

Determination of Coagulation Profile among Children with Sickle Cell: Anemia in Steady-State and Crisis

Abstract:

Background: Patients with Sickle Cell Disease (SCD) have been found to have an aberrant coagulation profile. One of the primary elements hypothesized to contribute to the vaso-occlusive crisis that characterizes sickle cell disease is coagulopathy (SCD)

Material and Methods: A total of 150 children were enrolled as follows, 50 children with Sickle Cell Anemia (SCA) in steady-state, 50 in crisis, and 50 with Hb AA genotype as control. 5 ml of Venous blood was collected. The platelets count was performed using Sysmex KX21N, the electrical impedance principle. The Mean Platelet Volume (MPV) is derived from the impedance platelet size distribution curve. STAGO PT31039352) semi-automated machine was used for estimation of PT, and APTT. **Result:** children with SCA have significantly a prolonged PT, and APTT compared with children with normal hemoglobin genotype ($P. value < 0.001$) the mean of PT was (16.64, and 12.6) respectively, and APTT (41.45 and 37.94) consequently. A significant increase in platelet count between patients with SCA when compared with control ($p. value 0.02$), however a significant prolonged in APTT when compared to steady-state ($P. value 0.005$). MPV among children with crises when compared with steady-state revealed a significant result ($p. value 0.006$) the mean of MPV in steady-state = 6.79 while the mean of MPV in crisis = 7.09. **Conclusion:** children with sickle cell anemia had a longer coagulation profile and marked variation in platelet count, which may increase the risk of thrombosis or bleeding.

Keywords: Sickle cell anemia; Coagulation Profile; Children; Sudan.

Introduction:

Sickle cell anemia (SCA) is a genetic hematological disorder characterized by red blood cells that assume an abnormal rigid, sickle shape. [1], this is hereditary disorder contributes the equivalent of 3.4% mortality in children aged under 5 worldwide or 6.4% in Africa. [2] SCA is associated with a hypercoagulable state that may certain morbidities such as vaso-occlusion and cerebrovascular accidents. It is noted that decreased levels of natural anticoagulant

proteins (Proteins C and S are two vitamin K-dependent plasma proteins that work in concert as a natural anticoagulant system) are observed in SCA and even more so in vaso-occlusive crisis [3]. These reduced levels may be a consequence of chronic consumption arising from increased thrombin generation which occurs in the vascular endothelium. Patients with this disease suffer from a variety of clinical events associated with small and large vessel occlusion, including vaso-occlusive painful episodes, strokes, and acute chest syndrome [4]. The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. Deoxygenation of HbS results in the normal conformational change of the tetramer hemoglobin exposing on its external surface a hydrophobic β_6 valine, instead of the hydrophilic glutamate of HbAA. This leads to decreased solubility hence polymerization. [5, 6] The rate and extent of polymerization are related to the intracellular concentration of HbS, the type and fractional content of other hemoglobin presents; particularly HbF, and percent oxygen saturation. There are factors such as endothelial damage with subsequent activation inflammatory and coagulation pathways that may trigger or complicate vaso- occlusion crisis [7, 8]. High levels of fetal Hb (HbSF) that may substantially reduce symptoms and clinical consequences [9, 10, 11]. This study, therefore, aims at determining the actual value of some coagulation profiles (PT, APTT, platelet count, and platelets indices) among Sudanese children with SCA in steady-state and crises and compare with subjects with normal hemoglobin genotype.

Materials and methods:

A case-control study was conducted among all sickle cell patients attending emergency pediatrics centers (Steady-state and crisis), during the period from April 2018 to October 2018. Sickle cell patients with any illness that affect coagulation profile such as (malaria, dengue fever, and leukemia), known inherited coagulation disorders, patients who were taking standard anticoagulant treatment, and patients with recent blood transfusion during the preceding 3 months were excluded. and informed consent was taken from the participant's parents. Ethical approval was taken from the institutional review board, Faculty of Medical Laboratory Sciences, Alzaeim Alazhari University.

A total of 50 children were enrolled as follows, children with SCA in steady-state, 50 in crisis, and 50 with Hb AA genotype as control. Case and control were matched for age and sex; their age range between 6 months and 15 years whom were classified into two groups: **Crisis state:** considered clinically to be in bone pain or joint pains in a single or multiple sites needing analgesics or hospitalization or had a hemolytic crisis (Hb less than baseline). **Steady-state:** The stable patients were those with HbSS who had been well for a minimum of 4 weeks. The Control group were healthy individuals, have Hb AA genotype, attending our patient's clinic.

Collection of blood samples:

Venous blood collected about 5 ml was collected from study subjects in Tri-sodium citrate for estimation of platelets, MPV, PT, and APTT.

Measurement of platelets count and MPV

The platelets count was performed using Sysmex KX21N, the electrical impedance principle. The MPV is derived from the impedance platelet size distribution curve. The MPV is very dependent on the technique of measurement and length and conditions of storage before testing the blood. Platelet histogram is analyzed using three discriminators: two discriminators lower discrimination (LD) and upper discrimination (UD) - Determined automatically between 2 - 6 fL and between 12 - 30 fL, respectively -and the fixed discriminator at 12 fL. Regarding PLT histogram, a check is made to see that there are no relative frequency errors at discriminators (LD) and (UD), distribution width error, and there is a single peak.

Measurement of PT, and APTT:

For PT estimation, 0.1 ml of plasma delivered into a glass tube placed in water bath and add 0.1 ml of thromboplastin . Then 0.1 ml of warmed CaCl_2 were added and start the stopwatch. Mix the content of the tube and record the end point. Carry out the test in duplicate on the patient's plasma and the control plasma when a number of sample are to be tested as a batch, the samples and controls must be suitably staggered to eliminate the time bias. Some thromboplastins contain calcium chloride, in which case 0.2 ml of thromboplastin is added to 0.1 ml plasma and timing is start immediately

For APTT measurement, equal volume of the phospholipids' reagents and the kaolin suspension was mixed and left in a glass tube in the water bath at 37°C. 0.1 ml of the plasma was measured into a second glass tube, and 0.2 ml of the kaolin- phospholipid solution were added to the plasma, mix the contents and start the stop watch simultaneously, at 37°C for 10 min with occasional shaking. At exactly 10 min, add 0.1 ml of pre-warmed CaCl₂ and start a second stopwatch. Record the time taken for the mixture to clot. The test repeated at least once on both the patient's plasma and the control plasma. It is possible to do four tests at 2-min intervals if sufficient stopwatches are available. The instrument (STAGO PT31039352) semi-automated machine was calibrated and the controlled by sample normal control (NC) and pathologic control (PC) was run at the beginning of each patch.

Statistical analysis:

Statistical evaluation was performed by SPSS. The data were expressed as mean in both control and test groups. The parameters were compared with the Independent T-test [*p. value less than 0.05*] was considered significant.

Result:

A total of 150 subjects participate in the study, 100 of them were patients with SCA (52 males and 48 females), their age range between 1 -15 years old with a mean of 7.5±SD and 50 subjects (28 females and 22 male) were the control group. Patients with sickle cell disease were subdivided into two groups 50 patients were a steady-state, and 50 patients were a crisis. Another 50 unrelated healthy individuals as control group were enrolled in this study, all patients & control were subjected for PLT count, MPV, PT & APTT. For the control group, blood transfusion represents a history of blood transfusion (trauma). All data were summarized in table 1.

Table 2 displayed the comparison of coagulation profile and platelets (count and MPV) between SCA patients and control, the study shows that children with SCA have significant a prolonged PT, and APTT compared with children with normal hemoglobin genotype (*P. value < 0.001*) the mean of PT was (16.64, and 12.6) respectively, and APTT (41.45 and 37.94) consequently. A significant increase in platelet count between patients with SCA when compared with control (*p. value 0.02*), while the insignificant difference in MPV between patients with SCA when

compared with control (*p. value* = 0.83) the mean of test = 6.94 while the mean of control = 6.96. Findings revealed that children with SCA in a steady-state have insignificant differences in PT when compared to the crisis. (*P.value* 0.08), however a significant prolonged in APTT when compared to steady-state (*P. value* 0.005). MPV among children with crises when compared with steady-state revealed a significant result (*p. value* 0.006) the mean of MPV in steady-state = 6.79 while the mean of MPV in crisis = 7.09. all data were summarized in table 3. Figure 1 displayed the Type of treatment for patients with sickle cell anemia.

Table 1: Demographic and Clinical data of patients and control

	Case n=100 (%)	Control n=50 (%)	P value
Gender			0.065
Male	52 (52%)	28 (56%)	
Female	48 (48%)	22 (44%)	
Regular follow up			-
Every month	21 (21%)	-	
Every 3 months	79 (79%)	-	
Blood transfusion			0.054
Yes	58 (58%)	2 (4%)	
No	42 (42%)	48 (96%)	
Tribe of participants			
Hawsa	60 (60%)	0	
Barno	15 (15%)	1 (2%)	

Falata	14 (14%)	2 (4%)	0.002
Mesaria	4 (4%)	10 (20%)	
Nuba	2 (2%)	11 (22%)	
Rubatab	1 (1%)	25 (50%)	
West of Sudan	4 (4%)	1 (2%)	

Table 2: Comparison of coagulation profile and platelets (count and MPV) between SCA patients and control

parameters	Cases Mean \pm SD	Control Mean \pm SD	P-value
Platelets	6.94 \pm 0.56	6.96 \pm 0.52	0.83
MPV	376.7 \pm 150.02	324.3 \pm 85.2	0.02
PT	16.64 \pm 1.86	12.6 \pm 1.04	0.001
APTT	41.45 \pm 5.78	37.94 \pm 3.72	0.001

* T-test was used to calculate p. value

p. value less than 0.05 was considered significant

Table 3: Comparison of coagulation profile and platelets (count and MPV) between crisis and steady-state

parameters	Steady-state Mean \pm SD	Crisis state Mean \pm SD	P-value
Platelets	6.79 \pm 0.52	7.09 \pm 0.56	0.006
MPV	387.48 \pm 138.72	365.92 \pm 161.22	0.47
PT	16.32 \pm 1.04	16.96 \pm 1.91	0.08
APTT	39.84 \pm 4.94	43.06 \pm 6.15	0.005

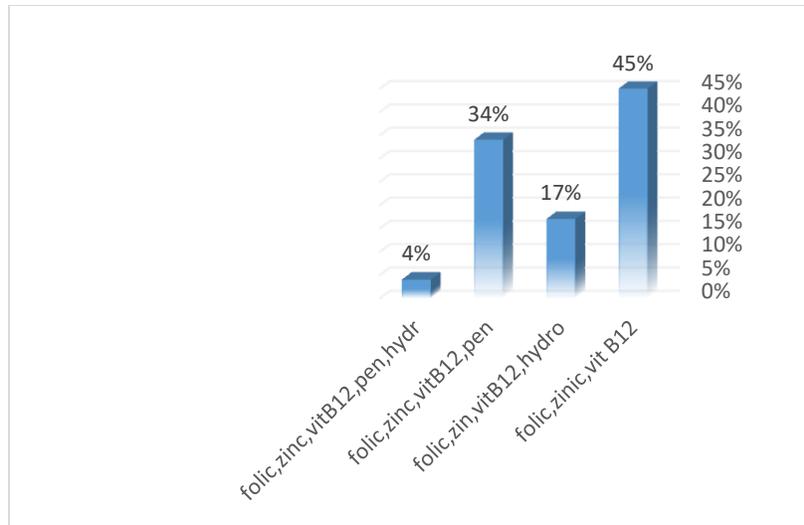


Figure 1: Type of treatment for patients with sickle cell anemia

Discussion:

Coagulation factor measurements have been shown to have some prognostic value for clinical outcomes. Hence present study aimed to determine coagulation profiles (PT, APTT, platelet count, and platelets indices) among Sudanese children with SCA in steady-state and crises, and the findings of this study provided further evidence of coagulation abnormalities among children with SCA.

In the present study majority of patients were (48%), their mean age, where (58%) have had a significant history of blood transfusion. 60% of patients group from tripe of Hawsaa, our findings were supported by Elderderly A et al [12] who noted that it was mainly widespread throughout Sudan's western tribes.

The study shows that children with SCA have significantly a prolonged PT when compared with children with normal hemoglobin genotype ($P. value=0.000$), and have signed a prolonged APTT when compared with children with normal hemoglobin genotype ($Value = 0.000$). Our finding is in agreement with Raffini LJ et al [13], who noted significant prolonged PT and APTT ($P value$ less than 0.05) in subjects with SCA when compared with normal hemoglobin genotype individuals. As well as with the findings of Antwi-Baffour S et al [14] who also noted significant prolonged PT, APTT and increased platelets counts in subjects with SCA when compared with normal hemoglobin genotype individuals. Our study revealed that the children with SCA in crisis

have significant a prolonged PT when compared with children with normal hemoglobin genotype (*P. value* 0.010) and significant a prolonged APTT (*P. value* 0.020), same findings documented by Chinawa JM, et al [15], nevertheless, a conflict was noted by Ajuwon MD *et al* [16], who conclude that there was insignificant difference in mean PT for their study groups.

On the other hand, our findings showed insignificant difference in PT of subjects in crises when compared to steady-state. (*P. value* 0.08), however, we revealed a significant difference in APTT (*P. value* 0.005) this was agreed with JM Chinawa *et al* [15] in PT with (*P. value* 0.35) and agreed with him in APTT (*P. value* 0.03). In addition, the study showed significant differences in platelet count between patients with SCA when compared with control (*p. value* 0.02), this is in keeping with the findings of Ataga KI *et al* [17], (*p. value* less than 0.05), and JM Chinawa *et al* (*P. value* 0.001) who conclude that thrombocytopenia is more frequent than thrombocytosis in serious sickle cell crises. However insignificant differences in platelet count between steady-state and control (*p. value* 0.07), which was disagreed with the finding of JM Chinawa *et al* [15] who revealed a significant correlation (*p. value* 0.001). Furthermore, our study found no significant difference between steady-state and crisis (*p. value* 0.47), which was similar to JM Chinawa *et al* [15] findings (*p. value* 0.19).

The study showed a significant difference between steady-state and crisis (*p. value* 0.006). But the insignificant difference in MPV between patients with SCA when compared with control (*p. value* 0.83). Also showed an insignificant difference between steady-state and control (*p. value* 1.0).

Recent evidence implies that coagulation stimulation may play a role in the pathophysiology of SCD, however, there is little information on the role of coagulation and platelet activation in SCD-related problems in people. New generations of anticoagulants and antiplatelet medicines should be tested in clinical trials employing a variety of clinical outcomes. Children with SCA should be screened for coagulation profile especially when in vaso-occlusive crises, or when they are prepared for surgical procedures [18].

We recommend that further studies are needed to focus deeply on coagulation tests, include (fibrinogen, D. dimer, coagulation inhibitors, and fibrinolytic tests). In addition, patients with

SCA who are not under treatment need to be investigated for coagulation profile to predict early the complication of the disease.

Conclusion:

The study confirmed that children with sickle cell anemia had a prolonged coagulation profile and marked variation in platelets count when compared with those with normal hemoglobin genotype, especially among crises patients and that may increase the risk for thrombosis or bleeding.

Data Availability: All datasets generated or analyzed during this study are included in the manuscript.

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