Original Article

Plasma level of von Willebrand factor: an indicator of severity in sickle cell disease

Nawal Eltayeb Omer[1], Maria M. Hamad Satti[2], Abdelrahim Osman Mohamed[1]

Abstract:

Background: Sickle cell anaemia is a congenital hemolytic disorder caused by mutation in the β-globin gene at position 6 with replacement of glutamic acid by valine. Patients who are homozygous for this mutation suffer from hemolytic anaemia and other serious complications. The underlying pathology of much of these complications is the occurrence of recurrent vasoocclusion due to microthrombi formation resulting in organs ischaemia.

Methods: In this study we investigated the role of vWF as a determinant of sickle cell disease severity through its contribution to the formation of such microthrombi. The clinical disease severity was determined using two different scoring methods, and vWF antigen level in the plasma was estimated by using ELISA technique.

Results and discussion: Seventy Sudanese patients were investigated in this study. They were 35 females and 35 males, the mean of their ages± standard deviation was 6.8 ± 4.7 years. Seventeen controls with normal haemoglobin were also included with mean age of 6.5 years. Thirty four patients presented during vasoocclusive crisis and 36 presented in steady state.

vWF was high (179.83%) among patients compared to controls (82.4%), $p < 0.001$. there was positive correlation between severity score and vWF level in the plasma in steady state in the two scoring methods used ($r = 0.79$, $p = 0.008$ for method I and $r = 0.78$, $p = 0.009$ for method II). So, Severity of sickle cell disease increases with elevation of vWF level in the plasma in the steady state.

Keywords: Keywords: sickle cell, disease severity, von Willebrand factor.

Sickle cell disease (SCD) is an inherited autosomal recessive disorder of the β-globin gene caused by mutation in position 6 with replacement of glutamate by valine. The disease is characterized by haemolytic anaemia, intermittent episodes of vascular occlusions that can cause both acute and chronic pain, increased susceptibility to infections and end organ damage[1]. There are several factors affecting the clinical severity of the disease which is extremely variable[2-4]. The frequency of painful crises is considered to be the major indicator of disease severity[5].

The pathophysiology of vaso-occlusive episodes is complex, involving not only polymerization of the mutant haemoglobin, but also interaction between sickled red blood cells, endothelium, platelets, leukocytes and plasma constituents[6].

Thromboembolic phenomenon is a well known feature of SCD with evidence of in vivo thrombin generation, endothelial activation and increased activation of coagulation and hence depletion of anticoagulants both in steady state and during vaso-occlusive crisis[7-9]. There is also activation of cellular elements including white cells and platelets[10-12].

As von Willebrand factor (vWF) plays an important role in platelet aggregation and thrombus formation[13] this study aimed to find a possible role for vWF in determining the clinical disease severity in patients with sickle cell disease.

1. Department of Pathology, Faculty of Medicine and Health Sciences, University of El Imam El Mahdi.
2[/] Department of Pathology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan
Corresponding Author: Prof Abdelrahim Osman Mohamed, MD, PhD. University of El Imam El Mahdi, Kosti, Sudan. Abdelrahim_osman@yahoo.com

Patients and Methods

This study was conducted at the sickle cell anaemia clinic at Khartoum Children's Emergency Hospital (KCEH) in Sudan. Seventy patients with homozygous sickle cell hemoglobin attending the clinic and 17 healthy controls were enrolled in the study after informed verbal consent of the patients or guardians of patients. All patients were known cases and haemoglobin electrophoresis was done previously. Patients who received blood transfusion less than 6 weeks before the time of sampling were excluded. A questionnaire addressing personal and socio-demographic data of the patients as well as history of conditions which determine the severity of the disease was used. Patients were grouped into those presenting during vaso-occlusive crisis and those presenting in steady state. The vaso-occlusive episode was defined as pain in the arms, legs, back, abdomen, chest or head that lasted at least two hours necessitating seeking medical care and analgesia.[14] All patients were subjected to physical examination. Two scoring methods for grading of disease severity were used; the first method (scoring method I) used by Ketty and colleagues[3] with a modification by omission of fundal examination. The second scoring method (scoring method II) considers the number of painful crisis per year (pain rate) irrespective of hospital admissions and administration of narcotics.[5]

Five ml of venous blood were obtained from each patient and control. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed on citrated platelet poor plasma. The rest of the plasma was stored at –20ºC to be used later for the determination of von Willebrand factor levels. Basic haematological parameters were obtained by an automated cell counter (Sysmex Kx-21, Japan). Haemogobin concentration in g/L (Hb), total white blood cell counts (TWBC x 109 /L) and platelet counts (x 109 /L) were measured using standard methods. Measurement of plasma vWF was performed by enzyme linked immunosorbant assay (ELISA). This is a sandwich ELISA using Asserachrom vWF kit (Diagnostica, Stago, France). The analysis was done following the manufacturer's instructions.

Statistical analysis

Data were analyzed by computer software statistical package for social science (SPSS) program. Pearson correlation, chi square test, t-test and one-way analysis of variance were used to compare the associated levels. P value < 0.05 was considered significant.

Results

Fifty percent of the patients were females. The ages ranged between 9 months and 17 years. The mean age± standard deviation was 6.8 ± 4.7 years with the age group 1-5 years comprising 35.7%.

Of the control group 47.06% were females. The ages ranged between 8 months and 16 years.

Thirty six patients (51.4%) had zero score when we used the scoring method I indicating mild form of the disease, 22.8% (n= 16) had score one, which indicated a moderate form of the disease, while 25.7% (n =18) scored 2 or more, which indicated a more severe form of the disease. Using the pain rate i.e. scoring method II as an indicator of disease severity, 23 patients (32.8%) had severe disease and the rest 67.1% (n= 47) had a mild disease.

The mean haemoglobin level among the patients was 71.1g/L, with most of the patients having a level ranging between 50 - 100g/L. The platelet counts ranged between 94-1025×109/ L indicating the presence of thrombocytopenia (5.7%) and thrombocytosis (41.4%) (Table1).

Table 1 Platelet count $(x10^9/L)$ distribution among the patients

Platelet count X 109/L	N0	(%)
Less than 150	4	(5.7)
150-400	37	(52.9)
More than 400	29	(41.4)
Total	70	(100)

The lower platelet counts were present mainly in patients presenting during vaso-occlusive crisis.

The control mean of vWF was 82.4% and the mean of the patient levels was 179.83%. Ninety-nine percent of the study group had vWF antigen level above that of the control mean. The difference between the means of the patients and controls was highly significant (P<0.001). Painful episodes were accompanied by the highest levels of vWF, and steady state with the lowest level. However, the difference was not statistically significant (P= 0.479).

Correlation between severity score and vWF levels in steady state revealed a strong direct correlation in both of the scoring methods used p< 0.01 for both (Fig. 1 and 2).
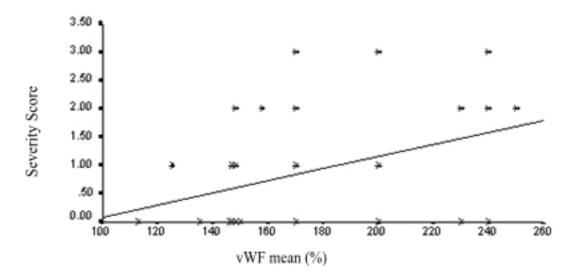

Fig. 1. Correlation between vWF mean (%) and severity score (method I) in steady state sickle disease, r =0.79, p =0.008
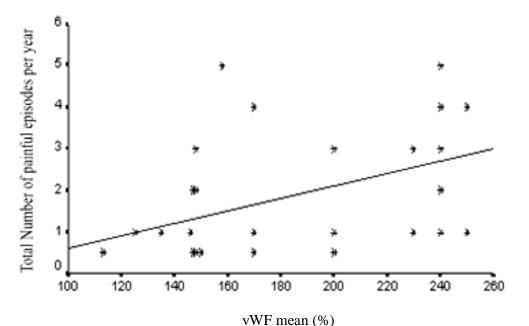

vWF mean (%)

Fig. 2. Correlation between vWF mean (%) and total number of painful episodes per year (Method II) in steady state sickle cell disease, r =0.78, p = 0.009.

There was also a direct correlation between clinical disease severity and TWBC when using scoring method II (P=0.017), but not when using scoring methodI. There was no correlation between clinical disease severity and haemoglobin levels in neither of the scoring methods. Low platelet counts were associated with the lowest vWF levels, normal counts with the highest levels and thrombocytosis with intermediate levels.

There was significantly prolonged APTT among patients presenting with painful crises (P=0.012) while the patients presenting with fever had significantly shorter APTT (P=0.018). APTT was directly correlated with vWF but inversely correlated with platelet counts (p< 0.001 for both). Prothrombin time (PT) was within normal range in the whole population of patients.

Discussion

Our results showed that patients with sickle cell anaemia had significantly (P<0.001) higher levels of vWF in the plasma compared to the controls and is in agreement with previous studies[7,15]. vWF mean levels during crises though higher than that of the steady state, did not show statistical significance (p= 0.479). There was a direct correlation between severity score and vWF level in the plasma in steady state in the two scoring methods used (Fig. 1 and 2). This correlation of vWF with the severity of sickle cell anaemia can be attributed to the fact thatt vWF is an acute phase protein and its level in the plasma can be elevated in a number of clinical situations specially those affecting the blood vessels. vWF is released in plasma due to the inflammatory process induced by the adhesion of sickled red blood cells to the endothelium leading to narrowing of the vessel lumen creating high sheer effect[16]. Adhesion of platelets to the adherent sickled cells and their activation will release more vWF. It has been previously reported that the level of vWF in the plasma is directly correlated with the extent of adhesion of sickled red blood cells to the endothelium which had been found to be correlated with

the severity of the disease[2,17]. A considerable number of our patients had high platelet counts (41.4%) particularly in steady state, in keeping with the results of Leslie et al [18,19] which would increase the interaction between the platelet receptors and vWF leading to enhancement of thrombus formation[20]. Another fact that can explain this correlation between the high vWF level and the disease severity is the direct correlation between factor VIII and vWF[21]. High levels of factor VIII during steady state of sickle cell anaemia were reported earlier[7,18,22]. Richardson reported also elevated vWF together with the elevation of factor VIII[7]. However, the author did not correlate the levels to each other. This elevated level of factor VIII with activation of platelets will put patients with sickle cell anaemia on a procoagulant state. Patients with vaso-occlusive crisis had shown significantly faster APTT (p = 0.018) which is an indicator of coagulation activation[23]. The sequences of events in sickle cell anaemia can be summarized as follows: in steady state there is thrombocytosis, normal APTT and vWF level at its lowest level but still higher than that of the controls. Early in crisis there is activation of platelets and coagulation leading to shortening of APTT. On progressing of the crisis, activation of the platelets becomes maximum and associated with elevation of vWF in the plasma. Later on in the crisis there is consumption of platelets and coagulation factors in the microthrombi formation resulting both in platelet count reduction and APTT prolongation. Reduction of the platelet count is associated with reduced release of vWF from α-granules and therefore, vWF levels go down gradually to the steady state level.

Elevated plasma vWF levels and high shear rate created by adherence of sickled erythrocytes to the endothelium will lead to accelerated platelet activation and aggregation and also a higher rate of thrombin generation. These conditions will consequently lead to fibrin and microthrombi formation leading to vaso-occlusion and organ ischaemia.

In conclusion high levels of vWF in the plasma of patients with sickle cell anaemia in steady state are associated with severe clinical disease.

Acknowledgements

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