

Full Length Research Paper

Thrombocytopenia and bleeding manifestations among patients with dengue virus infection in Port Sudan, Red Sea State of Sudan

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Dengue is a global endemic and most prevalent human arbovirus disease. This study aimed at evaluating thrombocytopenia and to find out platelet activation in dengue across the spectrum of the disease. A prospective study was conducted from February, 2013 to June, 2014 at Port Sudan Teaching Hospital, Red Sea State, Sudan. 334 cases of dengue caused by dengue virus along with 101 cases of control were enrolled. Laboratory-positive dengue cases were confirmed by immune chromatography and World Health Organization (WHO) criteria were used for classifying the dengue severity. Platelet counts (PLT) and platelet distribution width (PDW) were measured. Out of 334, 289 patients had dengue fever (DF) and 45 patients had dengue haemorrhagic fever (DHF). Thrombocytopenia was present in 234 (81%) DF cases and 45 (100%) DHF cases. Bleeding manifestation was seen in 35 (10.5%) patients. Haematuria (5.7%), haematemesis (0.3%), haemoptysis (0.3%), epistaxis (1.5%), and gum bleed (2.7%) are the common forms of bleeding. PLT and PDW were found to be significantly low and high in case of dengue ($P < 0.000$), respectively. Thrombocytopenia (low PLT) and high PDW shows considerable sensitivity for dengue virus infection.

Key words: Dengue, thrombocytopenia, bleeding manifestations, PDW, Port Sudan.

INTRODUCTION

Dengue is a member of the Flavivirus family, which includes yellow fever and various other mosquito and tick

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borne encephalitides (Cifu, 2008). It presents as four antigenically distinct serotypes (DENV1-4) (Khan et al., 2007). In recent years, dengue fever (DF) has become a major international health issue affecting tropical and sub-tropical regions around the world - especially urban and peri-urban areas (World Health Organization (WHO), 2012). The virus infects approximately 100 million people each year (Khan et al., 2007). Infection by the dengue virus is transmitted through the bite of the *Aedes aegypti* mosquito (Avila-Aguero et al., 2004). In the last years, Port Sudan faced many outbreaks, one was reported in 2005. The dengue virus (DENV) serotypes DENV1 and DENV2 were first reported in 1986 in Port Sudan (Hyams et al., 1986), while DENV-3 was recently identified in an outbreak (Malik et al., 2011). Since then, Port Sudan has become an endemic area with dengue virus (Ali and Abu, 2012). Thrombocytopenia is common in DF and constant finding in DHF/DSS. The pathogenesis of thrombocytopenia is poorly understood (Schexneider and Reedy, 2005; Lin et al., 2001; Huan et al., 2001).

The platelet counts usually drops to below $100,000/\text{mm}^3$ 1 to 2 days before defervescence and remain low for 3 to 5 days in most cases. The levels then increased rapidly to normal during convalescence. The platelet counts in shock cases are frequently below $50,000/\text{mm}^3$. Although the mean value of platelets in shock cases is around $20,000/\text{mm}^3$, this is not always associated with severe bleeding (Jameel et al., 2012; Nimmannitya, 1999). It was suggested that dengue-virus-induced bone marrow suppression depressed platelet synthesis and resulted in thrombocytopenia (Huan et al., 2001). Also found that the dengue-2 virus can bind to human platelets in the presence of virus-specific antibodies, and proposed that the immune-mediated clearance of platelets was involved in the pathogenesis of thrombocytopenia in DHF/DSS (Noisakran et al., 2008; Sun et al., 2007; Huan et al., 2001; Nimmannitya, 1999).

Surprisingly, we found IgM but no IgG anti-platelet autoantibodies in dengue patients. The titer of IgM anti-platelet antibodies is higher in DHF/DSS than in DF patients and was detectable even 8 to 9 months after convalescence. The presence of these autoantibodies not only induces platelet lysis via complement activation, but also inhibits adenosine diphosphate (ADP)-induced platelet aggregation (Chuansumrit and Chaiyaratana 2014; Lin et al., 2001; Huan et al., 2001). Preexisting dengue antibodies augment the binding of the virus to the platelet and destroyed the platelet by activating the complement on their surface. Thus, activation of platelets and its adherence to infected and damaged endothelium provides an important mechanism of removal of platelet from circulation without necessarily activating coagulation cascade (Saxena et al., 2011). Suggesting that factors in DHF/DSS sera lead to enhanced clearance of platelet by macrophage or phagocytes and complement-mediated lysis.

Clinical complication of thrombocytopenia

1. Platelet counts of $> 100,000/\mu\text{l}$ are usually asymptomatic and bleeding time (BT) remains normal.
2. Platelet count of $50,000/\mu\text{l}$ to $100,000/\mu\text{l}$ causes mild prolongation of the BT, bleeding occurs only after severe trauma.
3. Platelet count $< 50,000/\mu\text{l}$ has easy bruising, manifested by skin purpura after minor trauma. Platelet count of $< 20,000/\mu\text{l}$ have spontaneous bleeding, they usually have petechiae, and may have intracranial or spontaneous internal bleeding (Handian, 2001; Frankin et al., 1996).

Recently, a novel platelet index such as platelet distribution width (PDW) has been investigated as prospective platelet activation markers. The marker of platelet function and activity is measured as PDW by hematology analyzers. Platelets with increased number and size of pseudopodia differ in size, possibly affecting PDW which increases during platelet activation (Vagdatli et al., 2010). Dengue have been reported in different regions of the Sudan, which is the largest African country, including the study area Port Sudan (Ali et al., 2006). The most common hemorrhagic manifestations are mild and include a positive tourniquet test, skin hemorrhages (petechiae, hematomas), epistaxis (nose bleed), gingival bleeding (gum bleed), and microscopic hematuria. More serious types of hemorrhage include vaginal bleeding, hematemesis, melena and intracranial bleeding (Nimmannitya, 1999; Orsi et al., 2013). The present study was designed to find out variation in platelet parameters (PLT and PDW) and showed the bleeding manifestations.

MATERIALS AND METHODS

The present study was conducted prospectively for a period from February, 2013 to June, 2014 during the recent outbreak of dengue in Port Sudan teaching hospital, Red Sea State, Sudan. This study consisted of three hundred thirty four randomly selected patients positive with dengue infection. The inclusion criteria were all patients with clinical features and serologically positive dengue infection included. The exclusion criteria were patients with serologically negative dengue and if routine laboratory testing suggested a bacterial, parasite or any viral infection other than dengue infection or any other disease. Hundred and one, apparently healthy normal individuals with no clinical sign for dengue infection were selected randomly to be the control group. Blood samples were collected from all of the studied population. About 3 ml of blood was transferred to tri potassium ethylene diamine tetra acetic acid (EDTA) tube. Platelet count (PLT) and platelet distribution width (PDW) were done using semi automated hematology analyzer (Sysmex KX-21N, B 7151, and MF 9/2008). The specimens were analyzed within 1 h from venesection to avoid the problems occurring when EDTA collected samples are analyzed.

Criteria for dengue severity

Patients were classified as dengue fever, dengue hemorrhagic

fever or dengue shock syndrome according to WHO guidelines and laboratory diagnosis of dengue was established by demonstration of IgM and IgG immune chromatographic rapid strip test (BioTracer/BioFocus, REF: 17112, Exp.12/2015, Korea) sensitivity 95.6 and specificity 96.

Statistical analysis

Measurements of laboratory data platelet count and PDW of patients with DF, DHF were statistically tested by comparing mean and Chi-square test which ever was appropriate. A *p-value* less than 0.05 were considered statistically significant. The statistical package for social sciences (SPSS version 20.0, IBN. Chicago, USA) was used for data analysis.

Ethical considerations

This study was approved by the regional Ethical Review Committee (ERC) and written informed consent was obtained from all the patients.

RESULTS

This is a case control analytical study conducted in Port Sudan Teaching Hospital, Red Sea State, Sudan. The total number of the confirmed diagnosed dengue patients was 334. The age of the patients in this study was between 3 to 80 years (mean age 30 years). The control individual aged between 6 to 76 years (mean age 22 years). Of the 334 clinical patients, (217) 65% were males and (117) 35% were females. In control group, (64) 63.4% were males and (37) 36.6% were females. Table 1 shows the comparison of different characteristics between patients and controls. It shows that the Eastern part of the study area (Selalab) represented the highest incidence (27.2%) region affected by dengue virus infection and the students were the most common segment of occupation affected (34.4%), followed by traders (19.5%), and the house wife (18.0%). Table 1 illustrates that the overwhelming majority of dengue virus infection is among the Northern Sudan tribe (43.1%), followed by the Hadandwa tribe (21%), Bani amer tribe (18.3%), Western Sudan tribe (14.7%), and the immigrants tribe (3%). The clinical demographic manifestations of the studied were fever (334,100%), headache (282, 84.4%), joint pain (262, 78.2%), backache (198, 59.3%), myalgia (156, 46.7%), retro-orbital pain (69, 20.7%), and rash (28, 8.4%). According to the WHO classification system most of cases in the current study are dengue fever, followed by dengue haemorrhagic fever I (Table 1).

The difference between the patient group and the control group were found to be significant in platelet count and PDW. Platelet count was lower in the patient group than in the control group ($P < 0.000$). PDW was

found to be significant higher in patient group ($P < 0.000$) (Table 2). Thrombocytopenia was present in 234(81%) DF cases, 45(100%) DHF cases (Table 3). Severe thrombocytopenia was found more severe with bleeding (*P*.value 0.000) (Table 4). Bleeding manifestations were observed high in DHF than in DF patients (*P*.value 0.000) (Table 3). Severe bleeding was recorded in 35 (10.5%) cases of all dengue patients. Bleeding manifestations included haematuria in 19(54.3%) cases, haematemesis in 1 (2.9%), haemoptysis in 1 (2.9%), epistaxis in 5 (14.2%) cases, and gum bleed in 9 (25.7%) cases (Table 5). A high PDW which indicates as useful marker for platelet activation was seen in 28% of patients with DF and 26.7% in DHF patients (Table 6). Table 7 shows that low platelet and high PDW are comparatively more sensitive, specific, and positive predictive value for DF than DHF.

DISCUSSION

Dengue fever is one of the major public health problems in the studied area (Ali and Abu, 2012). The defects in DF/DHF are multifactorial mechanisms that include thrombopathy, coagulopathy and vasculopathy (Huang et al., 2001). Thrombopathy means the thrombocytopenia and platelet dysfunction. Thrombocytopenia is common in dengue fever, and is a constant finding in DHF/DSS (Gubler, 1998). The present study showed thrombocytopenia, and it was strongly associated with bleeding (*P*.value 0.000); raising the hypothesis that thrombocytopenia is not only a laboratory changing in DF but also an important cause of bleeding. Many factors can contribute to the onset of thrombocytopenia in DF from a reactive immune response against platelets to decreased platelet production (Wang et al., 1995; Saito et al., 2004). However, in this outbreak the majority of patients (279/334; 83.5%) had thrombocytopenia a finding similar to (Karoli et al., 2012; Khan et al., 2011; Chairulfatah et al, 2003; WHO, 1997; Sugianto et al., 1994; Natth, 1993). The depression in the bone marrow observed in dengue fever in the acute stage may account for thrombocytopenia. In addition, direct infection of megakaryocytes by dengue virus could lead to an increased destruction of platelet cells (Saito et al., 2004). The activation of the platelets causes some morphological alterations: the activated platelets seem larger by becoming spherical in shape and forming pseudopodia. As a result, platelets with enhanced number and size of pseudopodia will be different in size leading alterations in PDW (Vagdatli et al., 2010). In this study, PDW levels were higher in dengue fever patients compared to controls. This increase may be used to predict the disease. In addition, our findings was consistent with Gunawan et al. (2010).

Table 1. Characteristics of patients and control in the study.

Characteristics	Patients (n = 334)	Control (n = 101)	P-value
Age			
Mean \pm SD	30 \pm 15	22 \pm 6	0.000
Range	3 – 80 y	6 – 76 y	
Sex			
Male	217 (65%)	64 (63.4%)	0.726
Female	117 (35%)	37 (36.6%)	
Clinical diagnosis			
Dengue fever	289 (86.5%)	-	-
DHF grade I	31 (9.3%)	-	-
DHF grade II	12 (3.6%)	-	-
DHF grade III	2 (0.6%)	-	-
Clinical manifestations			
Rash (Purpura)	28 (8.4%)	-	0.003
Fever	334 (100%)	-	0.000
Joint pain	262 (78.4%)	-	0.000
Backache	198 (59.3%)	-	0.000
Headache	282 (84.4%)	-	0.000
Myalgia	156 (46.7%)	-	0.000
Retro-orbital pain	69 (20.7%)	-	0.000
Bleeding manifestations			
Epistaxis	5 (1.5%)	-	0.000
Haematemesis	1 (0.3%)	-	
Haemoptysis	1 (0.3%)	-	
Hematuria	19 (5.7%)	-	
Bleeding gum	9 (2.7%)	-	
Demographic data			
Residence			
Selalab	91 (27.2%)	23 (22.8%)	0.726
Diem alnour	44 (13.2%)	15 (14.9%)	
Al-thorat	62 (18.6%)	31 (30.7%)	
Al-diom alganobia	75 (22.5%)	17 (16.8%)	
Downtown	62 (18.6%)	15 (14.9%)	
Tribe			
Hadandwa	70 (21%)	13 (12.9%)	0.056
Bani amer	61 (18.3%)	13 (12.9%)	
Northern Sudan	144 (43.1%)	57 (56.4%)	
Western Sudan	49 (14.7%)	14 (13.9%)	
Immigrants	10 (3%)	4 (4%)	
Occupation			
Students	115 (34.4%)	40 (39.6%)	0.000

Table 1. Cont'd.

Traders	65 (19.5%)	21 (20.8%)	0.000
House wife	60 (18%)	14 (14.9%)	
Other jobs	94 (28.1%)	26 (25.7%)	

Table 2. The difference between test and control in studied parameters.

Parameters	Test group Mean \pm SD	Control group Mean \pm SD	Median test	Median control	Range test	Range control	P-value
Platelet count $\times 10^9/L$	95691 \pm 57	219099 \pm 59	95	214	3 - 443	93- 509	0.000
PDW	15.56 \pm 11	15.07 \pm 10.1	14.2	14	8.1-23	10-11.4	0.000

Table 3. Thrombocytopenia and bleeding among DF and DHF patients.

Parameter	DF n = 289	DHF n = 45	P-value
Thrombocytopenia	234 (81%)	45 (100%)	0.000
Bleeding manifest	10 (3.5%)	25 (55.6%)	0.000

Table 4. Association of bleeding with thrombocytopenia.

Platelet count $\times 10^9/L$	Patients without bleeding	Patients with bleeding	No. of patients
< 25.000	7 (29.2%)	17 (70.8%)	24 (7.2%)
25 - 50.000	47 (87%)	7 (13%)	54 (16.2%)
50 - 100.000	109 (94.8%)	6 (5.2%)	115 (34.4%)
> 100.000	136 (96.4%)	5 (3.6%)	141 (42.2%)
Total	299 (89.5%)	35 (10.5%)	334 (100%)

Table 5. Bleeding manifestations among thrombocytopenic patients.

Platelet count	Haematuria	Haematamesis	Haemoptysis	Epistaxis	Gum bleed	Total
<25.000	10 (58.8%)	1 (5.9%)	1 (5.9%)	2 (11.8%)	3 (17.6%)	17 (48.6%)
25 - 50.000	5 (71.4%)	0	0	1 (14.3%)	1 (14.3%)	7 (20%)
50 - 100.000	3 (50%)	0	0	0	3 (50%)	6 (17.2%)
>100.000	1 (20%)	0	0	2 (40%)	2 (40%)	5 (14.2%)
Total	19 (54.2%)	1 (2.9%)	1 (2.9%)	5 (14.3%)	9 (25.7%)	35 (100%)

Bleeding complication is the common complication reported in patients with dengue fever. Bleeding manife-

stations is highly variable, so factor like thrombocytopenia can synergistically cause bleeding in a dengue patient.

Table 6. Platelet distribution width (PDW) in the case group of the study.

Diagnosis	Low PDW	High PDW	Normal PDW	Total
DF	13 (4.5%)	81 (28%)	195 (67.5%)	289 (86.5%)
DHF	2 (4.4%)	12 (26.7%)	31 (68.9%)	45 (13.5%)
Total	15 (4.5%)	93 (27.8%)	226 (67.7%)	334 (100%)

Table 7. Statistical analysis of Platelet and PDW for diagnosis of Dengue fever (DF) and Dengue hemorrhagic fever (DHF).

Parameters	Sensitivity (%)	Specificity (%)	PPV	NPP	LR	95%CI
Low PLT						
DF	100	55	84	100	2.22	1.79-2.76
DHF	100	19	16	100	1.24	1.17-1.31
PDW >13fl						
DF	86	94	87	94	14.86	8.53-25.90
DHF	48	71	13	94	1.64	1.05-2.56

PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR, Likelihood Ratio; CI, Confidence Interval; PLT, Platelet Count; MPV, Mean Platelet Volume; PDW, Platelet Distribution Width

Severe bleeding is related to severe thrombocytopenia. In the present study, bleeding manifestations were present in 35 (10.5%) patients in which hematuria was the most common symptoms. Hematuria, bleeding gum and epistaxis were more commonly associated with 55.6% DHF patients during the acute phase of the illness in this study. These findings are also positively correlated with Shivbalan et al. (2004), Orsi et al. (2013), Ayyub et al. (2006), Sung et al. (2003) and Chairulfatah et al. (2003). Our results have demonstrated not only that cases of DF/DHF can manifest with bleeding, but also may be that these cases may be accompanied by changes in peripheral blood counts, coagulation and fibrinolysis that perhaps are not significantly detected in DF without this complication.

Conclusion

Dengue fever is an endemic disease in Port Sudan, Red Sea State, Sudan. Hemorrhage and thrombocytopenia induced by DENV infection may involve both viral factors and host factors. Viral factors such as virus infection. Host factors such as cytokines and autoantibodies induced DENV may also play a role in the disruption of the balance of coagulation and fibrinolysis as well as in the function of endothelial cells and platelets. Low PLT and high PDW may be used as probable indicators for

dengue in endemic area. Thrombocytopenia (low PLT) and high PDW shows considerable sensitivity for dengue virus infection. Moreover, understanding the bleeding disorders associated with dengue could lead to a new diagnosis in DF, since until recently, bleeding manifestations had been studied mainly in patients with DHF.

Conflicts of interest

Authors have none to declare.

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