International Journal of Hematological Disorders, 2015, Vol. 2, No. 1, 24-28
Available online at http://pubs.sciepub.com/ijhd/2/1/5

DOI:10.12691/ijhd-2-1-5

Partial Thromboplastin Time and Prothrombin Time as Predictors for Impaired Coagulation among Patients with Dengue Virus Infection in Red Sea State of Sudan

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Received February 12, 2015; Revised March 22, 2015; Accepted March 26, 2015

Abstract **Background** Impaired hemostasis, especially coagulopathy contribute to bleeding manifestations in dengue fever. Parameters such as partial thromboplastin time (PTT) and prothrombin time (PT) indicate the impact of dengue on coagulation system. **Objective** To identify the impact of dengue infection on the PT and PTT coagulation tests **Method** A prospective study was conducted which included 334 cases of dengue caused by dengue virus along with 101 cases of control. Patients who were reported for clinically dengue infection were enrolled. Laboratory-positive dengue cases were confirmed by immune chromatography and WHO criteria were used for classifying the dengue severity. Plasma PT and PTT were performed. **Results** There was a highly significant difference between the prolongations of PT and PTT in DF (P.value 0.000). Prolongations of PT and PTT were significantly higher in dengue fever (DF) 90% and 76.2% than dengue hemorrhagic fever (DHF) 10% and 23.8% patients respectively. The sensitivity of PT and PTT in predicting DF was 90% and 76% respectively. **Conclusion** PT and PTT parameters may be used to develop a future scoring system to predict and manage dengue infection severity early in the course of the illness.

Keywords: *PTT/PT, Bleeding, dengue fever, impaired coagulation, port sudan*

Cite This Article: Bashir A. Bashir, Osman K. Saeed, Babiker A. Mohammed, and Ali K. Ageep, "Partial Thromboplastin Time and Prothrombin Time as Predictors for Impaired Coagulation among Patients with Dengue Virus Infection in Red Sea State of Sudan." *International Journal of Hematological Disorders*, vol. 2, no. 1 (2015): 24-28. doi: 10.12691/ijhd-2-1-5.

1. Introduction

In the last decade, the incidence of dengue viral infection has increased dramatically, and the disease is becoming more severe. Dengue viral infection can be fatal [1]. The World Health Organization has estimated 50 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever occur each year, depending on the epidemic activity. Some 1.8 billion of the population at risk for dengue worldwide [2]. Dengue virus is a mosquito-borne Flavivirus that is transmitted by mosquitoes such as *Aedes aegypti* or *Aedes albopictus*. Based on the antigenic difference, dengue virus (DENV) can be divided into four different serotypes, DENV 1 – 4 [3]. In the last years, Port Sudan faced many outbreaks. The dengue virus serotypes DENV1 and DENV2 were first reported in the 1986 in Port Sudan [4], while DENV3 was recently identified in an outbreak [5]. Since then, frequent outbreaks reappear in our studied area [6]. Mechanisms of bleeding in dengue infection are vasculopathy, thrombocytopenia, coagulopathy, and disseminated intravascular coagulation (DIC). The coagulation system appears to be abnormal during infection manifesting as decreased fibrinogen levels, increase levels of fibrin degradation products (FDP), prolonged partial thromboplastin time and prothrombin time, low levels of coagulation factors VIII and XII [7]. In addition to the PTT prolongation, the fibrinolytic parameters such as tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) in dengue patients are also increased [8,9]. This study aimed to identify the impact of dengue infection on the PT and PTT coagulation tests.

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 randomly selected patients positive with dengue infection. The inclusion criteria were all patients with clinical features and serologically positive dengue

infection included. No hemostatic agents were administrated to the patients. The exclusion criteria were patients with serologically negative dengue, patients with underlying hemostatic disease, 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 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), and 3 ml in citrated buffer.

2.1. Predictor's Indicators

Patient characteristics of interest included: 1). Demographic: sex, age, residence, tribe, and occupation; 2). Mode of Presentation: presence of fever, joint pain, backache, headache, myalgia, retro-orbital pain and any bleeding episodes; 3) Hematological: Platelet count was done using automated hematology analyzer (Sysmex KX-21N, B 7151, and MF 9/2008 Japan); 4) Coagulation tests: PT and PTT 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). Coagulation tests were determined by (Biomed diagnostic reagent, Germany).

2.2. 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.

2.3. Statistical Analysis

Measurements of laboratory data coagulation tests of patients with DF, DHF were statistically 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. The sensitivity and specificity of relevant coagulation tests as predictors of dengue infection were also assessed by performing receptors operating curve (ROC) analysis.

2.4. Ethical Considerations

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

3. 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 – 80 years (mean age 30 years). 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. 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%). All of the Patients 334 presented was screened for PT and PTT. The normal and altered PT and PTT shows in (Table 2). Median PTT was 32.4" (mean ± SD 33.5 ± 9.7) (range 15.0" – 80.7") and median PT was 13.8" (mean ± SD 14.1 ± 2.1) (range 10.0" – 20.4"). PT and PTT was significantly higher in the DF (90%) and (72.7%) than DHF (10%) and (27.3%) of patients respectively. Prolongations of PT and PTT were observed in DF patients (P.value 0.000) (Table 2). Table 3 shows that prolonged PT and prolonged PTT are comparatively more sensitive, specific, and positive predictive value for DF than DHF (Figure 1).

Table 1. Characteristics of patients and control in the study

Characteristics	patients (*n=334*)	Control (*n=101*)	P. value
Age (mean ± SD)	30 ± 15	22 ± 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	35 (10.5%)		0.000
Epistaxis	5 (1.5%)		
Haematamesis	1 (0.3%)		
Haemoptysis	1 (0.3%)		
Hematuria	19 (5.7%)		
Bleeding gum	9 (2.7%)		
Demographic data			
Residence			0.726
Selalab	91 (27.2%)	23 (22.8%)	
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			0.056
Hadandwa	70 (21%)	13 (12.9%)	
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
Traders	65 (19.5%)	21 (20.8%)	
House wife	60 (18%)	14 (14.9%)	
Other jobs	94 (28.1%)	26 (25.7%)	

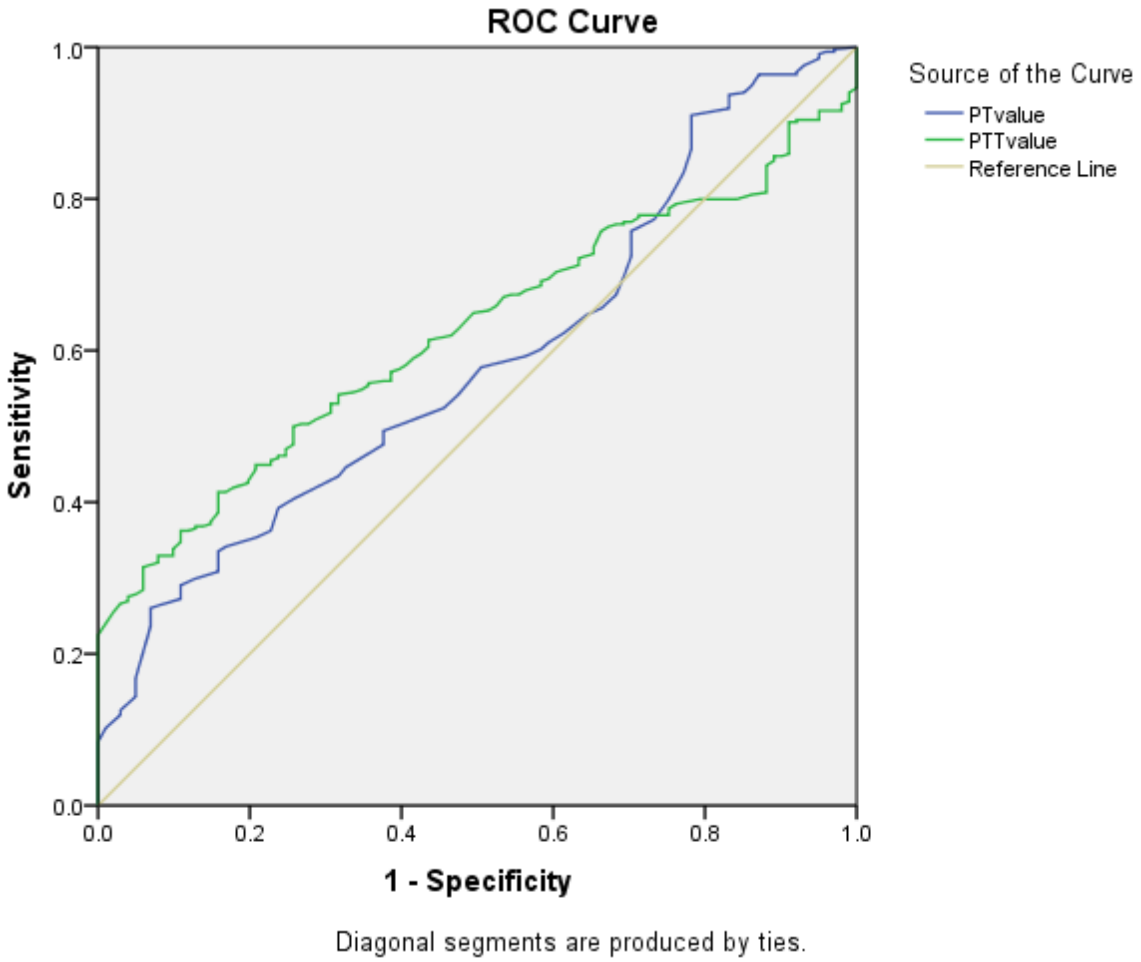

Figure 1. Receptor Operating Characteristic (ROC) curve of PT (prothrombin time) and PTT (partial thromboplastin time) for dengue virus infection

Table 2. PTT and PT results in comparison to DF/DHF

PTT	DF	DHF	TOTAL	P. VALUE
Prolong	32 (76.2%)	10 (23.8%)	42 (12.6%)	0.000
Short	18 (100%)	0	18 (5.4%)	
Normal	242 (88.3%)	32 (11.7%)	274 (82.0%)	
Total	292 (87.4%)	42 (12.6%)	334 (100%)	
PT	DF	DHF	TOTAL	
Prolong	27 (90.0%)	3 (10.0%)	30 (9.0%)	0.000
Normal	265 (87.2%)	39 (12.8%)	304 (91.0%)	
Total	292 (86.8%)	42 (12.6%)	334 (100%)	

PTT = partial thromboplastin time; PT = prothrombin time; DF = dengue fever
DHF = dengue hemorrhagic fever.

Table 3. Statistical analysis of PT and PTT for diagnosis of Dengue fever (DF) and Dengue hemorrhagic fever (DHF)

Parameters	Sensitivity (%)	Specificity (%)	PPV	NPP	LR	95%CI
Prolong PT						
DF	100	99	90	100	88.3	28.67-272
DHF	100	61	10	100	2.56	1.90-3.43
Prolong PTT						
DF	64	96	76	93	16.1	8.45-30.52
DHF	100	51	24	100	2.00	1.59-2.60

PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR, Likelihood Ratio; CI, Confidence Interval.

279 (83.5%) of the patients had thrombocytopenia. Thrombocytopenia was found more severe with bleeding individuals (P.value 0.000) (Table 4).

Table 4. Association of bleeding with thrombocytopenia

Platelet count	No 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%)

4. Discussion

The exact causes that can complicate the dengue infection are plasma leakage due to increased capillary permeability, and hemostasis impairment [7]. Bleeding manifestations in dengue infection are highly variable and ranging from a positive tourniquet test, mild bleeding from the skin such as petechiae, ecchymoses, purpura, or hematoma at the site of injection, gum bleed, epistaxis, hematuria, to severe bleeding such as haemoptysis and gastrointestinal tract bleeding. Gastrointestinal bleed (Haematamesis) is the most frequent severe bleeding that lead to mortality. This study shows that 35 patients (10.5%) had bleeding. Patients with mild bleeding manifestations such as epistaxis (5/35:14.2%), hematuria (19/35:54.3%), and gum bleed (9/35:25.7%), patients with severe bleeding such as haematamesis and haemoptysis (1/35:2.9%). A study by Phuong et al who performed a prospective study of DHF in Vietnam 1998 with total 319

DHF cases showed regarding to bleeding manifestations similar results to this study. In that study 11% showed spontaneous bleeding [10]. Hemostasis defects are caused by multiple factors such as a vasculopathy, thrombopathy, and defects in the coagulation system [11]. Hemostasis is maintained by the balance between the coagulation and fibrinolysis systems. During dengue infection, there is imbalance between these systems. Abnormal PT/PTT can occur as a consequence of imbalance in the coagulation system [12]. Plasma PT and PTT are indicators of extrinsic and intrinsic coagulation system during the cascade. This study found that there was a significant relation between prolonged PT /PTT and dengue fever (P.value 0.000). PT prolonged was seen in (30/334:9%) of patients with or without hemorrhage, while PTT prolongation was observed in (42/334:12.6%) of patients.

A prolonged PT/PTT values during the dengue infection indicates that there is a defect in coagulation cascade pathway. Accordingly, our study hypothesized that the coagulation system activated; we attribute this to the presence of dengue virus. Recently, we found nonstructural protein-1 (NS1) of dengue virus can bind to both thrombin and prothrombin. The thrombin activity is not altered when NS1 bind to thrombin, the binding of NS1 to prothrombin can inhibit it is activation which may contribute to prolongation of PTT in dengue patients [13]. This may explain why PTT abnormality occurs within 1st week of fever onset when antibodies are still under developed [14]. In addition, NS1 may also contribute to plasma leakage by mechanism without antibody involved. These results suggest that DENV secreted NS1 plays a direct and important role in vascular leakage and haemorrhage in DHF/DSS [15]. Regardless of the causes of the coagulopathy, our study showed that the coagulation activity may be impaired during dengue infection and that this disorder may associate with bleeding.

During the acute phase of the dengue viral infection, there is an increase in capillary permeability due to release of inflammatory mediators such as cytokines, Complement (C3a, C5a), tumor necrosis factor (TNF-α), interleukin (IL-2), IL-6, IL-10, interferon-α, and histamine. Thrombocytopenia and plasma leakage during dengue infection cause a decrease in fibrinogen and coagulation factors (factor II, V, VII, VIII, IX, X, and XII). In addition, liver cells can be damage by inflammation; this can be seen by an increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels. Damage of liver cells furthermore, decrease coagulation factors synthesis. AST/ALT may increases in dengue infection as well as the coagulation factors influenced due to low synthesis or high consumption; this will prolong PT/PTT during the acute stage of dengue infection [16]. Abnormal fibrinolysis can occur as a consequence of imbalance in the production of tissue-type plasminogen activator (tPA) and plasminogen activators inhibitors (PAI-1). Defect in coagulation and fibrinolysis system will appear as bleeding, intravascular thrombosis or both [12,16]. If the dengue infection accompanied by bleeding complication and there is possibility of DIC, this indicate that there are defect in intrinsic and extrinsic cascade.

Previous study that showed similar results was reported by Krishnamurit et al who conducted a prospective study on the bleeding mechanism during DHF without shock. Their study found that prolonged PTT had significant relationship with the dengue infection [17]. In addition, a study by Lui et al in Taiwan also showed similar results; a prolonged PTT was found in more than 20% dengue cases [18]. Wills et al studied coagulation defects during DHF in 167 children diagnosed with DSS in Vietnam between July 1998 and February 1999. In that study found prolonged PT and PTT in DSS patients with bleeding [7]. Their results different from ours finding because all cases included in their study had DSS with DIC complication. Further studies are needed to confirm our finding and to know specifically which factors may be involved.

Prolonged PT and prolonged PTT are too late to be used as prognostic indicators. Furthermore, PT and PTT are not routinely investigated in many hospitals, limiting their use in prognostic indicators.

5. Conclusion

In conclude the finding of prolonged PT/PTT in patients with dengue infection proved that the coagulation was impaired. These parameters may be used to develop a future scoring system to predict and manage dengue infection severity early in the course of the illness.

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