ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
Volume 4, Issue 1, 2015, 95-99

RESEARCH ARTICLE

# ASSESSMENT OF HYPERCOAGULABILITY STATE AMONG SUDANESE SICKLE CELL PATIENTS

**[1]Idris M. M. Hamid, [2] Rashad M. O. Mahmoud, [1]Ghada M. Merghani and [2*] Mahdi H. A. Abdalla (PhD)**

[1]Faculty of Medical Laboratory Sciences, Alneelain University, Sudan

[2]Department of Haematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan

**Received 25 January 2015; Accepted 15 February 2015**

**ABSTRACT**

Sickle cell anaemia is one of the causes of morbidity and mortality in Africa. This study aimed to determine the coagulation profile, including D-Dimer level, Fibrinogen level and TAT, and also to determine haematolgical feature of sickle cell anaemia in Sudan. The study included 100 Sudanese patients with sickle cell anaemia: 66 patients in a steady state and 34 in vaso-occlusive crisis. Their coagulation profiles and haematological parameters were measured and compared with 50 age and sex matched normal subjects as control, who attended Gaafer Ibn Auf hospital in Khartoum state, Sudan. Lower Hb level and higher TWBC count was observed among SCA patients when compared with controls (*p* value 0.000). Mean D-Dimer level, fibrinogen level and TAT were significantly higher among SCA cases in steady state than controls (*p* value 0.000, 0.000 and 0.005 respectivly). Further increases in D-Dimer level, fibrinogen level and TAT were observed among SCA patients with vaso-occlusive crisis than those in steady state (*p* value 0.000 for all parameters).

**Conclusion**: SCA is associated with reduction of Hb and increased of D-Dimer, fibrinogen and TAT level and may be used as probable indicator for hypercoagulability state which may lead to thrombotic complications.

**Key words:** SCA, D-Dimer, Fibrinogen, TAT. 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 property is due to a single nucleotide change in the β-globin gene leading to substitution of valine for glutamic acid at position 6 of the β-globin chain ($\beta^{6\ glu\rightarrow val}$) (2). The homozygosity of sickle cell genes (HbSS) results in SCA, while the heterozygosity results in other sickle cell diseases (SCD) which include sickle cell trait with one sickle cell gene and a normal haemoglobin gene (HbAS), and a double heterozygosity of a sickle cell gene with other abnormal haemoglobin variants gene (e.g HbSC)(3). SCD is characterized by chronic intravascular and extravascular haemolysis. Sickling- induced membrane fragmentation and complement-mediated lysis cause intravascular destruction of red cells. Membrane damage also leads to extravascular haemolysis through entrapment of poorly deformable cells or uptake by macrophages (4).

SCA is associated with a hypercoagulable state that may contribute to certain morbidities such as vaso-occlusion and cerebrovascular accidents (6). It is noted that decreased levels of natural anticoagulant proteins are observed in SCA and even more so in vaso-occlusive crisis (VOC) (7,8). These reduced levels may be a consequence of chronic consumption arising from increased thrombin generation which occurs in the vascular endothelium (9). Numerous studies provide laboratory evidence of a hypercoagulable state in patients with SCD (10,11,12,13). This hypercoagulable state has been documented by various abnormalities of cytokines, coagulation markers, and increased phosphatidylserine exposure (14). An important component of the hypercoagulable state is increased thrombin production. Recent evidence reveals that procoagulant microparticles play an important role in thrombin production (15). This accentuated thrombin production is supported by findings of an elevated D-Dimer, thrombin-antithrombin complex (TAT) and prothrombin fragment 1.2 in steady state (16). Subsequent studies have also determined that this hypercoagulable state increases further during sickle cell crisis (12). An increased plasma D-Dimer, prothrombin fragment 1.2, and TAT complexes during an acute vaso-occlusion are also suggestive of thrombin generation as a potential role in the pathophysiology of VOC (14).

The sickle mutant gene has the highest frequency of occurrence in Central Africa. SCA is particularly common among people whose ancestors come from Sub-Saharan



*Corresponding author: Mahdi H. A. Abdalla | E-mail: mahdi_razig@hotmail.com

Africa, South America, Cuba, Central America, Saudi Arabia, India, and Mediterranean countries such as Turkey, Greece, and Italy (4).

In Sudan, sickle cell anaemia is one of the major types of anaemia especially in western Sudan where the sickle cell gene is frequent especially in males of migrating West African tribes to Sudan particularly Hosa, Folani and Bargo. The high prevalence was detected among the Baggara tribe group that includes Hawazma and Meseria (5). Little is known about the clinical feature, diversity and severity of sickle cell anaemia among Sudanese patients. This study aimed to determine the coagulation profile (D-Dimer, Fibrinogen level and TAT) and haematolgical feature of sickle cell anaemia in Sudan

**MATERIALS AND METHODS:**

This is a prospective cross-sectional study included 100 patients with sickle cell anaemia either with VOC or in steady state (defined as a ≥ 4 weeks from an acute illness and ≥10 weeks post-transfusion) who have attended, or hospitalized in, Gaafer Ibn Auf hospital in Khartoum state, Sudan; and age and sex matched 60 apparently healthy controls (HbAA). Subjects with recent surgery, trauma, known history of diabetes mellitus, cardiopulmonary disease, autoimmune disease and malignancy were excluded from the study. Five ml of venous blood was collected from each subject: 2.5 ml in 3.8% trisodium citrate (9:1 vol/vol), kept on ice until centrifugation at 2500g for 30 minutes at 4°C, plasma samples were immediately frozen and stored at - 80°C for subsequent coagulation analysis; and 2.5 ml in EDTA for the blood count. Laboratory analysis was performed at the Department of Haematology, Faculty of Medical Laboratory Sciences, Alneelain University.

Blood cell count was performed by automated cell counter (Sysmex KX-21N).

Fibrinogen level was measured by Clauss modified method using a test kit (TECHNOCLONE GMBH, AUSTRIA). The method uses a functional assay based upon the time for fibrin clot formation, in brief, Diluted plasma is clotted with a high concentration of thrombin, and the concentration of fibrinogen is determined by comparing the plasma clotting time to a calibration curve of a reference plasma with a series of dilutions (1:5 –1:40). D-Dimer was measured using i-CHROMA$^{TM}$ system (Boditech–Korea). The test uses the sandwich immunodetection method. D-Dimer is bound with an antibody in buffer and the antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix. Signal intensity of fluorescence on detection antibody reflects the amount of the antigen captured and is processed by iCHROMA$^{TM}$ Reader to show D-Dimer concentration in the specimen. The working range of i-CHROMATM D-Dimer test is 50 – 10,000 ng/ml. TAT was measured by ELISA, affinity-purified antibody to human thrombin is used to capture thrombin and thrombin-inhibitor complexes in the sample. A peroxidase conjugated second antibody to ATIII is added to the plate to bind to the captured TAT complexes. The peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of $H_2SO_4$ and the colour produced is quantified using a microplate reader.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient's data was performed using the t-test. Results with p value < 0.05 were considered statistically significant.

**RESULTS:**

Patients included 38 males and 62 females; their median age was 4 years, with minimum age of 1 year and maximum of 33 years. All patients were tested for the blood cell count, D-Dimer level, Fibrinogen level and TAT.

The results of the blood count for SCA cases were as follows: Mean haemoglobin (Hb) concentration 64.6±11.3g/L; mean red blood cell (RBC) count 2.38±.46X$10^{12}$/L; mean packed cell volume (PCV) (20±3.5 %); mean cell volume (MCV) 86±7.8fl; mean cell haemoglobin (MCH) 27±2.8pg; and mean cell haemoglobin concentration (MCHC) 316±24 g/ L; mean total white cells (TWBC) count 19±9.4X$10^{9}$/L and mean platelets count 383 ±160X$10^{9}$/L. While for the control group were as follows: Mean Hb concentration 122±28 g/L; mean RBC count 4.3±1.1X$10^{12}$/L; mean PCV 36±8 %; MCV 88±3.8 fl; MCH 27±2 pg; mean MCHC 346±13 g/L; mean WBCs count 5.6 ±1.4 X$10^{9}$/L and mean platelets count 354 ±129 X$10^{9}$/L. All patients were anaemic, with maximum Hb level of 99 g/L. TWBCs was elevated in 88% among patients; mean WBCs count was significantly higher among cases than controls (*p* value 0.000). Platelets count was elevated in (29%) among patients, with no significant difference between cases and controls (*p* value 0.276). No significant differences were observed in the haematological parameters between patients with VOC and those in steady state (data were shown in table 1).

Mean D-Dimer level for patients in steady state was 1400.14±1040.45ng/ml, the levels were elevated in all patients, and were always normal among the control group with a mean level of 238±166 ng/ml. Mean D-Dimer level was significantly higher among the cases in steady state when compared with the control (*p* value 0.000). Further elevation in mean D-Dimer level was



 

observed among SCA patients with VOC than those in steady state with a mean value of 3512.91±3474.48 ng/ml (*P* value 0.000).

**Table 1: Blood count data between SCA patients with VOC and SCA patients in steady state**

| Parameter | Crsis | study | P.value |
|---|---|---|---|
| Hb mean±SD (g/l) | 64.5±12.5 | 64.5±10.7 | 0.233 |
| RBC mean±SD ($X10^{12}$/L) | 2.3±.51 | 2.3±0.43 | 0.701 |
| PCV mean±SD (%) | 20±4.3 | 20±3.0 | 0.316 |
| TWBC mean±SD ($X10^{9}$/L) | 19±9.6 | 20 ±9.4 | 0.369 |
| Platelets mean±SD ($X10^{9}$/L) | 383±138 | 383 ±145 | 0.982 |

Mean fibrinogen level for cases in steady state was 435.00±93.86 mg/dl, and for control was 323.50±47.51 mg/dl. Fibrinogen level was elevated in all patients, and always normal among the control with significant difference (*p* value 0.000). The level was also significantly higher among patients with VOC (mean 497.35±118.66 mg/dl) than patient is steady state (*P* value 0.000).

Mean level of (TAT) for cases in steady state was (2.55±2.04ng/mL), and for control was (0.58±0.08ng/mL). The level was significantly higher among cases than controls (*p* value 0.005), with further elevation among patients in crisis than those in steady state with a mean value of 5.21±5.03 ng/mL (*P* value 0.000).

**DISCUSSION:**

The present study included 100 Sudanese patients with sickle cell anaemia, 66 in a steady state and 34 in VOC. Their haematological parameters and coagulation profiles were measured and compared with 50 age and sex matched normal subjects as control. Most of patients (88%) have an elevated TWBC; mean TWBC was significantly higher among cases than controls. Leucocytosis was also noticed in previous study done in Sudan (17). Adegoke and Kute also reported an elevated TWBC count among SCD Nigerian children (18). This result was expected considering the degree of chronic haemolysis, vulnerability to overwhelming infections and chronic pain in sickle cell patients.

In this study we utilized a quantitative measurement for the determination of D-Dimer level, fibrinogen level and TAT. We observed a significant increase in mean D-Dimer level among SCA patients in steady state than controls, Abouh and Abdalla reported similar finding among Sudanese SCA patient in steady state (17) Mean D-Dimer level was increased by more than two fold among patients with crisis than those in steady state. This finding confirms that activation of the coagulation and fibrinolytic systems in the steady state is further escalated during VOC in patients with SCA.

Sickle cell anaemia is characterized by a hypercoagulable state with increased thrombin and fibrin generation, increased tissue factor procoagulant activity, and increased platelet activation, even when they are in a non-crisis, steady state. Furthermore, thrombosis may contribute to the pathogenesis of several SCA related complications. Thrombin generation is coupled with an increased fibrinolytic activity leading to increased D-Dimer levels (19). The present study showed increased fibrinogen level in patients with SCA in a comparison to controls, our finding is in agreement with previous study done in Jamaica (20). Our study showed increased TAT among patients in steady state than control, this finding is in agreement with previous report (21). Further increase in TAT was observed among patients in crisis. Our findings confirm a hypercoagulable state among SCA patients in the steady state with further escalation during VOC. No single mechanism explains the vaso-occlusion seen in SCD, and the complexity of the process of vaso-occlusion provides many possibilities for therapeutic intervention (22). External exposure of phosphatidylserine alters the adhesive properties of RBCs (23) and appears to be involved in the haemostatic changes observed in haemolytic anaemia, particularly SCD (24-27). Tissue factor is abnormally expressed in circulating endothelial cells in patients with SCD (28). Microparticles released during haemolysis may be tissue factor–positive (29).

**CONCLUSION:**

This study confirms the hypercoagulable state in SCA patients in steady state with higher D-Dimer levels, fibrinogen level and TAT with further escalation during VOC among the Sudanese population in a comparison with the control groups. The study highlights haematological reference values for Sudanese patients with SCA.



 

**ACKNOWLEDGEMENT:**
Special thanks to the Staff of Haematology Department, Faculty of Medical Laboratory Sciences, Alneelain University, ad to the staff of Gaafer Ibn Auf hospital in Khartoum state, Sudan.

**AUTHOR CONTRIBUTION:**
Idris M. M. Hamid and Mahdi H. A. Abdalla conceived the idea of the study, collected and analyzed samples and data and wrote the manuscript. Rashad M. O. Mahmoud and Ghada M. Merghani helped with samples analysis and manuscript drafting.

**REFERENCES:**

1. Quirolo K, Vichinsky E. Haemoglobin disorders. In: Behrman RE, Kliegman EM, Jenson HB editors. Nelson textbook of pediatrics. 17 th ed. Philadephia: WB Saunder. 2004; 1623-34.
2. Livingstone FB. Abnormal haemoglobins in human populations. 1975; 1-12.
3. Setty BN, Rao AK, and Stuart MJ. Thrombophilia in sickle cell disease: the red cell connection Blood. 2001; 98: 3228-3233.
4. A Victor Hoffbrand. Daniel Catovsky. Edward GD Tuddenham. Anthony R Green. Postgraduate Haematology. Sixth edition. 2010.
5. Nitin J. A Review of Clinical Profile in Sickle Cell Traits. Oman. Med. J;2010 25: 3-8.
6. Nduka N, Owhochuku SM, Odike P. Current observations on sickle cell genotype in Nigeria. East Afr Med J .1993;70:646-9.
7. Westerman MP, Green D, Gilman-Sachs A, Beaman K, Freels S, Boggio L, et al. Coagulation changes in individuals with sickle cell trait. Am J Hematol 2002;69:89-94.
8. Wright JG, Malia R, Cooper P, Thomas P, Preston FE, Serjeant GR. Protein C and S in homozygous sickle cell disease: Does hepatic dysfunction contribute to low levels? Br J Haematol 1997;98:627-31.
9. Lane PA, O'Connell JL, Marler RA. Erythrocyte membrane vesicles and irreversibly sickled cells binding protein S. Am J Hematol. 1994;47:295-300.
10. Ataga KI, Cappellini MD, Rachmilewitz EA. Beta-thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. Br J Haematol. 2007 ;139:3–13
11. Francis RB., Jr. Elevated fibrin D-dimer fragment in sickle cell anemia: evidence for activation of coagulation during the steady state as well as in painful crisis. Haemostasis. 1989;19:105–111.
12. Stuart MJ, Setty BN. Hemostatic alterations in sickle cell disease: relationships to disease pathophysiology.Pediatr Pathol Mol Med. 2001 ;20: 27–46.
13. Noubouossie DF, Le PQ, Corazza F, Debaugnies F, Rozen L, Ferster A, et al. Thrombin generation reveals high procoagulant potential in the plasma of sickle cell disease children. Am J Hematol. 2011.
14. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: new approaches to an old problem. Hematology Am Soc Hematol Educ Program. 2007:91–96.
15. Ardoin SP, Shanahan JC, Pisetsky DS. The role of microparticles in inflammation and thrombosis. Scand J Immunol. 2007 ;66:159–165.
16. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared with healthy black controls. Blood Coagul Fibrinolysis. 1995;6:93–99.
17. Alaeldin M. E. Abouh and Mahdi H. A. Abdalla. D-dimer level in sudanese children with sickle cell anaemia. 2014; 6:6599-6601
18. Adegoke SA and Kute BP..Evaluation of clinical severityof sickle cell anemia in Nigerian children. Journal ofApplied Hematology. 2013; 4: 58-64.
19. Fakunle EE, eteng KI, and Shokunbi WA. D-D dimer levels in patients with sickle cell disease during bone pain crises and in the steady state. Pathology and Laboratory Medicine International. 2012;4: 21-25.
20. Leslie J, Langler D, Serjeant GR, Serjeant BE, Desai P, Gordon YB. Coagulation changes during the steady state in homozygous sickle-cell disease in Jamaica. Br J Haematol 1975;30:159–166.
21. Colombatti R, De Bon E, Bertomoro A, Casonato A, Pontara E, Omenetto E, Saggiorato G, Steffan A, Damian T, Cella G, Teso S, Manara R, Rampazzo P,Meneghetti G, Basso G, Sartori MT, Sainati L. Coagulation activation in children with sickle cell disease is associated with cerebral small vessel vasculopathy . 2013 ; 25:8-78801.
22. Kotila TR, Fawole OI, Shokunbi WA. The role of Haemoglobin F on the clinical severity of sickle cell disease in Nigerian patients. Afr J Med Med Sci. 2000; 29:229–231.
23. Setty BN, Kulkani S, Stuart MJ. Role of erythrocyte phosphatidylserine in sickle red cell-endothelial adhesion. Blood. 2002;99:1564–1571.
24. Setty BN, Rao AK, Stuart MJ. Thrombophilia in sickle cell disease: the red cell connection. Blood. 2001 ; 98:3228–3233.
25. Setty BN, Kulkani S, Rao AK, Stuart MJ. Fetal hemoglobin in sickle cell disease. Relationship to erythrocyte phosphatidylserine exposure and coagulation activation. Blood. 2000;96:1119–1124.
26. Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, Rachmilewitz EA, Eldor A. Phosphatidylserine in the



 

outer leaflet of red blood cells from β-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. Am J Hematol. 1993;44:63–65.

**27.** Wandersee NJ, Lee JC, Deveau SA, Barker JE. Reduced incidence of thrombosis in mice with hereditary spherocytosis following neonatal treatment with normal hematopoietic cells. Blood. 2001;97:3972–3975.

**28.** Key NS, Slungaard A, Dandelet L, et al. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. Blood. 1998; 91: 4216–4223.

**29.** Shet AS, Aras O, Gupta K, et al. Sickle blood contains tissue factorpositive microparticles derived from endothelial cells and monocytes. Blood. 2003; 102: 2678–2683.



 