme-linked immunosorbent assay (ELISA) was performed for detection of IgM antibody and 250 individuals were found to be seropositive. Platelet counts were performed on whole blood of seropositive patients using Sysmex XE-5000 Automated Hematology Analyzer. Among 250 dengue patients, 2% had severe thrombocytopenia, 65.2% were found to have mild to moderate thrombocytopenia and 32.8% had normal platelet counts. Bleeding was related to the severity of thrombocytopenia as 80% of patients having platelet count lower than 25000/ μ l showed bleeding manifestations.

Key words: Dengue, thrombocytopenia, seropositive, immunoglobulin M (IgM), enzyme-linked immunosorbent assay (ELISA), platelet count.

INTRODUCTION

Dengue viral infection is currently amongst the most critical arthropod-borne infections from the public health view point. Concerning the incidence of dengue all over the world, the graph has risen up noticeably in recent decades and over 40% of the world's population is now at risk from dengue. It has been estimated that there may be 50 to 100 million dengue infections globally per year (World Health Organization (WHO), 2013).

Four distinct serotypes of dengue virus are known to

cause the disease (DEN-1, DEN-2, DEN-3 and DEN-4) and *Aedes aegypti* mosquito is the primary vector. Recovery from infection by one serotype offers lasting immunity against that particular serotype, but subsequent infections by other serotypes increase the risk of developing severe dengue (Centers for Disease Control and Prevention (CDC), 2000).

Dengue fever is a severe, flu-like sickness in which high grade fever (104°F) is accompanied by severe

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headache, pain behind the eyes, joint pains, vomiting and rashes on body, but is rarely fatal. However, severe dengue (previously referred as Dengue Haemorrhagic Fever) is a potentially lethal complication characterized by plasma leaking, fluid accumulation, severe bleeding, or organ impairment (WHO, 2013).

In Pakistan, dengue has been around for the past 20 years. The first major outbreak in Pakistan was reported in 1994 to 1995. During 2005 to 2006, there was an unexpected spread of virus in the country. The recent (2011 to 2012) wave of dengue fever hitting Pakistan's eastern province of Punjab killed at least 365 people and 21597 cases of dengue fever have been reported, making it the world's biggest epidemic of DF ever (Shakoor et al., 2012).

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 (Dacie and Lewis, 2006). Thrombocytopenia is a common problem in dengue, which causes concern for the patients and treating doctors (Halstead, 2007). With the advances in medical research, it is now evident that activation of immune process and direct marrow suppression by the viral particles is responsible for decline in platelets (Gubler, 2002). 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 (Cines and McMillan, 2005).

Thrombocytopenia in dengue infection raises concerns about bleeding risk. The aim of this study was to assess the incidence of thrombocytopenia in seropositive dengue patients. Bleeding manifestation was also inspected in seropositive dengue patients to evaluate its association with the severity of thrombocytopenia

MATERIALS AND METHODS

Sample collection

Samples were taken from 750 individuals who presented to Excel Diagnostic Laboratory, Islamabad for serological screening test of dengue from 1st of September 2011 to 15th of January, 2012. Patient having any hematological malignancy (which may interfere with platelet counts) or bleeding disorders such as VWB disease were excluded from the study. After informed consent and written performa was taken from each patient, 2 ml of blood was drawn into gel vial for serum separation and another 2 ml was drawn into an ethylenediaminetetraacetic acid (EDTA)-filled tube for platelet count. Blood tubes were transported on ice within 2 h to the Biochemistry Department of ARID Agriculture University, Rawalpindi. Samples were processed within 6 h of the initial sample collection.

Serological screening of dengue infection

The Calbiotech Dengue virus IgM ELISA Kit (Catalog #DE051M) was used for the detection of IgM antibody to dengue virus in serum samples. IgM ELISA was performed according to the protocol given

by the manufacturer. In brief, 100 µl of patient serum was added to wells in microtitre plate coated with purified antigen. An incubation of 20 min was given to allow specific IgM, if present in patient sample, to bind to the coated antigen. Microtitre plate was then washed to remove all unbound materials and 100 µl of the enzyme conjugate was added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate was washed off and 100 µl of substrate was added. The plate was then incubated to allow the hydrolysis of the substrate by the enzyme. The optical density at 450 nm was read using ELISA reader.

Platelet counts

Platelet counts were performed on whole blood of those individuals who were found seropositive for dengue infection. Platelet counts were performed using Sysmex XE-5000 Automated Hematology Analyzer.

Statistical analysis

The data was entered and analyzed by using IBM SPSS Statistics version 20 (IBM Corp, Armonk, NY). Frequency and percentages were calculated for qualitative variables like IgM positivity, age group, platelets counts groups and bleeding manifestation were expressed. Pearson Chi square and Fisher's exact tests were used to observe the association between qualitative variables. A p-value ≤ 0.05 was considered as statistical significant.

RESULTS

In this study, 750 individuals were screened for dengue infection and 250 of them were found to be seropositive. Amongst 250 seropositive patients, 155 were males and 95 were females. They were divided into 3 age groups. Age group of less than 15 years contained 101 patients, whereas 92 of them were between 15 and 50 years and 57 were above 50 years of age. The data obtained from the study shows that dengue is more prevalent in children (p-value 0.002) (Figure 1).

In this study, out of 250 seropositive cases, 82 patients show platelet counts within normal range. In 5 patients, platelet count was found to be severely low, that is, <25,000/µl. In 106 patients, platelet count was found to be moderately low, that is, 25,000/µl to 100,000/µl. 57 cases were borderline, that is, having platelet counts of 100,000/µl to 150,000/µl (Table1). There was no statistically significant association found between gender and platelet group (p-value 0.875), or age and platelet group (p-value 0.960).

Bleeding manifestations were equally common in both genders. There was no predilection for any age group among the patients who developed bleeding manifestations. Bleeding was significantly related to thrombocytopenia (Table2).

DISCUSSION

Dengue fever outbreaks have become a cyclical nightmare

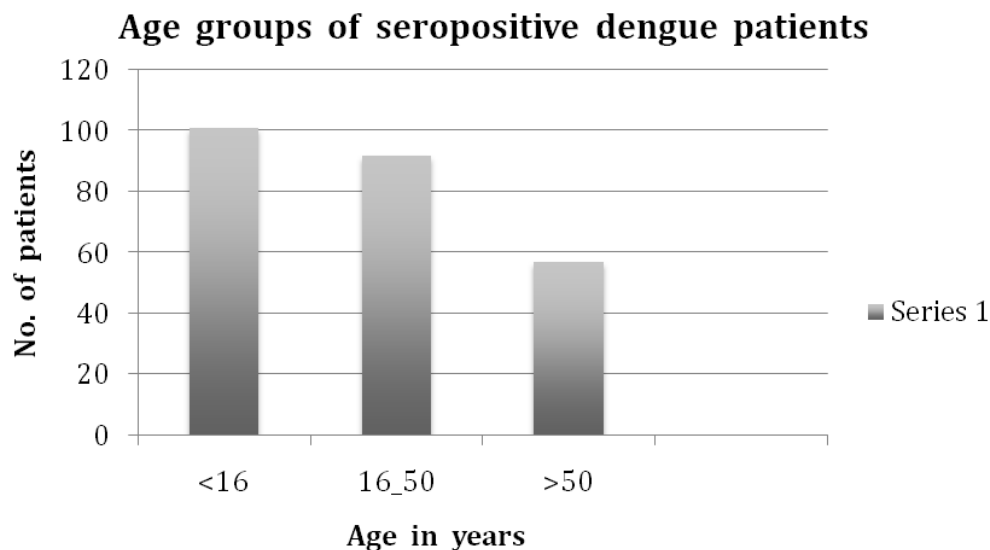


Figure 1. Age groups of patients.

Table 1. Association of gender and age groups with platelet counts.

Characteristic	Group	Platelet count				Total	p-value
		Normal (%)	Border line (%)	Moderately low (%)	Severely low (%)		
Gender	Male	53 (34.2)	33 (21.3)	66 (42.6)	3 (1.9)	155	0.875
	Female	29 (30.5)	24 (25.3)	40 (42.1)	2 (2.1)		
Age	≤15	37 (36.6)	22(21.8)	40 (39.6)	2 (2.0)	101	0.960
	16-50	29 (31.9)	21(23.1)	39 (42.9)	2 (2.2)	91	
	>50	16 (27.6)	14(24.1)	27 (46.6)	1 (1.7)	58	
Total	n (%)	82 (32.8)	57 (22.8)	106(42.4)	5 (2.0)	250	

Table 2. Association of bleeding manifestation with gender, age and platelet counts.

Characteristic	Group	Bleeding manifestation		p-value
		Present (%)	Absent (%)	
Gender	Male	18 (11.6)	137 (88.4)	0.597
	Female	9 (9.5)	86 (90.5)	
Age	≤15	14 (13.9)	87 (86.1)	0.124
	16-50	5 (5.5)	86 (94.5)	
	>50	8 (13.8)	50 (86.2)	
Platelet counts	Normal	0	82 (100)	< 0.001
	Borderline	0	57 (100)	
	Moderately low	83 (78.3)	23 (21.7)	
	Severely low	4 (80)	1 (20)	

in Pakistan for the last several years. This study showed that the majority of dengue positive patients were children, that is, in accordance with a study conducted in

Indonesia (Chairulfatah et al., 2003) and another study conducted in India (Narayanan et al., 2003).

Thrombocytopenia is common in dengue infection. Severe

bleeding is related to severe thrombocytopenia. Out of 250 dengue patients in this study, 32.8% had normal platelet counts, whereas 2% had severe thrombocytopenia and the remaining 65.2% were found to have mild to moderate thrombocytopenia. These results are similar to the findings of Narayanan et al. (2003), who reported that in the serologically-confirmed dengue cases, the prevalence of thrombocytopenia was 58% on admission and 83% during hospitalization. The results of this study also correlate with the findings of Sumarmo (1983).

Bleeding was significantly related to the severity of thrombocytopenia as 80% of patients having platelet count lower than 25000/ μ l showed bleeding manifestations and 23% of patients showing moderately low platelets also manifested bleeding. There was no predilection for any age group or gender for thrombocytopenia or bleeding among the dengue patients. These results are in accordance with a study conducted in India by Narayanan et al. (2003).

During an epidemic, people of effected community should be screened serologically for dengue infection. If positive, people may keep themselves within a safe zone of platelet count by proper care, therapy and management since all serologically positive cases do not develop thrombocytopenia as seen in current study as well.

Patients with significantly low levels of platelets bleed and may need platelet transfusion. Since neither cure nor vaccine exists for dengue fever, prevention is the only option to control the human and economic cost of the epidemic.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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