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The Distinct Pattern of DIC among the Patients with Dengue Virus Infection, Red Sea State, Sudan

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Abstract: ***The clinical spectrum of dengue fever (DF) varies from acute febrile course to refractory shock and bleeding with increase mortality. Disseminated intravascular coagulation (DIC) is the most serious mechanism involved in the pathogenesis of bleeding. A case control study was conducted to detect the DIC in dengue infection in Red Sea State. Platelet count, Coagulation tests and D-Dimer were sequentially measured. Out of 334 patients with dengue virus infection, 101(30.2%) had DIC. DIC was significantly higher in DF 81(80.2%) than in dengue hemorrhagic fever (DHF) 20(19.8%) of patients. 56.4% diagnosed as non-overt DIC, and 43.6% diagnosed as overt DIC. Nearly one-third of patients had DIC. Non-overt DIC was the distinct pattern present in the study. D-Dimer is a marker for DIC may predict the clinical course of the disease.***

Keyword: DIC, D-Dimer, dengue, Port Sudan

1. Introduction

Dengue infection is a global concern as it is an expanding public health problem in the tropical and subtropical world. Reports suggest 2.5 billion people are at risk for dengue with 50 - 100 million dengue virus infections each year and more than 25,000 reported deaths annually [1]. In recent years, dengue fever (DF) has become a major international health issue [2]. 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 the 1986 in Port Sudan [3], while DENV3 was recently identified in an outbreak [4]. Since then, frequent outbreaks reappear in our studied area [5]. Dengue virus is a mosquito-borne Flavivirus that is transmitted by mosquitoes such as *Aedes aegypti* or *Aedes albopictus*. Based on the antigenic difference, DENV can be divided into four different serotypes, DENV 1 – 4. DENV might lead to an influenza-like illness, which is called dengue fever or cause more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF is a severe febrile disease characterized by abnormalities in hemostasis and increased capillary leakage that can progress to blood pressure decrease, and hypovolemic shock (DSS)[6]. Mechanisms of bleeding in dengue infection are vasculopathy, thrombocytopenia, coagulopathy, and disseminated intravascular coagulation (DIC). The coagulation fibrinolysis system appears to be abnormal during infection manifesting as decreased fibrinogen levels, increase levels of fibrin degradation products (FDP), prolonged partial thromboplastin time, low levels of coagulation factors VIII and XII [7]. The presence of the D-dimer (DD) indicated activation of the coagulation system resulting from the destruction of cross-linked fibrin and reflects clot formation and lysis [8,9]. Thus the D-dimer assay, a specific marker for cross-linked fibrin, is often used as a marker for DIC [10]. The diagnosis of DIC should take into account both the clinical presentation as well as laboratory findings. It is important to appreciate that DIC is a syndrome that is always secondary to another underlying pathological condition and that there is no single diagnostic laboratory test for DIC [11]. DIC is associated with an acquired deficiency of naturally occurring anticoagulants, particularly antithrombin (III) and protein C [12]. A diagnostic scoring using widely available coagulation tests has been proposed by the DIC Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis (ISTH). The design of this scoring system has a pathophysiologic basis, incorporating the concept of “overt” (decompensated) and “non-overt” (compensated) DIC [11]. This study focused on coagulation disorders as indicator for severity of dengue infection. The aim of this study is to detect the DIC in patients with dengue virus infection.

2. Material and Method

This 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 patients positive for dengue infection. The inclusion criteria were all patients with clinical features and serologically positive dengue infection. No hemostatic agents were administrated to the patients. The exclusion criteria include patients’ serologically negative dengue and if routine laboratory testing suggested a bacterial, parasite or any viral infection other than dengue virus or any other known disease. Hundred and one, apparently healthy normal individuals with no any clinical sign for dengue infection were selected randomly to be the control group. Blood sample were collected from all of the studied population. About 3 ml blood was placed in potassium ethylene diamine tetra acetic acid (EDTA), 3 ml in citrated buffer. Platelet count was done using automated hematology analyzer (Sysmex KX-21N, B 7151, and MF 9/2008). Coagulation tests were examined within 4 hours of collection using

a semi-automated blood coagulation analyzer (bio bas-1 manufactured by RAL for SPINREACT, SN 536, Spain-European Community). 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. Dengue virus infection was the underlying disease referred to the DIC scoring system for this study. The DIC scoring system used is shown in Table 1and 2 adopted from Taylor FB et al [11]. The DIC scoring system evaluates the following parameters: the underlying disease, platelet count, fibrinogen, prothrombin time, partial thromboplastin time, protein C, antithrombin and fibrin degradation product (FDP). Reagents of (Tulip diagnostic, India) were used in the established of laboratory coagulation tests. FDP was determined by NycoCard® method using NycoCard®READER II (SN 67498, Axis-Shield PoC AS, Oslo, Norway).

2.1 Statistical Analysis

Differences in laboratory data between patients with DF, DHF and DIC scores were tested by compare 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 20.0 version, IBN. Chicago, USA) was used for data analysis.

2.2 Ethical Considerations

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

Table 1: Diagnostic scoring system for overt DIC

Items	Test result	Score
Platelet count	> 100 x 10^9/l	0
	50 – 100 x	1
	< 50 x 10^9/l	2
FDP	No increase	0
	Moderate	1
	Strong increase	2
Prolonged PT	< 3s	0
	3 – 6s	1
	> 6s	2
Fibrinogen level	> 1.0 g/l	0
	< 1.0 g/l	1
If score ≥ 5 compatible with overt DIC		

Table 2: Diagnostic scoring system for non-overt DIC

Criteria	Test result	Score
Underlying disease	Yes	2
	No	0
Platelet count	> 100 x 10^9/l	0
	< 100 x 10^9/l	1
PT	< 3s	0
	> 3s	1
FDP	Normal	0
	Raised	1
Antithrombin	Normal	1
	Low	1
Protein C	Normal	1
	Low	1
Total score		

3. Result

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 – 80 years (mean age 30 years). 101 individuals, age and sex matched, were selected as control group. The control individual aged between 6 – 76 years (mean age 22 years). Of the 334 clinical patients, (217) 65% were males and (117) 35% were female. In control group, (64) 63.4% were males and (37) 36.6% were females. Demographics data were obtained from patients with dengue virus infection include residence, tribe, and occupation. The eastern part of the study area (Selalab) represented the highest incidence (27.2%) region affected by dengue virus infection. The student was the most common segment of occupation affected (34.74%), followed by traders (18.73%), and the house wife (18.13%) (Figure 1). 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%).Out of 334 subjects confirmed positive dengue virus infection, 101 (30.2%) had disseminated intravascular coagulation (Table 3). 44(43.6%) diagnosed as overt DIC (classic) score ≥ 5, and 57(56.4%) diagnosed as non-overt DIC score ≥ 6 (Table 4). The non-overt DIC was significant higher in the patients of the study (Table 5). In our study, the DIC patients (101), 93 (92.1%) had thrombocytopenia; an altered coagulation profile prolonged prothrombin time (PT) in 30 (29.7%); prolonged partial thromboplastin time (PTT) found in 42 (41.6%); reduced fibrinogen (FB) found in 61 (60.3%), normal FB in 9(8.9%) and high FB in 31 (30.7%); 92 (91%) had low Protein C (PC); 62 (61.4%) had low Protein S (PS), 27 (26.7%) normal PS and 12 (11.9%) high PS; 97 (96%) had low antithrombin (AT) of the patients shows in (Table 6). D-Dimer was found to be positive in 87 (86.1%) of 101 DIC patients and 70 (86.4%) of 81 DF and 17(85%) of 20 DHF patients. The sensitivity and specificity of D-Dimer was 97% and 39% respectively and the positive value of D-Dimer in predicting DF was 80%.

Interestingly, DIC was significantly higher in DF 81(80.2%) patients than the DHF 20 (19.8%) patients. Bleeding manifestations in DIC patients was observed in DHF 15 (75%) than in DF 6 (7.4%) of patients (Table 7)(P.value 0.083 and 0.058 respectively). D-Dimer also was relatively higher in overt DIC 44(51%) than in non-overt DIC 43(49%) (P.value 0.000) (Figure 2). D-Dimer, PTT, and FB were strong correlated with DIC (P.value 0.000). Protein C was correlated positively with DIC (P.value 0.040). Antithrombin and Protein S were negatively correlated with DIC (P.value 0.412 and 0.447 respectively).

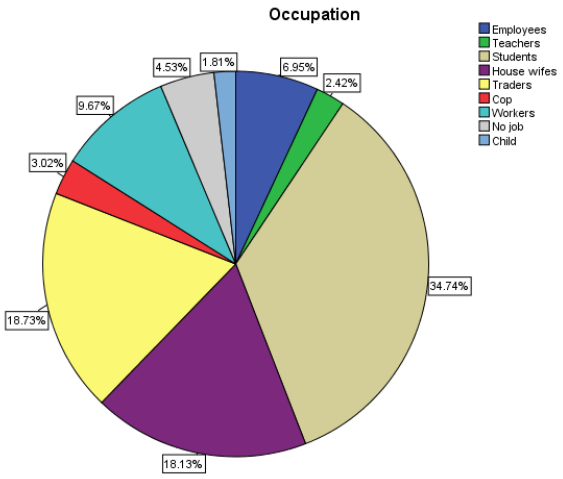

Figure 1: Frequency of the occupational status for the individuals included in the test group of the study

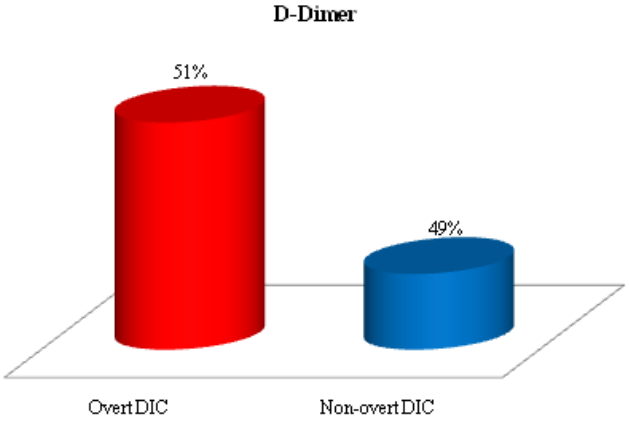

Figure 2: D-Dimer results among DIC type

Table 3: Frequency of DIC in studied population

Diagnosis	*DF*	*DHF*	*TOTAL*
Patients with DIC	81 (80.2%)	20 (19.8%)	101 (30.2%)
Patients without DIC	208 (89.3%)	25 (10.7%)	233 (69.8%)
Total	289 (86.5%)	45 (13.5%)	334 (100%)

Table 4: Summary of DIC score results

Parameters	*Score*	*Overt DIC n = 44*	*Non-overt DIC n = 57*	*P.value*
Platelet score	0	4 (9.1%)	16 (28.1%)	0.000
	1	14 (31.8%)	41 (71.9%)	
	2	26 (59.1%)	—	
PT score	0	32 (72.7%)	39 (68.4%)	0.667
	1	4 (9.1%)	18 (31.6%)	
	2	8 (18.2%)	—	
FDP score	0	4 (9.1%)	14 (24.6%)	0.000
	1	12 (27.3%)	43 (75.4%)	
	2	28 (63.6%)	—	
Fibrinogen score	0	11 (25%)	—	0.000
	1	33 (75%)	—	
Protein C (low)	1	43 (98%)	49 (86%)	0.040
(normal)	1	1 (2%)	8 (14%)	
Antithrombin(low)	1	43 (97.7%)	54 (94.7%)	0.412
(normal)	1	1 (2.3%)	3 (5.3%)	
Total score		≥ 5	≥ 6	

Table 5: DIC results in comparison to DF/DHF

DIC	DF	DHF	TOTAL
Overt DIC	34 (77.3%)	10 (22.7%)	44 (43.6%)
Non-overt DIC	47 (82.5%)	10 (17.5%)	57(56.4%)
Total	81(80.2%)	20 (19.8%)	101 (100%)

Table 5: Coagulation parameters of DIC patients among DF and DHF

Parameters	DF n=81	DHF n=20	P.value
PT	25 (31%)	5 (25%)	0.628
PTT	33 (40.7%)	9 (45%)	0.916
FB	47 (58%)	14 (70%)	0.464
Thrombocytopenia	76 (93.8%)	17 (85%)	0.301
Protein C	72 (89%)	20 (100%)	0.295
Protein S	46 (56.8%)	16 (80%)	0.158
Antithrombin	79 (97.5%)	18 (90%)	0.226
D-Dimer	70 (86.4%)	17 (85%)	0.287

Table 7: Hemorrhagic manifestations of DIC patients among DF and DHF

Diagnosis	Bleeding manifestations		Total	P.value
DF	6 (7.4%)	75(92.6%)	81(80.2%)	0.058
DHF	15(75%)	5(25%)	20(19.8%)	0.083
Total	21 (20.8%)	80 (79.2%)	101	

4. Discussion

This is the first study determined the pattern of DIC in our region. No previous data regarding the DIC was reported. Several studies showed abnormal hemostasis including DIC [10]. In this study, we attempted to apply the diagnostic criteria of DIC to 334 patients with dengue virus infection. Our study showed significantly higher D-Dimer level in DF patients compared with DHF with the sensitivity of D-Dimer in predicting DF of 80%. D-Dimer was also found to be positively correlated with DIC (P.value 0.000). Detection of D-Dimer in patients with dengue infection may be beneficial for predicting the clinical course of the disease. This helps the clinicians in predicting the disease severity before the patients' progress into toxic stage. Detection of D-Dimer suggests that DIC and activation of fibrinolytic system occur early in patients with dengue virus infection before onset of severe hemorrhagic manifestations. On the other hand, excessive fibrinolysis was associated with bleeding in our patients with DHF and DF. The stimulus of fibrinolysis in dengue was occurred secondary to DIC [6, 7, 13, and 14]. Moreover, prolongation of prothrombin and partial thromboplastin time in dengue infection have been reported by Wills BA et al, Kolitha H. Sellahewa, and Nimmannitya S a findings which are agreeing with our results [7, 15 - 17]. Reduced fibrinogen level in patients with dengue infection was reported by Wills BA et al which was similar to our finding [7]. With respect to Plasma levels of the naturally occurring anticoagulant proteins C, S and antithrombin were significantly reduced at presentation, the anticoagulant proteins C, S and antithrombin are predominantly synthesized in the liver, the low circulating levels probably reflect capillary leakage alone. An increase in the rate of consumption of these proteins may be a contributing factor in the severity of disease [7]. Dengue virus may downregulate thrombomodulin-thrombin-protein C complex

formation thus reducing activated protein C [18]. Low concentrations of plasma anticoagulant proteins C and antithrombin III have been detected in severe dengue but have not been associated with clinical thrombosis [19]. The low circulating levels of protein C reported by Wills BA et al and low antithrombin reported by Jong JB et al were similar to our result and both correlated with the severity of dengue infection [20].

Collectively, our data suggest the coagulation abnormalities for these proteins due to the presence of DIC.

5. Conclusion

Nearly one-third of patients had DIC. Non-overt DIC was the distinct pattern present in the study. D-Dimer is a marker for presence DIC, activity of fibrinolysis and may predict the clinical course of the disease.

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