BIOMARKERS OF COAGULATION ACTIVATION IN CHILDREN WITH SICKLE CELL DISEASE

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ABSTRACT

Sickle cell disease (SCD) is a multisystem disease, characterized by its multi-phenotypic diversity although being a disease caused by a single nucleotide substitution.In the present study, we measured biomarkers of coagulation activation in children with SCD. The study was performed on 50 Egyptian children with SCD and 41 age- and sex-matched controls. Complete blood picture (CBC), International normalized ratio (INR)and Partial thromboplastin time (PTT) levels were measured. Thrombin-antithrombin (TAT) plasma levels was estimated by enzyme-linked immunosorbent assay (ELISA). A statically significant increase in all of INR, PTT and TAT plasma levels were detected. We agree with the previous studies that children with SCD exhibit a hypercoagulable state.

Key words: Sickle cell disease – Hypercoagulability– Thrombin-Antithrombin.

1.Introduction

Sickle cell disease (SCD) is the first human inherited disease shown to be linked to a single amino acid mutation caused by a single nucleotide substitution in a known gene (Kato, 2015). Glutamic acid is replaced by valine on the β globin chain of hemoglobin molecule results in production of an abnormal sickle hemoglobin (HbS),HbS polymerized into rigid fibers when deoxygenated that cause deformation of the erythrocytes **(Elias *et al*., 2012)**.

Sickle cell abnormal shape causes various complications in patients with SCD, haemolysisand Vaso-occlusion are the most common complications. Haemolysis results in high free hemoglobin levels within circulation which causes reduction in Nitric oxide (NO), a vasodilator, bioavailability and contributing to the vaso-occlusivion**(Elias *et al*., 2012)**. SCD is associated with increased procoagulant factor as circulating tissue factor (TF), increased markers of thrombin generation, decreased levels of natural anticoagulant proteins, and evidence of platelet and fibrinolytic system activation **(Key *et al*., 1998; Nishank*et al*., 2013)**.

Exposure of phosphatidylserine (PS) on sickle cell surface functions as a recognition signal for cell removal during apoptosis of nucleated cells, a docking site for enzymatic complexes involved in coagulation and anticoagulation pathways and alters the adhesive properties of sickle RBC **(Stowell*et al*., 2009; Uchida *et al*., 2011; Artemenko*et al*., 2016)**. Over expression of TF on the surface of each platelet, endothelial cells and circulating microparticles (MP), derived from endothelial cells and monocytes. MP, small membrane–derived vesicles released by cells following activation or apoptosis, may be derived from RBC, platelets, endothelial cells and monocytes according to its origin they express TF or PS on their surfaces on both cases a state of coagulation activation occurs **(Shet*et al*., 2003)**. In our study we investigate the hypercoagulable state in Egypchildren with SCD.

Materials and methods

Study subjects

This study included fifty children (24 males and 26 females) with SCD, their ages range from 2 to 18 years. Patients were

diagnosed as SCD patients according to hemoglobin electrophoresis pattern. Referring patients were invited to participate in the study during their regular follow-up visits in Pediatric Hematology Clinic, New Children Hospital, Cairo University. Patients participated after informed consents were freely obtained from their guardians. Forty one age- and sex-matched healthy children free from hematological disorders were studied as a control group.Our study was approved by the Ethical Committee of Kasr Al-Ainy School of Medicine, Cairo University.

Blood sampling

Venous blood samples were withdrawn from both patients and control under aseptic conditions and divided into two tubes, ethylenediaminetetraacetic acid (K-EDTA) tube for complete blood cell (CBC) count, and other sodium citrated tube for coagulation profile.

Hematological analysis

CBC was performed by PENTRA-80 automated blood cell analyzer (ABX Diagnostics, Montpellier, France).

Coagulation profile

Plasma was obtained by centrifugation of citrated tube at 4ºC for 20 minutes at 2,200 g (within 15 min after blood collection). International Normalized Ratio (INR) and Partial thromboplastin time (PTT) were tested by Stago fully automated instrument (STA Compact; DiagnosticaStago S.A.S., Gennevilliers, Paris, France) using commercial reagents and following the standard procedures for each test [thromboplastin reagent (STA – Neoplastine CI Plus 10) for prothrombin time (PT) and STA – C.K. PREST 5 for activated partial thromboplastin time (APTT)].

For TAT measurement, Citrated plasma was stored at -20ºC for AT antigen measurement which quantified by thrombin-antithrombin complex-derived human enzyme-linked immunosorbent assay kit (AbCam China, Shanghai, China).

Results

In the present study, 50 children with sickle cell disease and 41 free from hematological disorders individuals as control group were enrolled.In **Table 1** both control and SCD subjects' demographic parameters were illustrated.

Table 1. Demographic parameter of SCD patients and control subjects.

Parameter	**Control n=41**	**SCD n=50**	**p-value**
Gender (*Male/female*)	15/26	24/26	NS
Age Mean ± SD	9 ± 5	9 ± 6	NS

Significance p< 0.05, no significance (NS)

Hematological parameters in both SCD patients and control subjects were shown in **Table 2**. In which, highly statically significant reduction in red blood cells (RBCs) count, hemoglobin (Hb) concentration and hematocrit (HCT) levels ($p<0.001$) with negative correlation in RBCs count($r= -0.791$, $p<0.001$), Hb concentration ($r= -0.791$, $p<0.001$) and HCT ($r= -0.791$, $p<0.001$) were observed in SCD patients.

Where a remarkable increase in RBCs indices; mean corpuscular volume (MCV) ($p<0.01$), mean corpuscular hemoglobin (MCH) ($p<0.001$) and mean corpuscular hemoglobin concentration (MCHC) levels ($p<0.01$) with positive correlation ($r= 0.314$, $p<0.01$; $r= 0.379$, $p<0.001$; $r= 0.268$, $p<0.01$, respectively) were detected in SCD patients compared with controls. Significant increase in WBCs ($p<0.01$), monocytes ($p<0.001$) and granulocytes count ($p<0.01$) in SCD patients than those health control with positive correlation ($r= 0.396$, $p<0.001$; $r= 0.640$, $p<0.001$; $r= 0.357$, $p<0.01$, respectively) was observed.

Table 2. Hematological parameters of SCD patients and control subjects.

Parameters	**Control (n=41)**	**SCD (n=50)**	**p-value**	**Correlation with SCD**
RBC (10^6/µl)	4.61±0.34	2.85±0.87	<0.001	r= -0.791 p<0.001
Hb (g/dl)	12.21±0.97	8.25±2.37	<0.001	r= -0.791 p<0.001
HCT (%)	37.67±2.8	24.46±6.62	<0.001	r= -0.790 p<0.001
MCV (fL)	81.36±4.37	87.69±11.58	<0.01	r= 0.314 p<0.01
MCH (pg)	26.42±1.93	29.47±4.27	<0.001	r= 0.379 p<0.001
MCHC (g/dl)	32.58±1.13	33.67±1.99	<0.01	r= 0.268 p=0.01
Leukocytes (10^3/µl)	7.58±2.43	12.18±9.85	<0.01	r= 0.396 p<0.001
Lymphocytes (10^3/µl)	3.48±1.2	4.29±2.25	<0.05	NS
Monocytes (10^3/µl)	0.37±0.14	1.13±1.11	<0.001	r= 0.640 p<0.001
Granulocytes (10^3/µl)	3.72±1.99	5.76±3.34	<0.01	r= 0.357 P<0.01

All data are presented as mean ± SD (Sinificance p< 0.05, no significance (NS)

Coagulation function parameters' values; platelets count, international normalized ratio (INR), activated partial thromboplastin time (APTT) and thrombin-antithrombin (TAT) were as shown in **Table 3**. A highly measured for patients and control subjects significant increase in each INR, PTT and TAT levels in SCD patients (r=0.565, p<0.00; r=0.741, p<0.001; r=0.234, p<0.05), respectively. Platelets count was insignificant among our both grou

Table 3. Coagulation parameters of SCD patients and control subjects.

Parameter	Control n=41	SCD n=50	p-value	Correlation with SCD
Platelets (10^3/µl)	310.58 ± 82.53	324.08 ± 158.69	NS	NS
INR	1.032 ± 0.034	1.186 ± 0.15	<0.001	r= 0.565 p<0.001
PTT (s)	26.2 ± 2.72	33.71 ± 7.16	<0.001	r= 0.741 p<0.001
TAT (ng/l)	21.19 ± 20.14	32.19 ± 16.32	<0.01	r= 0.234 p<0.01

All data are presented as mean ± SD (Significance p< 0.05).

DISCUSSION

Sickle cell disease is characterized by a chronic inflammatory state**(Platt, 2000; Hebbel *et al*., 2004)**. Patients exhibit elevated leukocyte counts, abnormal activation of granulocytes, monocytes, and endothelial cells**(Soloveyet *al*., 1997; Belcher *et al*., 2000; Inwald*et al*., 2000)**, and increased levels of multiple inflammatory mediators**(Graido-Gonzalez *et al*., 1998; Hebbel *et al*., 2004; Pathare*et al*., 2004; Lee *et al*., 2006)**. In this study,

we found an increase in all leukocytes count and their types; lymphocytes, monocytes and granulocytes in SCD patients compared to control subjects.

In addition, SCD is often referred to as a hypercoagulable state**(Francis Jr, 1991)** because patients manifest increased thrombin and fibrin generation**(Westerman*et al*., 1999; Tomer*et al*., 2001)**increased tissue factor procoagulant activity**(Singh *et al*., 2012)**and increased platelet activation**(Inwald*et al*., 2000; Tomer*et al*., 2001; Lee *et al*., 2006)** even when they are in a non-crisis, steady state. Furthermore, thrombosis may contribute to the pathogenesis of several SCD-related complications. For example, stroke, caused by large vessel obstruction with superimposed thrombosis, often occurs in SCD patients **(Prengler*et al*., 2002)**. Both pulmonary embolism and pregnancy-related venous thromboembolism appear to occur more commonly in SCD patients than in appropriate control patients**(James*et al*., 2006; Stein *et al*., 2006)**. In our cohort, Plasma levels of TAT were shown to be increased in patients with SCD compared to the levels in healthy controls. May be as a result of hypercoagulable state represented in SCD patients a significant increase in INR and APPT values were detected.

Several factors, including abnormal red blood cell phospholipid membrane asymmetry, with increased expression of phosphatidylserine**(Zwaal&Schroit, 1997)** and ischemia-reperfusion injury**(Solovey*et al*., 2004)** appear to contribute to the hypercoagulability observed in SCD patients.Data suggest that type II phosphatidylserine red blood cells (highly phosphatidylserine-positive and including dense sickle cells) cause a 2-fold increase in endothelial tissue factor expression. This appears to be due to the increased hemolysis of these cells rather than to the physical interaction of type II phosphatidylserine red blood cells with the endothelium in patients with SCD**(Setty*et al*., 2006)**. We found a reduction in RBCs count, Hb concentration and hematocrit level, increase in RBCs indices (MCV, MCH and MCHC) in SCD patients compared

to our health subjects.

CONCLUSION

Egyptian children with sickle cell disease indeed exhibit a hypercoagulable state with increase of

thrombin generation factors.

CONFLICT OF INTEREST

Authors declare that no conflict of interest.

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