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Original Research Article

Association of hemolysis markers and cortisol level with different severity of sickle cell disease among Sudanese patients

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ABSTRACT

Background: Sickle cell anemia (SCA) is an inherited disorder of hemoglobin. Several biomarkers have been identified, which is essential in the different clinical presentations of the disease. This study aimed to determine the association between hemolysis markers and cortisol level with varying severity groups of Sudanese patients with SCA.
Methods: This descriptive cross-sectional study included 100 patients with sickle cell disease between February 2016 and April 2017. According to Hedo et al scoring, medical history was obtained to conduct disease severity. A total of 3 ml of venous blood was collected from each patient. A complete hemogram was performed using an automated hematology analyzer (Sysmix®-KX-21N). Bilirubin and lactate dehydrogenase (LDH) were estimated using a spectrophotometer, while cortisol was measured using the Elecsys® system 2010 E170. The reticulocyte count was performed manually. Data were analyzed using statistical package for the social sciences (SPSS) version 21 computer software program.
Results: Disease severity was variable and was categorized into; eighteen (18%) patients had mild symptoms, while 70 (70%) patients had moderate disease, and 12 (12%) patients had severe disease. The analysis of variance (ANOVA) test showed that hemoglobin, reticulocyte count, LDH, and direct bilirubin were positively correlated with disease severity, p value: 0.001, 0.04, 0.00, and 0.02, respectively. While indirect bilirubin, total bilirubin, and cortisol did not correlate with disease severity, the p value was (0.248, 0.083, and 0.868, respectively).
Conclusions: This study confirmed that the hemolysis markers (Hb, reticulocyte count, direct bilirubin and LDH) were positively associated with disease severity. In contrast, indirect bilirubin, total bilirubin, and cortisol levels were not associated with the disease severity.

Keywords: Sickle cell disease, Hemolysis markers, Cortisol, Disease severity

INTRODUCTION

SCD is an autosomal recessive disorder resulting from the presence of a mutated form of hemoglobin S (HbS).[1] The clinical course of the disease varies significantly from patient to patient, depending on age, the prognosis of the disease and health status. It includes chronic hemolysis, inflammation and vascular adhesion, recurrent pain

episodes, organ damage, and early death. Hemolysis is the most clinical feature of SCA (HbSS). It is defined as abnormal destruction of red blood cells (RBC) and shortening RBC survival, leading to anemia and pigment gallstones.

Recently, intravascular hemolysis has been discovered to be contributed to a number of other complications of HbSS, such as pulmonary hypertension, priapism, leg ulcers and stroke.[2]

Many studies estimate hemolysis's effect on SCA patients by studying the hemolysis biomarkers. The biomarkers indicate normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention.[3] Biomarkers for hemolysis include: firstly, hemoglobin (Hb) concentration, a useful biomarker in SCD. Low Hb is associated with anemia and other complicated clinical features such as stroke and premature death.[4] The second biomarker is RBC survival. In SCD, the RBC survival is variable ranging from15-17 days; it is not measured for regular clinical use because it is measured by chromium labelling of red cells, which is radioactivity.[5]

Reticulocyte count is another biomarker of hemolysis. It is an indicator of the compensatory response of the bone marrow and correlates well with the RBC life span.[6] Also, serum lactate dehydrogenase (LDH) level is a good biomarker for SCD, and it increases in patients with leg ulcers priapism. LDH levels also tend to increase in erythropoiesis with the premature death of RBCs as in SCD.[7] In addition, haptoglobin is one of the hemolysis biomarkers and is a liver-synthesized glycoprotein that binds freely circulating hemoglobin released by hemolysis or normal erythrocyte turnover.[8] Haptoglobin is decreased in SCD due to increased free plasma Hb.[9] Bilirubin is another biomarker for extravascular and intravascular hemolysis. In SCD, the breakdown of red blood cells results in high bilirubin levels.[10]

Individuals with SCA have stress biomarkers associated with pain intensity.[11] It includes cortisol, which aids in the metabolism, mediates immune response, and the sleep/wake cycle.[12] High cortisol levels likely reflect more severe stress.[13] The severity of SCD was classified into mild, moderate and severe groups according to the degree of anemia, frequency of crisis, and the number of organ complications.[14] Despite the risk of the severity of the disease, information about hemolysis and stress biomarkers in the population suffering from SCA in Sudan are unknown. This study aimed to investigate and determine the association of hemolysis biomarkers and cortisol (one of the stress biomarkers) with disease severity in Sudanese SCA patients.

METHODS

A prospective cross-sectional descriptive study was conducted between February 2016 and April 2017 in Elobied (Sudan). One hundred patients (57% male and 43% female) with sickle cell disease diagnosed by Hb electrophoresis and attended to Sudan SCA center (SSCAC) were enrolled in this study. Their ages ranged from (3.5 month-30 years) with the most affected group ≥3 years.

The study had been approved by the ethical committee of the center. Before sample collection, written informed consent was obtained from all patients or their parents/caregivers. Only patients who refused to donate blood were excluded from the study.

Demographic data was collected from patients' parents to determine the severity of the disease by scoring the clinical presentation according to Hedo et al. The severity score includes the number of crises and previous blood transfusion per year, the concentration of hemogloin and the presence or absence of complications such as pneumonia; osteomyelitis; chest syndrome; heart failure; avascular necrosis of femoral head; renal failure; pigment gallstone and jaundice; liver failure; seizure; growth retardation; dehydrated; acute splenic sequestration; generalized or localized vaso-occlusive crises. The patients were classified into mild, moderate, or severe SCA in the evaluation.

A total of 3 ml of venous blood was collected from each patient under completely aseptic condition, divided into 1.5 ml in EDTA anticoagulant containers for complete blood count determination with Sysmex KX-21N® automated hematology analyzer, and reticulocyte count with the manual method. Then the blood samples were centrifuged to get the plasma for cortisol assay, which was estimated using Elecsys® system 2010 E170 use competition test by using a polyclonal antibody directed against cortisol, and the analyzer automatically calculate the cortisol concentration of each sample in microgram\dl.

The remaining blood was placed into heparin anticoagulant container for LDH and bilirubin estimation using spectrophotometer.

Data were analyzed using statistical package for the social sciences (SPSS) software version 21. The mean of the LDH, bilirubin and cortisol levels in patients of different severity grades was determined using an independent t-test. The correlation of LDH, bilirubin and cortisol level in different severity groups' conditions was determined using analysis of variance (ANOVA). The p value was considered a significant difference at 0.05 and a 95% confidence level.

RESULTS

This study included 100 patients with sickle cell anaemia, of whom 57 (57%) were male, and 43 (43%) were female. Their ages range from (3.5 month to 30 years) with the most affected group being 4 to 6 years. According to the tribe classification, twenty-six (26%) patients were Afro-Asiatic, and an equal number 26 (26%) patients were from

Niger-Congo while 48 (48%) patients were from sub-Saharan origin.

Statistical analysis of the hematological parameters showed that the hemoglobin concentration of the majority of the studied patients, 87% (87/100) had hemoglobin concentration below the normal range also, the descriptive statistical analysis showed that the mean and S.D. of the hemoglobin was (7.69 g/dl±1.71), while 82% (82/100) patients had an abnormal RBCs count (2.87×10^6/l±0.74). The patient's TWBC count in 68% (68/10) was above the normal range. Mean, and standard deviation (SD) of WBC count was (18.63×10^6/l±8.77), while the platelet count was within the normal range in 86% (86/100) patients (450.11×10^6/l±176.02).

Disease severity was variable in the study population using Hedo scoring. 18 (18%) patients had mild disease, while 70 (70%) patients had moderate disease, and only 12 (12%) patients had severe disease.

If hemolysis markers correlate with the severity of the disease; the ANOVA test showed that hemoglobin, reticulocyte count, LDH and direct bilirubin were positively correlated with the P value of disease severity: 0.001, 0.04, 0.00 and 0.02 respectively (Tables 1-3).

Table 1: Correlation of hemoglobin to the severity groups.

Haemoglobin	Mean	Standard deviation	P value
Mild	8.8	2.1	0.001
Moderate	7.9	1.6	
Severe	6.51	1.01	

Table 2: Correlation of reticulocyte count with the severity groups.

Reticulocyte	Mean	Standard deviation	P value
Mild	7.64	1.44	0.04
Moderate	11.80	2.63	
Severe	15.51	4.01	

Table 3: Correlation of LDH to the severity groups.

LDH	Mean	Standard deviation	P value
Mild	459.78	78.51	0.021
Moderate	619.34	63.23	
Severe	782	55.20	

While indirect bilirubin, total bilirubin and cortisol did not correlate with the disease severity, p value (0.248, 0.083 and 0.868 respectively) (Table 4).

Table 4: Correlation of bilirubin and cortisol level with the severity groups.

Parameters	Mean	Standard deviation	P value
D. bilirubin			
Mild	0.53	0.42	0.021
Moderate	0.47	0.33	
Severe	1.20	0.99	
Total	0.50	0.37	
In. bilirubin			
Mild	1.23	0.32	0.248
Moderate	1.40	0.41	
Severe	1.45	0.49	
Total	1.37	0.39	
T. bilirubin			
Mild	1.71	0.49	0.083
Moderate	1.88	0.60	
Severe	2.65	0.49	
Total	1.87	0.59	
Cortisol			
Mild	391	51	0.868
Moderate	378	65.3	
Severe	401	78	
Total	298	55	

DISCUSSION

SCD is a heterogeneous disease with different clinical presentations. Although the disease has been previously described and intensive research has been done on SCA, SCA still poses a major health problem for patients. The relationship between biomarkers and disease severity in Sudanese patients was still unclear. This study was designed to determine the hemolysis markers in different severity groups. This study showed that Hb, PCV and red blood cells were low, a finding consistent with the study by Akinbani et al.[15] The low Hb, PCV and RBCs in SCA are due to chronic hemolysis. The result of the leukocyte count was significantly high in our patients. The high WBC counts in the SCD population could be due to infections or drug reactions.[16] The study was in agreement with Ahmed et al. He found a high WBC count among sickle patients with cough two times than patients without cough.[17] The platelets counts were significantly high in this study, consistent with the study done by Charles et al.[18] The platelet count may increase in response to the vaso-occlusive crisis; hyposplenism in SCA contributes significantly to the high mean of platelets count.[19] Our study showed high reticulocyte count, suggesting increased hemolysis and indicative of sickling crisis. Our result agrees with the study by Tite et al.[20] In this study and oxidative stress associated with hemolysis, serum LDH levels were higher in our patients in contrast to Tite et al.[20]

Our study showed that patients had a significant decrease in Hb levels between the different severity groups (mild, moderate and severe). The low Hb rate in sickle cell

patients is due to chronic hemolysis, which gradually increases with severity. Our findings were in agreement with Tite et al.[20]

In the different clinical phenotype, LDH and direct bilirubin level were elevated, which may be due to intravascular hemolysis; in which RBCs simultaneously release LDH and bilirubin into the bloodstream.[21] Our result was consistent with Aleluia et al.[22]

In this study, cortisol levels were within the normal range. They were not associated with the disease severity in sickle cell disease, and there were no significant differences in mean cortisol levels in men and women. Our result was in agreement with a study done by Marwa in which she found normal Cortisol levels in people with SCA.[23] In contrast, our finding disagreed with Akinlade who found that the cortisol was significantly higher in subjects with severe SCA than mild and moderate one.[24] These differences may be due to ethnic background. So it is important to determine the level of biomarkers in SCA patients and investigate its association with clinical presentation.

CONCLUSION

The study concluded that the hemolysis marker (Hb, reticulocyte count, direct bilirubin and LDH) were positively associated with the disease severity. In contrast, indirect bilirubin, total bilirubin and cortisol level were associated with the disease severity.

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