

## Research Article

# Coagulation Profile, Platelets and Endothelial Activation Markers among Patients with Steady State Sickle Cell Anemia

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**Background and Objective:** Sickle Cell Anemia (SCA) causes multiple organ damage resulted from small blood vessels that leads to many vascular complications as acute chest syndrome, cerebral vascular accidents and avascular necrosis; this study designed to measure coagulation profile, platelets and endothelial activation markers among patients with steady state SCA.

**Material and Methods:** Sixty sickle cell anemia Saudi patients and sixty apparently healthy age- and sex-matched non-sickle cell disease subjects were involved in this study.

**Results:** The mean values of the coagulation parameters (PT, APTT, TT, Platelet count, Bleeding time) were significantly elevated in stable-state SCA patients when compared with normal subjects. While, the mean value of platelets activation markers (Soluble CD40L, Soluble F1.2, Serum thromboxane and Soluble P-Selectin) were significantly elevated in stable-state SCA patients when compared with healthy subjects. Also, the mean value of endothelial activation and inflammation markers (ICAM-1, VCAM-1, E-selectin and sCD40) were significantly elevated in stable-state SCA patients when compared with healthy subjects.

**Conclusion:** This study approved that Saudi patients in steady state SCA have prolonged coagulation indices and altered markers of platelets and endothelial activation when compared with normal subjects.

**Keywords:** Coagulation profile; Platelets activation markers, Endothelial activation markers; Sickle cell anemia; Steady state

**Introduction**

Sickle Cell Anemia (SCA) is a hematologic disorder leads to multiple organs irreversible damage [1]. However, recurrent vascular occlusion and chronic hemolysis that enhanced by leukocyte and red blood cells adhesion has been reported in patients with SCA [2]. Moreover, disorders of blood coagulation profile, abnormal inflammatory cytokines and endothelial dysfunction were found to be associated with SCA [1-3]. The severity of clinical presentation ranges from mild degree to life-threatening degree [4].

Sickle cell disease is characterized with prolonged Prothrombin Time (PT) and Activated Thromboplastin Time (APTT) [5,6]. In addition, platelet counts and platelets activation markers (P-selectin and CD40L) are usually increased among SCA in steady state [7-9]. While, endothelial activation biomarkers (vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1 and E-selectin) are usually elevated among patients with SCA [10-12].

Microvascular occlusion is the main cause of organ damage and recurrent attacked of painful crises in SCA. However, systemic inflammatory stimuli and endothelial dysfunction that induced by sickle cells restrict the microcirculation [13]. More over increased levels of endothelial function biomarkers as intercellular adhesion

molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and E-selectin play a pivotal role in painful SCA crises [12,14].

However, Sickle cell anemia causes multiple organ damage resulted from small blood vessels that leads to many vascular complications as acute chest syndrome, cerebral vascular accidents and avascular necrosis[18]. Many changes in the hemostatic system in SCA patients has been reported as fibrinolysis activation and excess in thrombin generation [13]. These changes are seen in SCA both in vaso-occlusive crises (VOC) and steady state [15,16]. Some documented abnormalities in fibrinolytic system in SCA include reduced plasminogen concentration [17], elevated D-dimer [18] and defective release of tissue plasminogen activator (tPA) [19]. Moreover, excessive thrombin generation, activation of platelet, decreased circulating anticoagulants levels and contact factors has been reported [15].

This study designed to measure coagulation profile, platelets and endothelial activation markers among patients with steady state SCA.

**Subjects and Methods****Subjects**

One hundred twenty subjects were enrolled; sixty healthy subjects and sixty patients with SCA in steady state were selected from

**Table 1:** Comparison of demographic and hematologic variables between patients with SCA (group A) and healthy subjects group (B).

	Group (A)	Group (B)	Significance
Age (year)	32.83 ± 6.12	35.16 ± 4.97	0.1672
BMI (kg/m <sup>2</sup> )	18.85 ± 4.92	20.26 ± 4.35	0.0695
Hemoglobin (g/dL)	7.45 ± 3.62 <sup>*</sup>	12.92 ± 3.17	0.0046
Red blood cells (10 <sup>12</sup> L)	2.36 ± 1.22 <sup>*</sup>	4.82 ± 2.31	0.0017
white blood cells (10 <sup>9</sup> L)	11.42 ± 3.78 <sup>*</sup>	5.36 ± 1.96	0.0003

BMI: Body Mass Index; (\*) indicates a significant difference between the two groups,  $P < 0.05$ .

Department of Hematology, King Abdulaziz University Hospital. The mean age was 35.16±4.97 years for healthy subjects (range 23-45 years) and 32.83±6.12 years for patients with SCD (range 22-43 years). Diagnosis of SCA participants was confirmed by using hemoglobin electrophoresis equipment, however, steady state of Sickle cell anemia was confirmed if the patient did not receive blood transfusion during the previous 120 days and not have acute episodes (vaso-occlusive or infective crisis) for at least 30 days before participation in the study [20]. Exclusion criteria included cancer, hypertension, pregnancy, contraceptive pills, anticoagulant medications, cardiopulmonary disorders, diabetes mellitus and patients received blood transfusion within the previous 120 days. All participants signed a written informed consent and ethical approval from the ethical committee, Faculty of Applied Medical Sciences, King Abdulaziz University has been obtained. All participants were enrolled equally in group (A) patients with SCA and group (B) healthy subjects.

## Measurements

**A. Determination of coagulation profile:** Both plasma level of prothrombin time was detected by adding 0.1 ml of both plasma placed in a water bath to 0.1 ml of thromboplastin and calcium. However, activated partial thromboplastin time in kaolin was detected by mixing equal volumes of kaolin suspension and the phospholipids reagent. Moreover, hemoglobin concentration and platelet count was measured using automated Sysmex KX-21N model [21].

**B. Determination of platelets activation markers:** Flow cytometer (FACS Calibur cytometer and Cell Quest Pro software, San Jose, CA) was used to determine platelets activation markers. Soluble CD40L (Quantikine Human CD40 Ligand Immunoassay, R&D Systems, Minneapolis, MN) and P-selectin (Human P-Selectin ELISA, R&D Systems, Minneapolis, MN) were assessed in plasma prepared from blood samples collected into ethylenediamine tetra acetic acid (EDTA) and centrifuged at 1000 g for 15 minutes within 30 minutes of collection. Samples for the CD40L assay were centrifuged for an additional 10 minutes at 10,000g [22,23].

**C. Determination of endothelial activation markers:** The serum samples was stored at -80°C to be used by ELISAs in order to measure levels of ICAM-1 and VCAM-1, and E-selectin, (R&D Systems) that considered as endothelial activation markers.

## Statistical analysis

SPSS version 17 (Chicago, IL, USA) was used for statistical analysis via independent “t” test to compare the investigated parameters between both groups ( $P < 0.05$ ).

**Table 2:** Mean value and significance of coagulation profile of patients with sickle cell anemia (group A) and healthy subjects group (B).

	Group (A)	Group (B)	Significance
Prothrombin time (seconds)	12.87±2.41 <sup>*</sup>	10.67±2.33	0.0268
APTT (seconds)	41.98±5.75 <sup>*</sup>	37.65±5.42	0.0312
Thrombin time (seconds)	12.36±2.17 <sup>*</sup>	9.73±2.18	0.0153
Bleeding time (minutes)	3.21±1.15 <sup>*</sup>	3.94±1.23	0.0278
Platelet Count (×10 <sup>9</sup> )	298.43±36.72 <sup>*</sup>	217.26±28.22	0.0114

APTT: Activated Partial Thromboplastin Time; (\*) indicates a significant difference between the two groups,  $P < 0.05$ .

## Results

One hundred twenty subjects were enrolled; sixty healthy subjects and sixty patients with steady state SCA. The mean age was 35.16 ± 4.97 years for healthy subjects (range 23-45 years) and 32.83 ± 6.12 years for patients with SCD (range 22-43 years), the two groups were considered homogeneous regarding the demographic variables (Table 1). There was significant differences in hemoglobin, red blood cells and white blood cells between both groups.

The mean value of coagulation profiles (PT, APTT, TT, Platelet count, Bleeding time) were significantly elevated in stable-state SCA patients when compared with normal subjects (Table 2). Also, the mean value of platelets activation markers (Soluble CD40L, Soluble F1.2, Serum thromboxane and Soluble P-Selectin) were significantly elevated in stable-state sickle cell anemia patients when compared with normal controls (Table 3). Moreover, the mean value of endothelial activation and inflammation markers (ICAM-1, VCAM-1, E-selectin and sCD40) were significantly elevated in stable-state sickle cell anemia patients when compared with normal subjects (Table 4).

Organ damage and painful crises is a common problem facing patients with SCA [6]. Elevation in platelet activation and thrombin generation along with reduced level of circulating anticoagulants are the main changes in the hemostatic system in SCA [13]. Results of this study proved that patients with SCA in steady state have prolonged coagulation indices and altered markers of platelets and endothelial activation when compared with those with normal subjects. These findings agreed with prior studies of SCA patients in the non-crisis steady state relative to subject of normal hemoglobin genotype [24-26].

Our results showed a prolonged thrombin time and increased platelets count among SCA patients when compared with control subjects. This agreed with Ataga et al. and Noubouossie, et al found

**Table 3:** Mean value and significance of platelet activation markers (Soluble CD40L, Soluble F1.2, Serum thromboxane and Soluble P-Selectin) of patients with SCA (group A) and healthy subjects group (B).

	Group (A)	Group (B)	Significance
Soluble CD40L (pg/ml)	583.31±64.26 <sup>*</sup>	275.14±37.82	0.0143
Soluble F1.2 (nmol/L)	1645.81±174.12 <sup>*</sup>	287.63±31.54	0.0188
Serum thromboxane B2 (ng/mL)	218.51±23.26 <sup>*</sup>	119.27±15.92	0.0216
Soluble P-Selectin (ng/ml)	39.45± 6.72 <sup>*</sup>	27.13±5.28	0.0274

CD40L: CD40 ligand; F1.2: prothrombin fragment 1.2; (\*) indicates a significant difference between the two groups,  $P < 0.05$ .

**Table 4:** Mean value and significance of endothelial activation and inflammation markers of patients with SCA (group A) and healthy subjects group (B).

	Group (A)	Group (B)	Significance
ICAM-1	14.82 ± 3.75 <sup>*</sup>	13.11 ± 3.16	0.0145
VCAM-1	18.94 ± 4.82 <sup>*</sup>	10.51 ± 2.71	0.0261
E-selectin	4.87 ± 1.63 <sup>*</sup>	1.77 ± 0.68	0.0079
sCD40	953.16 ± 42.51 <sup>*</sup>	537.22 ± 25.13	0.0116

ICAM-1: Intercellular Adhesion Molecule; VCAM-1: Vascular Cell Adhesion Molecule; (\*) indicates a significant difference between the two groups,  $P < 0.05$ .

increased formation rate of thrombin among SCA patients in the non-crisis steady state relative to subject of normal hemoglobin genotype [3,27]. Also, Nilesh et al. stated that fibrinogen levels were found to be raised and prolonged Prothrombin Time (PT) and Activated Thromboplastin Time (APTT) in addition to larger platelets are more thrombogenic in SCA patients in the non-crisis steady state relative to subject of normal hemoglobin genotype [5]. While, Freedman and Karpat kin reported that platelets count was increased among 8 SCA adult patients [28].

Platelets activation markers were founded to be increased among patients with SCA relative to normal subjects, these findings agreed with prior studies of patients with SCA in the non-crisis steady state that included biomarkers of platelet activation and coagulation found similar results [29-31]. Similar to Wun et al. found increased level of P-selectin and CD40L in flow cytometric studies of patients with SCA [7,32]. We also observed elevations in levels of soluble F1.2 and TXB2 in patients with SCA that agreed with Stuart and Setty found that level of F1.2 and Thrombin Anti-Thrombin (TAT) complexes increased among SCA patients [13].

Finally, our results confirmed SCA patients had increased endothelial activation markers relative to healthy subjects, these findings are consistent with prior studies of Setty et al. and Ataga et al. reported increased level of VCAM, E-select in and P-select in among SCA patients relative to normal population [13,33]. Also, Blum et al. proved that the level of CD40 was increased among SCA patients [34]. Similarly, Sakamoto et al. stated that steady-state SCA patients had abnormal level of adhesive and inflammatory biomarkers in comparison to from healthy control individuals [10]. The possible explanation of increased activation of endothelial cells among SCA patients in steady state is that Monocytes from sickle cell patients are highly activated that induce more expression of endothelial activation markers[35].

## Conclusion

This study approved that Saudi patients in steady state SCA have prolonged coagulation indices and altered markers of platelets and endothelial activation when compared with normal subjects.

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