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 50 children were enrolled as follow, children with SCA in steady state, 50 in crisis, and 50 with Hb AA genotype as control. 5 ml of Venous blood was collected. Platelets count was performed using Sysmex KX21N, electrical impedance principle. The MPV is derived from the impedance platelet size distribution curve. STAGO PT31039352) semi-automated machine was used for stimation of PT, and APTT. **Result:** 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 significance increase in platelets 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 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; Steady State; Crisis state; 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 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 into the normal conformational change of the tetramer hemoglobin exposing on its external surface a hydrophobic β6 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 present; particularly HbF, and percent oxygen saturation. There are factors such as endothelial damage with subsequent activation inflammatory and coagulation pathways may trigger or complicate vaso- occlusion [7, 8]. High levels of fetal Hb (HbSF) 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 stickler's patients attending emergency pediatrics center (Steady state and crises), 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 inform consent was taken from participant's parent's. 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 follow, 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 hemolytic crisis (Hb less than base line). **Steady state:** The stable patients were those with HbSS who had been apparently well for a minimum of 4 weeks

Collection of blood sample:

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

Platelets count was performed using Sysmex KX21N, electrical impedance principle. The MPV is derived from the impedance platelet size distribution curve. The MPV very dependent on the technique of measurement and on length and conditions of storage prior to 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, 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:

The reagent was bringing to room temperature (RT) for pre warmed and mixes do not shake. To the test tube add 50ul of test plasma and 50ul from BioCelin reagent (R1) then incubated for 3 min at 37C. Then add 50ul from calcium chloride (cacl) following incubation and record the time. Repeat for duplicate test using same test plasma. Found the average from duplicate test values. 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 begging of each patch.

Statistical analysis:

Statistical evaluation was performed by SPSS. The data was expressed as mean in both control

and test groups. The parameters were compared with 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 female), their age range between 1 -15 years old with mean 7.5±SD and 50 subjects (28 females and 22 male) were control group. Patients with sickle cell disease were subdivided in two group 50 patients were steady state, and 50 patients were crisis. Another 50 unrelated apparently healthy individual as control group were enrolled in this study, all patients & control were subjected for PLT count, MPV, PT & APTT.

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** significance increase in platelets count between patients with SCA when compared with control (*p. value* 0.02), while 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 steady state have insignificant different in PT when compared to 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 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 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			
Male	52 (52%)	28 (56%)	0.065
Female	48 (48%)	22 (44%)	
Regular follow up			
Every month	21 (21%)	-	-
Every 3 months	79 (79%)	-	
Blood transfusion			
Yes	58 (58%)	2 (4%)	0.054
No	42 (42%)	48 (96%)	
Tripe of participants			
Hawsa	60 (60%)	0	
Barno	15 (15%)	1 (2%)	
Falata	14 (14%)	2 (4%)	
Mesaria	4 (4%)	10 (20%)	0.002
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 ±SD	Control Mean ±SD	P value
Platelets	6.94±0.56	6.96±0.52	0.83
MPV	376.7±150.02	324.3±85.2	0.02
PT	16.64±1.86	12.6±1.04	0.001
APTT	41.45±5.78	37.94±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 ±SD	Crisis state Mean ±SD	P value
Platelets	6.79±0.52	7.09±0.56	0.006
MPV	387.48±138.72	365.92±161.22	0.47
PT	16.32±1.04	16.96±1.91	0.08
APTT	39.84±4.94	43.06±6.15	0.005

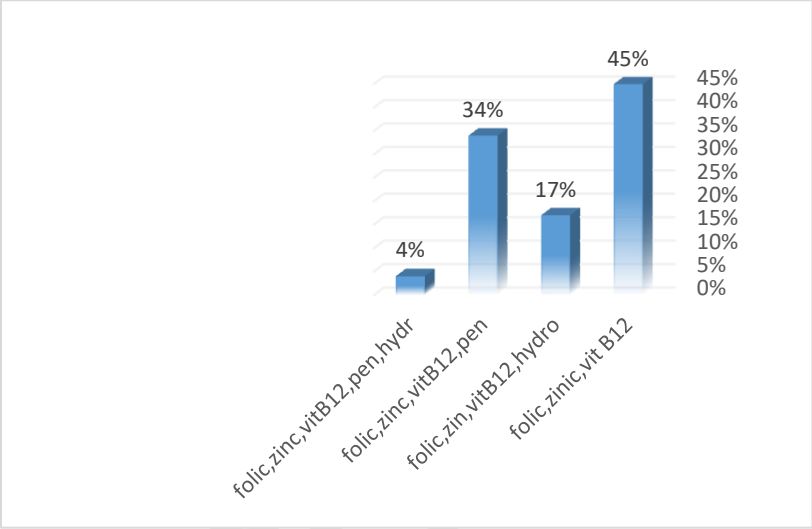

Figure 1: Type of treatment for patients with sickle cell anemia

Discussion:

Coagulation factor measurements have been shown to have some prognostic value for clinical outcome. 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 significantly history of blood transfusion. 60% of patients group from tripe of Hawsaa, our findings were supported by Elderdery **A** et al [12] who noted that it was mainly widespread throughout Sudan's western tribes.

The study shows that children with SCA have significant a prolonged PT when compared with children with normal hemoglobin genotype (*P. value*=0.000), and have significant 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 insignificance 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 significance differences in platelets count between patient 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 platelets count between steady state and control (*p. value* 0.07), which was disagreed to the finding of *JM Chinawa et al* [15] who revealed 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 significant difference between steady state and crisis (*p. value* 0.006). But insignificant difference in MPV between patients with SCA when compared with control (*p.*

value 0.83). Also showed insignificant difference between steady state and control (*p. value* 1.0).

Recent evidence imply 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 in 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 in order to predict early the complication of disease.

Conclusion:

The study confirmed that children with sickle cell anemia had 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 risk for thrombosis or bleeding.

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

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