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RESEARCH ARTICLE

D-DIMER LEVEL IN SUDANESE CHILDREN WITH SICKLE CELL ANAEMIA

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ABSTRACT

Sickle cell anaemia (SCA) is an inherited blood disorder that is characterized by chronic haemolysis and episodes of acute clinical complication. SCA is associated with hypercoagulable state with increased thrombin generation and elevated D-Dimer level which is reported as a marker for SCA related complication. This study aimed to determine the D-Dimer level in Sudanese children with SCA in a steady state and to correlate it with the haematological parameter. Following informed consent, one hundred and one subjects; fourty one children with SCA in steady state, and age and sex matched sixty healthy subjects as controls were enrolled. Blood count was performed by automated cell counter (Sysmex KX-21N). D-dimer was measured using i-CHROMA™. Mean D-Dimer level was significantly higher among SCA cases when compared with the controls (p value 0.000). Mean TWBC count and mean platelets count were significantly higher in the SCA patients than in controls (p value 0.000 and 0.005 respectively). There was no significant correlation between D-Dimer level and all haematological parameters. In conclusion, the study confirms the hypercoagulable state in SCA. The study highlights haematological reference values for Sudanese patients with SCA.

INTRODUCTION

Sickle cell anaemia (SCA) is an inherited blood disorder in which the clinical manifestations arise from the tendency of abnormal haemoglobin (Sickle haemoglobin or HbS) to polymerize and deform red blood cells into a characteristic sickle shape (Bolanos-Meade *et al.*, 1999). This property is due to a single nucleotide change in the β - globin gene leading to a substitution of valine for glutamic acid at position 6 of the β - globin chain (Bunn, 1997). 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 (eg HbSC) (Setty *et al.*, 2001). HbS polymerization is associated with a reduction in cell ion and water content (cell dehydration), and increased red cell density with persistent membrane damage and haemolysis (De *et al.*, 2011). Pathophysiological studies have shown that the dense, dehydrated red cells play a central role in acute and chronic clinical manifestations of SCA, in which intravascular sickling in capillaries and small vessels leads to vaso-occlusion and impaired blood flow (Steinberg, 1999; Solovey *et al.*, 2001). Numerous studies provide laboratory evidence of a hypercoagulable state in sickle cell patients (Francis, 1989; Stuart and Setty, 2001; Noubouossie *et al.*, 2012). This hypercoagulable state has been documented by various abnormalities of cytokines, coagulation markers, and increased phosphatidylserine exposure (Ataga and Key, 2007). An important component of the hypercoagulable state is increased thrombin production (Ardoin *et al.*, 2007). Thrombin generation is coupled with increased fibrinolytic activity leading to increased D-dimer levels and plasmin-anti-plasmin complexes. Levels of the D-dimer are raised in both the acute sickle cell crisis as well as during the steady state and are reportedly markers of sickle cell disease related complications (Nsiri *et al.*, 1996; Tomer *et al.*, 2001; Hagger *et al.*, 1995). Genetic heterogeneity is associated not only with the degree of anemia, but also with many other clinical complications in SCA including pain crisis, prevalence of stroke, leg ulcers, pulmonary hypertension, osteonecrosis, hepatobiliary complications and priapism, among other several clinical aspects (Steinberg and Adewoye, 2006; Steinberg, 2005; Steinberg, 2009). Sickle cell disease is one of the most common inherited diseases worldwide (Ohaeri and Shokunbi, 2001). This disease is particularly common among people whose ancestors come from sub-Saharan Africa, Spanish-speaking regions (South America, Cuba, Central America), Saudi Arabia, Oman, India, and Mediterranean countries such as Turkey, Greece, and Italy (Nitin 2010). Little is known about the clinical feature, diversity and severity of the disease related complications among Sudanese patients. This study aimed to determine the level of D-dimer in Sudanese children with sickle cell anaemia.

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MATERIALS AND METHODS

Following informed consent, one hundred and one children were enrolled: fourty one known sickle cell patients (HbSS) in a non-crisis steady state, defined as a ≥4 weeks from an acute illness and ≥10 weeks post-transfusion; and age and sex matched sixty 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). D-dimer was measured using i-CHROMA™ 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 *i*-CHROMA™ Reader to show D-Dimer concentration in the specimen. The working range of *i*-CHROMA™ D-Dimer test is 50 – 10,000 ng/ml. Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient's data was performed using the t-test and Pearson correlation test. Results with p value < 0.05 were considered statistically significant.

RESULTS

The male: female ratio of the patients was 1.4 and the median age was 11 year, with minimum age of 7 and maximum of 14 years. All patients were tested for the blood cell count and D-Dimer level. Mean D-Dimer level for the patients was 1847.47±2004.92ng/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 when compared with the controls (p value 0.000). The results of the blood count for SCA cases were as follows: Mean haemoglobin (Hb) concentration 60.8±23.4 gram /L; mean red blood cell (RBC) count 2.7±2.0 $X10^{12}$/L; mean packed cell volume (PCV) 20±8 %; mean cell volume (MCV) 73±33fl; mean cell haemoglobin (MCH) 23±9pg; and mean cell haemoglobin concentration (MCHC) 311±46 g/l. While for the control group: Mean Hb concentration 132±26.6 gram /L; mean RBC count 4.8±1.1 $X10^{12}$/L; mean PCV 40±8 %; MCV 83±8 fl; MCH 27±2 pg; and MCHC 320±38 g/l. (Table 1). Mean total white cells (TWBC) count for SCA cases was 15.7 ±14.0 X109/L, and for the controls was 10 ±9 X109/L. Twenty one (51%) of the cases had an elevated TWBC count (11.2 X109/L – 40.9 X109/L). Mean TWBC count was significantly higher in SCA patients when compared with the control group (p value 0.000). Mean platelets count for SCA cases was 317 ±250 X109/L, and for the controls was 271 ±138 X109/L. Although most of the cases 37 of 41 (96%) had a platelets count less than 450 X109/L (within the normal range), mean platelets count was significantly higher among SCA patients when compared with the controls (p value 0.040). There was no significant correlation between D-Dimer level and all haematological parameters.

Table 1. Blood count data between SCA patients and controls

Parameter	Cases	Controls	P value
Hb mean±SD (g/l)	60.8±23.4	132±26.6	0.000
RBC mean±SD ($X10^{12}$/L)	2.7±2.0	4.8±1.1	0.000
PCV mean±SD (%)	20±8	40±8	0.000
MCV mean±SD (fl)	73±33	83±8	0.001
MCH mean±SD (pg)	23±9	27±2	0.000
MCHC mean±SD (g/l)	311±46	320±38	0.022
TWBC mean±SD ($X10^{9}$/L)	15.7 ±14.0	10 ±9	0.000
Platelets mean±SD ($X10^{9}$/L)	317 ±250	271 ±138	0.005

DISCUSSION

In this study we utilized a quantitative approach for the determination of D-dimer level. The study included 41 Sudanese children with sickle cell anaemia in a steady state, their D-dimer levels and complete blood count were measured and compared with 60 age and sex matched normal subjects as control. We observed a significant increase in the mean of the D-dimer level among SCA patients, when compared with the controls. Similar findings, with higher D-Dimer level, in other patient populations have previously been reported (Francis, Jr., 1989; Shah *et al.*, 2012; Fakunle *et al.*, 2012; Dar *et al.*, 2010; Ataga *et al.*, 2012; Colombatti *et al.*, 2013). 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 (Fakunle *et al.*, 2012; Dar *et al.*, 2010). Most of the patients (71%) were severely anaemic with haemoglobin value less than 70 g/l, and maximum haemoglobin value of 85.5g/l. The mean Hb and PCV values are in agreement with previous study done in Sudan (Abbas, 2014). White blood cells count was significantly higher among SCA patients than controls. This result was expected considering the degree of chronic haemolysis, vulnerability to overwhelming infections and chronic pain in sickle cell patients. Leucocytosis was also noticed in SCD Nigerian children and found to be related to the disease severity (Adegoke and Kute, 2013). The mean platelets count is in agreement with previous study (Abbas, 2014). Although most of the cases had a normal platelets count, mean platelets count was significantly higher among the cases when compared with controls. Reduced or absent splenic sequestration of platelets as a result of hyposplenism in SCA may contribute significantly to higher mean platelet counts in SCA. In addition of determining D-Dimer level, this study highlights haematological reference values for Sudanese patients with SCA.

Conclusion

This study confirms the hypercoagulable state in SCA with higher D-Dimer levels among the Sudanese population, in a

comparison with the control groups. The study highlights haematological reference values for Sudanese patients with SCA.

Recommendation

Further studies are needed with an extended coagulation analysis in a correlation with the clinical manifestation to determine the feature and the severity of SCA related complication among the Sudanese population.

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Authors contributions

A.M.E. About and M.H.A. Abdalla conceived the idea of the study, collected and analyzed samples and data and wrote the manuscript.

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