International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Von Willebrand Factor Level among Sudanese Patients with Dengue Fever Disease

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Abstract: ***Backgrounds and Aims**: Remarkable fluctuations in von willebrand factor (VWF) was observed in dengue infection. The knowledge of VWF in patients with dengue is not clear. Due to the increased recurrence of hemorrhage this study was undertaken. A cross-sectional study was conducted to investigate quantitative aspects of VWF: Ag in dengue infected patients in Port Sudan Teaching Hospital, Red Sea State, Sudan. **Materials and Methods**: 101 patients with dengue wereenrolled in this study. Laboratory-positive dengue cases were confirmed by IgM enzyme-linked immunosorbent assay test and WHO criteria were used for classifying the dengue severity. Platelet count (PLT), Plasma Prothrombin time (PT), activated partial thromboplastin time (APTT), Fibrinogen (FB), and VWF: Ag were performed. **Results**: 81 patients (80.2%) had classic dengue fever and 20 patients (19.8%) had dengue hemorrhagic fever (DHF), classified as 5 patients (5.0%) hadDHF I; 13 patients (12.9%) had DHF II and two of them developed dengue shock syndrome (DSS) and died (2.0%). 92.1% had thrombocytopenia. PT, APTT, and FB were found to be statistically significant higher in patients when compared to the controls (P< 0.000 and 0.001 respectively). PT was prolonged in 29.7%, APTT was prolonged in 42.6%, hypofibrinogenemia in 18.3% and hyperfibrinogenemia in 67.4% of the patients. VWF: Ag was low (57.4%) among patients compared to controls (7.9%) (P< 0.001), a significant reduction in VWF: Ag levels among patients presenting DF 44 (43.7%) and entire group of DHF 14 (13.9%). **Conclusion**: This study indicates a positive correlation between low platelet count and the remarkable reduction of VWF: Ag. However, VWF may have abnormalities when associated with dengue infection.*

Keywords: VWF, Dengue, Bleeding, Port Sudan, Sudan

1. Introduction

Dengue has become a major global public health concern with up to 100 million annual cases worldwide. It always manifests as a non-specific febrile illness, but it's may become complicated in association with bleeding and a transient plasma leakage that may ultimately lead to shock and death [1]. Dengue virus (DV) is a mosquito-borne transmitted by mosquitoes such as *Aedes aegypti* or *Aedes albopictus*. DV is a single positive-stranded RNA with envelope. There are five serotypes of dengue virus, namely DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5. They belong to the genus *Flavivirus*, family *Flaviviridae*. DV infection might lead to an influenza-like illness, which is called classic dengue fever (DF) or cause more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [2]. The von Willebrand factor (VWF) is a large glycoprotein present in megakaryocytes, platelets, endothelial cells, and plasma. It plays two essential hemostatic roles: (a) binding of platelets to the subendothelium at sites of vascular injury, and (b) stabilization and protection of FVIII which is the anti-hemophilic factor (AHF). A reduction or qualitative defect of VWF results in von Willebrand disease (VWD), the most common inherited human bleeding disorder. There is also an evidence to implicate increased plasma VWF levels as a risk factor for thrombotic disease [3].Endothelial dysfunction is usually followed by an increase in procoagulant activation, decrease in anticoagulant activation, and increase in synthesis and secretion of von Willebrand factor as well as its propeptide [4]. Injury or activation of the endothelial leads to a rapid secretion of equimolar amounts of stored VWF and VWF propeptide, and both proteins are regarded as markers of endothelial cell injury [1]. Krishnamurti et al studied platelet and endothelial cell activation in dengue fever patients. The results revealed that the ratio of P-selectin to the real plateletcounts increased in the acute phase specimens with increasing disease severity. Theysuggest that hemorrhagic diathesis and the severe thrombocytopenia in dengue withoutshock are probably due to platelet activation (high level of P-selectin) andits consumption and that the endothelial cell activation may play some role [5]. From the evidences as mention above, malfunction of vascular endothelial cells may be the main factor involving in transient leakage syndromeand/ orvasculopathy in dengue infected patients [6]. The malfunction of endothelial cells during dengue virus infection mayaffect to quantitative and/or qualitative VWF production, which can result inbleeding and hemostatic disorders in patients with dengue virus infection [20].The objective of this study is to investigate the quantitative aspects of VWF and determine the relationship of clinical outcome in dengue infection.

2. Materials and Methods

This study was carried out prospectively in the hematology laboratory department at Port Sudan Teaching Hospital in Sudan. Hundred-one patients with dengue infection attending the laboratory and 101 healthy controls were enrolled in the study.

Inclusion and exclusion

All patients serologically positive dengue was included. Patients who had serologically negative dengue or other diseases and administrative hemostatic agents or blood transfusion were excluded.

Study subjects

The data of all patients studied include age, sex, clinical findings encompassing the bleeding tendency. The tests

conducted include platelet count, coagulation tests (PT, APTT and FB), and von Willebrand factor Ag level.

Study sample

Samples of patient and control was collected into three blood containers. The first was K_3EDTA (tri- potassium ethylene diamine tetra acetic acid) blood containers for platelet count using semi-automated hematology analyzer (Sysmex KX-21N, B 7151, and MF 9/2008 Japan), the second was the tri-sodium citrate containers from which plasma was used to detect PT, APTT, FB, and polyclonal anti-VWF specific antibodies by enzyme-linked immunosorbent assays (ELISA test using the sensitive TECHNOZYM® VWF: Ag Actibind kit (Technoclone GmbH, Austria). The third was plain containers into which serum was extracted for assessing anti-dengue immunoglobulins (IgM) by ELISA.

Criteria for dengue severity

According to WHO guidelines, patients were classified as dengue fever, dengue hemorrhagic fever or dengue shock disease and the laboratory diagnosis of dengue was established by demonstration of anti-dengue immunoglobulins IgM (ELISA test Nova Tec Germany). The test had 98% sensitivity and 95% specificity.

Statistical analysis

Differences in laboratory data between patients with DF, DHF and controlswere tested by Independent-sample t test, scatter/dot and Pearson Chi-square test, whichever 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.

Ethical consideration

All protocols were approved by the ethics committee of the institution (Ethics Committee, Port Sudan Teaching Hospital, Sudan) before the initiation of the study.

3. Results

A total number of 101 patients with dengue infection were enrolled, of whom 81 were classified as having DF and 20 as DHF (grade I, II, and III). 63.4% of the patients were males and 36.6% were females. The ages ranged between 4 – 75 years. In addition, 101 healthy controls were enrolled. Of the controls 59.4% were males and 40.6% were females and the ages ranged between 6 – 76 years.

The clinical characteristics of the patients are summarized in (Table 1). According to the WHO classification system most of the cases in the present study were dengue fever (80.2%), followed by dengue hemorrhagic fever (19.8%).Of DHF, 5 patients (5.0%) hadDHF I; 13 patients (12.9%) had DHF II and two of them developed dengue shock syndrome (DSS) and died (2.0%).

All the patients 101 presented were screened for PLT, PT, APTT, FB, and von Willebrand factor antigen level. The difference between patient and control were found to be significant in PLT, PT, and APTT (P< 0.000). In addition, FB and VWF: Ag levels were found to be significant (P< 0.001). The platelet counts ranged between 9 – 278×10^9/l indicates the phenomenon of thrombocytopenia (93/101: 92.1%). 93.8% (76/81) presented in DF cases and 85% (17/20) presented in DHF cases (Table 2).

Bleeding manifestations were significantly recorded in 17 (16.82%) cases (P< 0.000). Bleeding episodes included hematuria presented in 7 (6.9%) cases, hemoptysis presented in 1 (1.0%), hematemesis presented in 1 (1.0%), gum bleeds presented in 3 (2.97%) cases, epistaxis presented in 5 (4.95%) cases as well as rash 8 (7.9%) in the whole population of the patients (Table 1 and 3).

Prothrombin time was significant prolonged in 30 (29.7%) of the patients and normal level in 71 (70.3%). Activated partial thromboplastin time also was significant prolonged in 42 (41.6%) of the patients, shortened in 7 (6.93%) of patients, and normal in 52 (51.5%) of the patients. There was significant hypofibrinogenemia detected in 61 (60.4%), 15 (14.9%) had normal fibrinogen level, and 25 (24.8%) patients had hypofibrinogenemia (Table 3). A significant reduction in VWF: Ag levels among patients presenting DF (43.7%) and 4 (20%) DHF I, 8 (40%) DHF II patients. There were only 2 cases comprise 10% expressed DHF III and those patients died. Normal VWF: Ag level was seen in 29 (28.7%) DF patients and high VWF: Ag level was observed in 8 (7.9%) DF patients (Figure 1). However, the difference of VWF: Ag in DF/DHF was not statistically significant (P< 0.634).

VWF: Ag level was directly proportion with platelets (Figure 2) and inversely correlated with PT, APTT, and FB (P< 0.213, 0.139, and 0.889 respectively).

Table 1: Patients characteristics and baseline data with DF and DHF

Characteristics	DF n = 81	DHF (I,II, and III) n = 20
Sex		
Male	50 (49.5%)	14 (13.9%)
Female	31 (30.7%)	6 (6.1%)
Age (mean ± SD)	30±14.3	41±20.2
Clinical findings		
Fever	81 (100%)	20 (100%)
Headache	66 (81.5%)	14 (70%)
Joint pain	59 (72.8%)	15 (75%)
Backache	50 (61.7%)	9 (45%)
Retro-orbital pain	18 (22.2%)	6 (30%)
Bleeding manifestations		
Hemoptysis	0 (0.0%)	1 (5.0%)
Hematemesis	0 (0.0%)	1 (5.0%)
Epistaxis	5 (6.25%)	0 (0.0%)
Hematuria	6 (7.50%)	1 (5.0%)
Gum bleeds	2 (2.50%)	1 (5.0%)
Rash (Purpura)	7 (8.75%)	1 (5.0%)
Mortality rate	0 (0.0%)	2 (10.0%)

Table 2: The difference between test and control in studying parameters

Parameters	Test group Mean ± SD	Control group Mean ± SD	Median Test	Median Control	Range Test	Range Control	P.value
PLT×10^9/l	97.64 ± 59.7	219 ± 58.9	92	214	9-278	93-509	0.000
PT second	14.76 ± 2.7	13.34 ± 1.6	14.2	13.5	10–20.4	10-16.5	0.000
APTT second	39.3 ± 13.4	29.64 ± 4.5	40.3	28.9	15–80.7	20.1-39	0.000
FB g/dl	4.27 ± 8.37	3.26 ± 1.44	1.40	2.9	0.34– 56.3	1.37-10.2	0.001
VWF: AgU/l	0.61 ± 0.56	0.83 ± 0.37	0.39	0.75	0.08-2.98	0.15-2.40	0.001

Table 3: General parameter among DF and DHF patients

Parameter	DF *n = 81*	DHF *n = 20*	P. value
Thrombocytopenia	76 (81%)	17 (85%)	0.000
Bleeding manifest	13 (16.04%)	4 (20.0%)	0.000
Prolong PT	25 (30.86%)	5 (25.0%)	0.067
Prolong APTT	33 (40.7%)	9 (45.0%)	0.066
Hypofibrinogenemia	47 (16.6%)	14 (28.9%)	0.763
Hyperfibrinogenemia	21 (13.8%)	4 (17.8%)	0.653
Low VWF: Ag	44 (54.3%)	14 (70%)	0.634

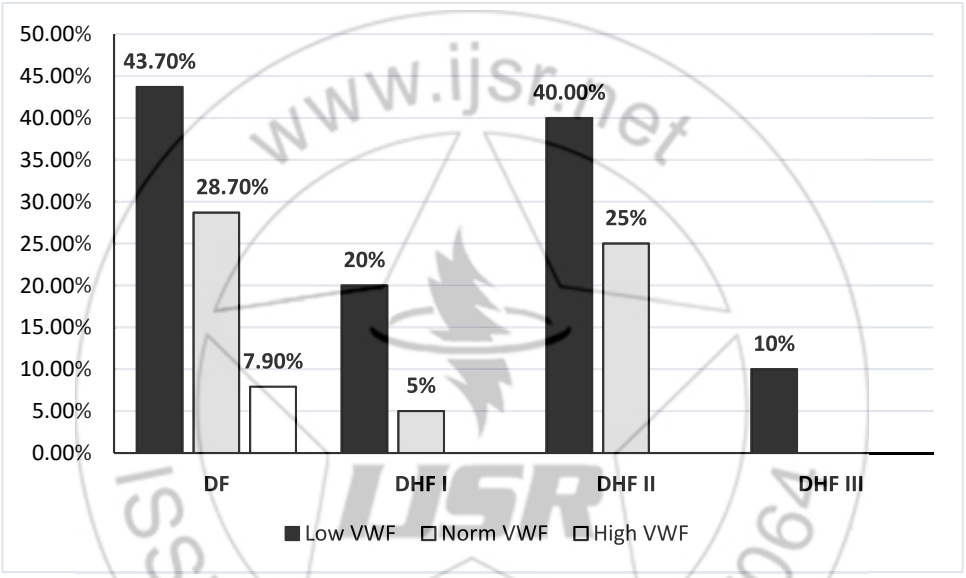

Figure 1: VWF status indengue infection categories

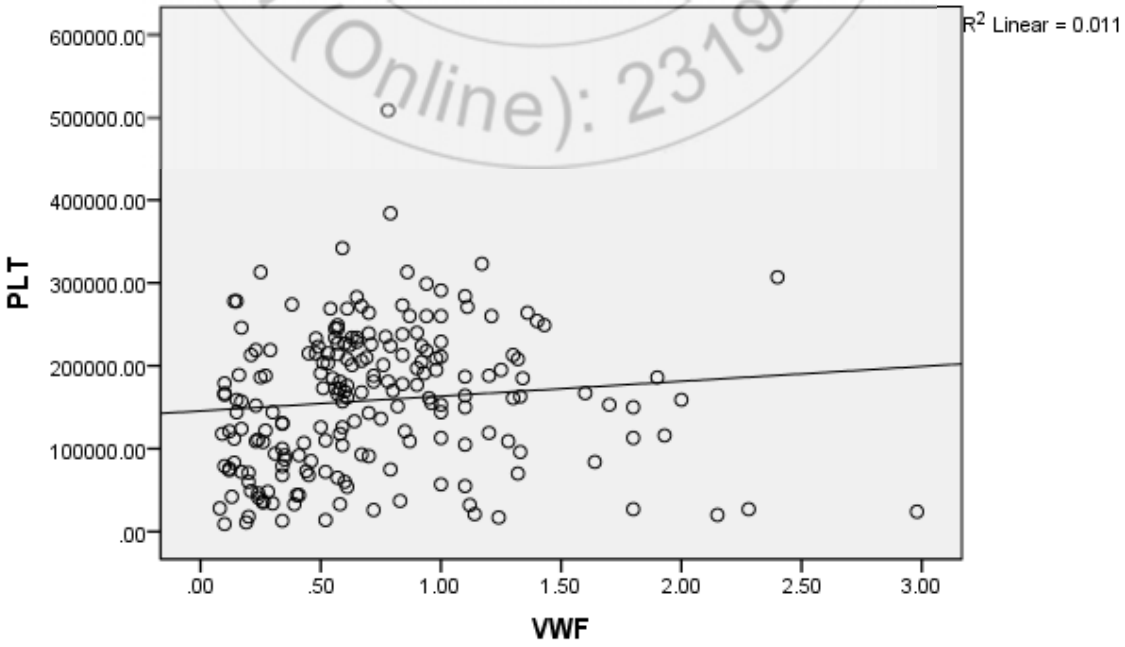

Figure 2: Correlation between VWF and platelet count

4. Discussion

Dengue infection is one of the most severe form of viral hemorrhagic infection in many developing countries including our areas. It is still remaining a major health problem in Sudan [8]. The mechanism of bleeding in dengue infection is not known, but some degrees of disseminated intravascular coagulation, liver derangement, and thrombocytopenia may operate synergistically [4]. Mechanisms of bleeding that may be involved in dengue infection, as proposed by other authors, include thrombocytopenia, endothelial injury, reduced levels of coagulation factors, disseminated intravascular coagulation (DIC) [9].

Multiple signs of bleeding were also seen in this study along with thrombocytopenia and reduced level of VWF. This could have been due to the fact that, although mechanisms of bleeding have not been well defined, thrombocytopenia and VWF were not the only contributors.

This study has highlighted that von willbebrand factor antigen (VWF: Ag) in patients with dengue fever and dengue hemorrhagic fever have significantly lower levels. The pathogenesis of the disease belonged to VWF antigen is not well understood [1].

A considerable number of patients had thrombocytopenia (92.1%) particularly in patients with DF. A finding similar to previous studies [10, 11]. Moreover, in the current study thrombocytopenia was directly correlated with von willbebrand factor antigen ($P< 0.003$).

Their sequence of events in dengue infection can be summarized as follows: there is thrombocytopenia, prolonged PT, APTT, high/low FB and VWF level at its lowest level but still higher than that of the controls. VWF a marker of endothelial cell injuring was studied. Our results are similar to a previous result study by Basuki et al [4]. So, our results have demonstrated that the level of VWF: Ag was reduced among the DHF and DF patients, these findings suggest that there is consumption of VWF in dengue infection. Consequently, this remarkable reduction of VWF: Ag is consistent with an acquired von Willebrand deficiency. Our finding is consistent with the result ofDjamiatun K, et al [1]. Previous studies in other populations described an increased level of VWF: Ag, a finding which is different from ours [12, 13], the differing results may be explained by the difference in the dengue virus type involved in their outbreak or the type of blood group. Elevated level of VWF: Ag with VWF: Rcof may be used as predictors for DSS during the febrile stage [14].

Several limitations to our study should be considered. First, enrollment in our study was restricted to the patients admitted to Port Sudan teaching hospital. Second, the limit number of dengue infected cases. Third, VWF: Ag level has been only able to measure in this study. Regrettably, due to short facilities VWF multimers, ristocetin cofactor activity (VWF: RcoF), platelet functions, and biomarkers of endothelial cell injury (P-selectin – L-selectin) were not assessed in this study.

5. Conclusion

Therefore, VWF may have associated with the abnormalities of dengueviral infection. The studies of VWF in dengue virus infection may provide new insights into the roleof VWF in the pathogenesis of disease. Furthermore, the reduction in VWF: Ag in dengue might involve in the mechanisms underlying the hemorrhagic tendencies associated with dengue infection. This study indicates a positive correlation between low platelet count and the remarkable reduction of VWF: Ag.

6. Conflict of Interest

The author declare that they have no conflicts of interest.

7. Acknowledgements

The author would like to thanks the staff of hematology department, Port Sudan Teaching Hospital, Sudan for assistance and support in this research.

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