

Determination of Sick Cell Crisis in Sudanese Children with Sick Cell Anemia

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ABSTRACT

Background: Sick Cell Anemia (SCA) is a genetic hematological disorder characterized by red blood cells that assume an abnormal rigid, sickle shape. This hereditary disorder contributes the equivalent of 3.4% mortality in children aged under 5 worldwide or 6.4% in Africa.

Methods: Some of red blood cell profile (Hb, PCV, RBCs count and RBCs indices), WBCs count and differential count and platelet count and platelet indices and Hb electrophoresis were determined by the automated Hematology Analyzer (SysmexBC-3000plus) and reticulocyte count was counted using supravital stain.

Results: The study was performed on diagnosed 260 children patients with sickle cell disease. Found in this study Frequency of gender among study population in female more than male and insignificant result when Comparison of age, white cells counts, red cells count, hemoglobin, Hematocrit, red cells indices, reticulocyte count and absolute Reticulocyte count among gender of sickle children. Also show insignificant result when Comparison of age, white cells counts, red cells count, hemoglobin, red cells indices, reticulocyte count and absolute Reticulocyte count among age sickle children, however it revealed a significant association with Hematocrit. Significant result show when Comparison of reticulocyte count among hemoglobin group and insignificant absolute Reticulocyte count among hemoglobin group. The Comparison of hemoglobin, reticulocyte count and absolute Reticulocyte count among WBCs. The result show Insignificant. The result were represented significant when Correlation between haemoglobin and reticulocyte count (%).

Conclusion: Insignificant result show when comparison of age, white cells counts, red cells count, hemoglobin, Hematocrit, red cells indices, reticulocyte count and absolute Reticulocyte count among gender of sickle children. The Comparison of white cells counts, red cells count, hemoglobin, red cells indices, reticulocyte count and absolute Reticulocyte count among age group result show insignificant. The Comparison of hemoglobin, reticulocyte count and absolute Reticulocyte count among WBCs. The result show insignificant and also insignificant when correlation between haemoglobin and absolute reticulocyte count. This study concluded to the significant relation between antibiotic use and Monocytes and Basophils. The study Frequency of duration of disease acute, and chronic, and The Frequency of no antibiotic use and antibiotic use. The significant relation between Eosinophils and the symptom and significant relation observed between the Eosinophils and duration of disease. The study showed insignificant correlation between platelet count and platelet indices depending on age, sex and duration of the disease. There was statistically significant Hb F, Hb S in sickle cell individuals.

Keywords: Sick cell anemia; Crisis; Sick cell disease; Sudanese children patients

Background

Anemia is a reduction in the hemoglobin concentration of the blood. Although normal values can vary between laboratories, typical values would be less than 13.5 g/dL in adult males and less than 11.5 g/dL in adult females. From the age of 2 years to puberty, less than 11.0 g/dL indicates anemia. As newborn infants have a high hemoglobin level, 14.0 g/dL is taken as the lower limit at birth. Reduction of hemoglobin is usually accompanied by a fall in

red cell count and Packed Cell Volume (PCV) [1]. Morphologic classification of anemia: Hypochromic microcytic anemia (low mean cell volume (MCV) low Mean Cell Hemoglobin (MCH)), Normochromic normocytic anemia (normal (MCV), normal (MCH) and Macrocytic anaemia (high MCV). Sick Cell Anemia (SCA) is a genetic hematological disorder characterized by red blood cells that assume an abnormal rigid, sickle shape [2]. This hereditary disorder contributes the equivalent of 3.4% mortality in children aged under 5 worldwide or 6.4%

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in Africa. Sickle cell disorders were originally found in the tropics and subtropics but are now common worldwide due to migration of people from the tropical to temperate zone [3,4]. The most common haemoglobin variant in this group is sickle haemoglobin or Hb S. As a result of the replacement of glutamic acid by valine in position 6 of the β chain, Hb S has poor solubility in the deoxygenated state and can polymerize within the red cells. The red cell shows a characteristic shape change because of polymer formation and becomes distorted and rigid, the so-called sickle cell. In addition, intracellular polymers lead to red cell membrane changes, generation of oxidant substances, and abnormal adherence of red cells to vascular endothelium [5].

Sickle cell disease caused by a single point mutation at codon 6 of the globin gene those results in valine, glutamine substitution. Deoxygenated because valine can dock with complementary sites on adjacent globin chains [6]. The abnormal Hb S is soluble and gives no problem when oxygenated. When the oxygen tension decreases, the S hemoglobin polymerizes, forming "tactoids" or "fluid crystals" [7]. The hemoglob⁺⁺⁺ in realigns and distorts the red cell into the characteristic sickle shape. The sickling process works in a vicious cycle, once triggered, the cycle builds on itself. The sickle cells in circulation increase the viscosity of the blood, which slows circulation thereby increasing the time of exposure to a hypoxic environment. This promotes further sickling. The formation of rigid sickle cells is likely to plug small circulation,

further lowering the PH and oxygen tension and increasing the number of sickled cells [7].

Materials and Methods

■ Study participants

The study was performed on diagnosed 260 children patients with sickle cell disease in both gender.

■ Sample preparation and methods

Vein puncture blood sample was collected from the group of study and then putted in EDTA container immediately then was mixed gently. Some of red blood cell profile (Hb, PCV, RBCs count and RBCs indices), WBCs count and differential count and platelete count and platelete indices and Hb eletrophoresis were determined by the automated Hematology Analyzer (SysmexBC-3000plus) and reticulocyte count was counted using supravital stain.

■ Statistical analysis

Statistical assessment was carried out with Statistical Package for Social Sciences (SPSS) version 17.0 for windows statistical software.

Results

The study showed that among children with sickle cell anemia in Khartoum State, all RBCs indices were low with a marked increase in reticulocyte count (Table 1). This study also showed that the frequency of hemoglobin among the different age groups is less than 7 g/

Table 1: Descriptive statistics of study variables.

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	260	1	14	8.5	3.6
WBCs ($\times 10^9/l$)	260	5.2	25.4	13.9	4.9
RBCs ($\times 10^{12}/l$)	260	1.52	4.11	2.7	0.6
Haemoglobin (g/dl)	260	4	9.9	7.5	1.3
PCV (%)	260	14.2	29.6	23.3	3.4
MCV (fl)	260	21.5	109.7	88.3	12.2
MCH (pg)	260	19	36.7	28.7	3.5
MCHC (g/l)	260	26.2	35	32.1	1.8
Reticulocyte count (%)	260	0.3	89	21.5	17.3
Absolute Reticulocyte count ($\times 10^9/l$)	260	0.7	158.3	51.9	36.9
TWBCs ($\times 10^9/l$)	260	3.3	16.1	7.1	2.4
Neutrophils ($\times 10^9/l$)	260	0.8	8.9	3.6	1.7
Lymphocytes ($\times 10^9/l$)	260	1.4	7.2	3.1	1.2
Monocytes ($\times 10^9/l$)	260	0.1	1.6	0.5	0.3
Eosinophils ($\times 10^9/l$)	260	0.01	1.3	0.3	0.2
Basophils ($\times 10^9/l$)	260	0	0.2	0.03	0.02

dl in 54 subjects of the age group of <8 years old and more than 7 g/dl in 139 subjects in the same age group. Less than 7 g/dl was found in 114 subjects of the age group of >8 years old and more Than 7 g/dl in 119 subjects in the same age group (**Figure 1**). The study also revealed that the frequency of WBCs among sickle cell disease patients, 113 patient WBCs less than 7, 137 patients WBCs from 7-15 and 10 patients WBCs more than 15 (**Figure 2**). There was insignificant association (P value >0.05) between gender and white cells counts, red cells count, hemoglobin, hematocrit, red cells indices, reticulocyte count and absolute reticulocyte count among sickle patients (**Table 2**). The association of age with

Frequency of gender among sickle patients

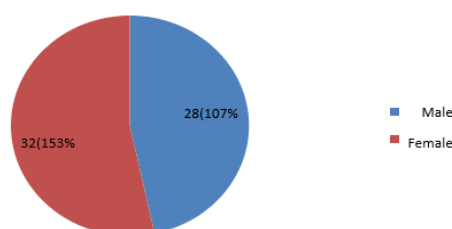


Figure 1: Frequency of gender among study population.

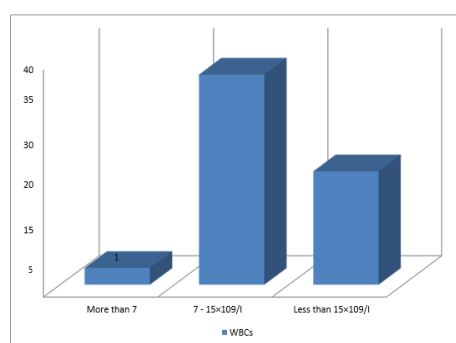


Figure 2: Frequency of WBCs among sickle cell disease patients.

white cells counts, red cells count, hemoglobin, red cells indices, reticulocyte count and absolute Reticulocyte count was found to be insignificant (p-value >0.05), However it revealed a significant association with hematocrit (p-value 0.039) (**Table 3**). A significant association (p-value 0.016) was also found between reticulocyte count and hemoglobin level and insignificant absolute reticulocyte count among hemoglobin group (**Table 4**). The association of hemoglobin, reticulocyte count, absolute reticulocyte count and WBCs was found to be insignificant (p-value>0.05). The result were represented significant (p-value 0.002) when correlation between haemoglobin and reticulocyte count (%). However it revealed an insignificant when correlation between haemoglobin and absolute reticulocyte count. The result show the mean of age among study group (7.4 year), and Mean of TWBCs ($7.1 \times 10^9/l$), in this study the mean of neutrophils ($3 \times 10^9/l$), lymphocytes ($3.1 \times 10^9/l$), monocytes ($0.5 \times 10^9/l$) (0.5), eosinophils ($0.3 \times 10^9/l$) and mean of basophils ($0.03 \times 10^9/l$) (**Table 1**). In this study the frequency of duration of disease acute (140) Percent (57.1), and chronic (120) (42.9) and the frequency of no Antibiotic use (140) percent (57.1), antibiotic use (120) percent (42.9). The significant relation observed between the eosinophils (p-value 0.023) and duration of disease and the significant relation between antibiotic use and Monocytes (p-value 0.005), and basophils (p-value 0.046) (**Table 5**), the result according to antibiotic use show insignificant in age (p-value 0.160) and show insignificant in TWBCs (p-value 0.692). The significant relation between eosinophils (p-value 0.003) and the symptoms. The frequency of the platelet count in sickle cell was founded in 126 patients less than $400 (\times 10^9 /l)$ and more than $400 (\times 10^9 /l)$ in the remaining

Table 2: Comparison of white cells counts red cells count, hemoglobin, Hematocrit, red cells indices, reticulocyte count and absolute Reticulocyte count among gender of sickle patients.

Parameters	Gender (Mean $\pm$ SD)		p-value
	Male (n = 28)	Female (n = 32)	
WBCs ($\times 10^9/l$)	13.8 $\pm$ 5.1	15.4 $\pm$ 9.7	0.477
RBCs ($\times 10^{12}/l$)	2.5 $\pm$ 0.5	2.7 $\pm$ 0.7	0.222
Haemoglobin (g/dl)	7.5 $\pm$ 1.4	7.6 $\pm$ 1.2	0.854
HCT (%)	22.9 $\pm$ 3.7	23.7 $\pm$ 3.2	0.44
MCV (fl)	90.2 $\pm$ 7.3	86.6 $\pm$ 15.1	0.243
MCH (pg)	29.3 $\pm$ 2.6	28.0 $\pm$ 4.1	0.191
MCHC (g/l)	32.5 $\pm$ 1.4	31.8 $\pm$ 2.1	0.169
Reticulocyte count (%)	21.1 $\pm$ 11.2	21.6 $\pm$ 13.2	0.932
Absolute Reticulocyte count ($\times 10^9/l$)	45.6 $\pm$ 36.9	57.4 $\pm$ 36.4	0.222

Table 3: Comparison of white cells counts, red cells count, hemoglobin, Hematocrit red cells indices, reticulocyte count and absolute Reticulocyte count among age group.

Parameters	Age group		p-value
	(Mean ± SD)		
	Less than 8 years (n=27)	More than 8years (n = 33)	
WBCs (× 10 ⁹ /l)	14.8 ± 5.0	14.4 ± 9.7	0.875
RBCs (× 10 ¹² /l)	2.8 ± 0.6	2.6 ± 0.5	0.101
Haemoglobin (g/dl)	7.8 ± 1.1	7.2 ± 1.3	0.108
HCT (%)	24.4 ± 3.4	22.6 ± 3.3	0.039
MCV (fl)	88.7 ± 9.3	87.9 ± 14.0	0.813
MCH (pg)	28.8 ± 3.8	28.8 ± 3.2	0.829
MCHC (g/l)	32.1 ± 1.7	32.2 ± 1.9	0.865
Reticulocyte count (%)	21.9 ± 10.8	21.1 ± 15.1	0.869
Absolute Reticulocyte count (× 10 ⁹ /l)	52.9 ± 35.4	51.2 ± 38.5	0.858

Table 4: Comparison of reticulocyte count and absolute Reticulocyte count with hemoglobin group.

Parameters	Hemoglobin (Mean $\pm$ SD)		p-value
	Less than 7 g/dl (n=20)	More than 7 g/dl (n=40)	
Reticulocyte count (%)	28.9 $\pm$ 21.3	17.7 $\pm$ 13.8	0.016
Absolute Reticulocyte count ($\times 10^9/l$)	55.0 $\pm$ 34.4	50.4 $\pm$ 38.4	0.654

Table 5: Comparison of duration of disease and white blood cells.

Parameters	Duration of disease (Mean $\pm$ SD)		p-value
	Acute (n=140)	Chronic (n=120)	
Age (years)	7.2 $\pm$ 3.8	7.5 $\pm$ 3.3	0.747
TWBCs ($\times 10^9/l$)	7.3 $\pm$ 2.8	6.7 $\pm$ 1.8	0.37
Neutrophils ($\times 10^9/l$)	3.2 $\pm$ 1.9	2.7 $\pm$ 1.2	0.275
Lymphocytes ($\times 10^9/l$)	3.1 $\pm$ 1.3	3.2 $\pm$ 1.2	0.818
Monocytes ($\times 10^9/l$)	0.55 $\pm$ 0.25	0.51 $\pm$ 0.25	0.61
Eosinophils ($\times 10^9/l$)	0.33 $\pm$ 0.26	0.22 $\pm$ 0.12	0.023
Basophils ($\times 10^9/l$)	0.03 $\pm$ 0.01	0.03 $\pm$ 0.02	0.922

134 patients. The mean of platelet count was insignificant=440.6, MPV (ft)=7.6, P LCR% (11.4) and PDW (ft)=9.2. Comparison of age, platelets count, and platelets indices among gender of sickle patients (**Table 6**), male=(440.3 $\pm$ 194.8); female=(458.3 $\pm$ 230.6); p-value=0.497. MPV (ft) in male=(7.6 $\pm$ 0.8); in female (7.6 $\pm$ 0.9); p-value=0.705. P LCR%, in male (11.4 $\pm$ 6.2); female (11.5 $\pm$ 5.9); p-value=(0.917). PDW (ft) in male (9.4 $\pm$ 1.7); female (9.0 $\pm$ 1.3); p-value=(0.250). Comparison of platelets count, and platelets indices among age group, the patients less than 8 years; platelet count ($\times 10^9/L$)=(464.3 $\pm$ 244.6) and patents equal or more than 8 years platelet count (421.1 $\pm$ 205.8), p-value=(0.44). MPV (ft) less than 8 years (7.5 $\pm$ 0.8), equal or more than 8 years (7.7 $\pm$ 0.9), p-value (0.429). P. LCR%, less than 8 years (10.9 $\pm$ 5.7) equal or more than 8 years (11.8 $\pm$ 6.3),

p-value (0.560). PDW (ft) less than 8 years (8.7 $\pm$ 1.2) and equal or more than 8 years (9.7 $\pm$ 1.6), p-value (0.010). The results of platelet count and in SCD were represent insignificant different with age the p-value (>0.05). Comparison of the platelet indices among the age group were represent insignificant difference with the MPV and P-LCR, but significant with PDW. p-value (0.01). The result of platelet count were represent insignificant value when correlated with age the p-value (0.170). The result of the PMV showed insignificant correlation with age, The p-value (0.225). P-LCR (the p-value (0.273, and significant with PDW (the p-value 0.069). The results were compared according to the objectives of the study as illustrated below (**Tables 7 and 8**). It show mean and SD of HbF, HbS and HbA2 (12.5 $\pm$ 7.5), (84.1 $\pm$ 7.3) and (3.0 $\pm$ 0.4) respectively, highly significant

Table 6: Correlation of age group and platelet count and platelet indices.

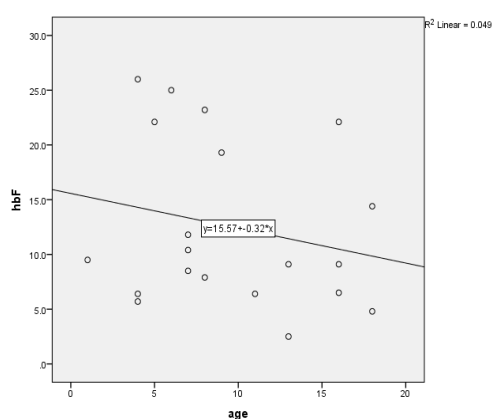
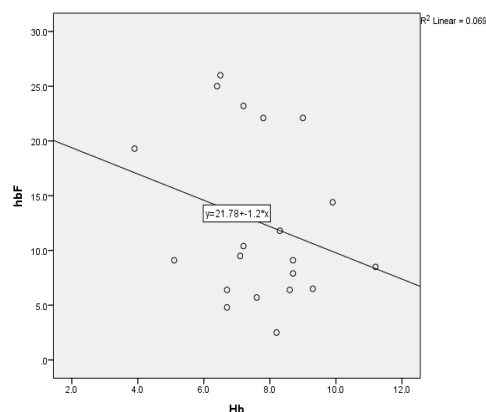
Parameters	Age group (Mean $\pm$ SD)		p-value
	Less than 8 years (n=127)	Equal or more than 8 years (n=133)	
Platelets Count ($\times 10^9/l$)	464.3 $\pm$ 244.6	421.1 $\pm$ 205.8	0.441
MPV (fl)	7.5 $\pm$ 0.8	7.7 $\pm$ 0.9	0.429
P-LCR (%)	10.9 $\pm$ 5.7	11.8 $\pm$ 6.3	0.56
PDW (fl)	8.7 $\pm$ 1.2	9.7 $\pm$ 1.6	0.01

Table 7: Mean and standard deviation of different hemoglobin types.

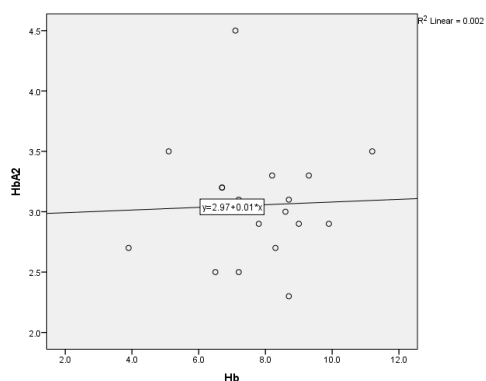
Variable	Mean $\pm$ SD Deviation
HbF	12.5 $\pm$ 7.5
HbS	84.1 $\pm$ 7.3
HbA2	3.0 $\pm$ 0.4

Table 8: Comparison of different hemoglobin types in both genders.

Variable	Male	Female	p-value
	Mean $\pm$ Std Deviation	Mean $\pm$ Std Deviation	
HbF	14.7 $\pm$ 7.2	7.7 $\pm$ 3.3	0.02
HbS	81.7 $\pm$ 7.5	89.5 $\pm$ 3.2	0.03
HbA2	3.0 $\pm$ 0.53	3.1 $\pm$ 0.26	0.55

**Figure 3:** Correlation between age and HbF among patients.**Figure 4:** Scatter plot of correlation between Hb and HbF among patients.

increase of HbF and HbS according to gender of patients with sickle cell anemia (p-value 0.02, 0.03 respectively). The results show significant positive correlation between the age and HbF ($r=0.022$ p-value 0.02) (Figure 3) and between the HbF and Hb levels ($r = 0.2$, p-value 0.03) and significant negative correlation between the HbA2 and Hb levels ($r=0.04$, p-value 0.55) (Figures 4 and 5).

**Figure 5:** Scatter plot of correlation between Hb and HbA2 among patients.

Discussion

The study showed that among children with sickle cell anemia in Khartoum State, all RBCs indices were low with a marked increase in reticulocyte count. This agrees with result shows, overall mean haemoglobin concentration, packed cell volume, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration in case is low when comparison with control and agrees with [8,9]. Result shows a majority of subjects had demonstrable anemia with increased reticulocytosis. Only increased absolute reticulocyte levels during early infancy were associated with a significant increase in hospitalization during the first three years of life and agrees with [10,11]. Result shows level of WBC was, however significantly higher in patients with HbSS VOC among the SCD, (WBCs are noted in various status of SCD), low in Hb and RBC. There was insignificant association between gender and white cells counts, red cells count, hemoglobin, hematocrit, red cells indices, reticulocyte count and absolute reticulocyte count among sickle patients. This disagrees with [12] result shows mean MCV was higher among HbS subjects but it was only among females and when the result was analyzed irrespective of gender. The association of age with white cells counts, red cells count, hemoglobin, red cells indices, reticulocyte count and absolute reticulocyte count was found to be insignificant, however it revealed a significant association with hematocrit. This disagrees with the result show mean age at diagnosis was significantly lower among males than female [13].

The study showed that the mean of WBCs was not significant associated with present or absence of infection. This agree with the mean WBCs was not significantly different according to the presence of infection or not, in Regression analyses, the presence of infection was not associated with an increase in total and differential WBCs. Also that white blood cell count is not enough to diagnose severe sickle cell disease this is in an agreement with the study of Derek findings further highlight the poor utility of WBC count by failing to demonstrate an association between WBC count and disease outcomes. Thus, while complete blood counts may help identify complications in those with severe sickle cell diseases, reliance on the WBC count to help guide management decisions

should be discouraged. In this study show statistical significant of Eosinophils this result Agree with result of patients with asthma with higher blood Eosinophil counts experienced more asthma attacks than those with lower eosinophil counts. In this study the platelet count and platelet indices showed insignificant deference, and agreed with the results of [14-18]. Also in our study the platelet count and platelet indices showed insignificant deference, and agreed with the results of. However the platelet count and platelet indices showed significant deference, and disagreed with the results of the study. Platelet function was measured on 29 occasions in 16 adult patients in the asymptomatic steady state of sickle-cell anaemia. There was a significant increase in platelet number and micro-aggregate formation, and a lower aggregation threshold with adenosine diphosphate, compared with 23 healthy controls. Similar changes were found, however, in 12 splenectomised patients without sickle-cell diseases. The platelet hyperactivity of the sickle-cell steady state therefore reflects an increased circulating population of young, metabolically active platelet resulting from previous autosplenectomy. The mean of HbS is the larger indicate that most patients have large level of HbS which is the diagnostic hemoglobin for sickle cell anemia. The mean of HbF is increased, while HbA2 is decreased. HbF, HbS and HbA2 show different means and standard deviations in males and females as HbF% in males (14.7 ± 7.2) while in females (7.7 ± 3.3); p-value (0.02); HbS% in males (81.7 ± 7.5) while in females (89.5 ± 3.2); p-value (0.03); HbA2 (%) in males (3.0 ± 0.53) while in females (3.1 ± 0.26); p-value (0.55). This study similar to study which done by Khair FM (Khartoum University) at 2002 in Sudan among sickle cell anemic patient.

Males recorded a higher mean level than females that similar to study. Also, our study agreement with another study of the same fetal hemoglobin is the major genetic modulator of the hematologic and clinical features of sickle cell disease.

Conclusion

A significant association was also found between reticulocyte count and hemoglobin level and insignificant absolute Reticulocyte count among hemoglobin group. The association of hemoglobin, reticulocyte count, absolute

Reticulocyte count and WBCs was found to be insignificant. The result were represented significant Correlation between haemoglobin and reticulocyte count (%). This study concluded the importance of screening the white blood cells Count and differential count for children with sickle cell disease when the results of this study are consistent with some previous studies, Significant relation between antibiotic use and Monocytes and Basophils. Significant relation between Eosinophils and the symptom and significant Relation observed between the Eosinophils and duration of disease. The study showed insignificant correlation between sickle cell anemia.

The study showed insignificant correlation between platelet count and platelet indices depending on age, sex and duration of the disease. HbF in sickle cell anemia patients was found to be 12.5% (raised). HbS in sickle cell anemia patients was found to be 84.1%. HbA2 in sickle cell anemia patients was found to be 3.0% (normal).

Declarations

■ Ethical approval and consent to participant

Approval of this study was obtained from hematology department of medical laboratory

science (MLS), Alwatania University, and ministry of health issued by the local ethical committee, Khartoum State, Sudan. Written consent was taken from each member of the study.

■ Consent for publication

Not applicable.

■ Availability of data and materials

The datasets generated during and or analyzed in this study are not publicly available due to Bahri Hospital center ethical policy in order to protect participant confidentiality.

■ Competing interest

The authors declare that they have no competing interests.

■ Funding

No funding was obtained for this study

■ Author's contributions

AH and AA contributed in literature search and manuscript writing. AH had the main idea of the study and contributed in manuscript writing, AA contributed to clinic work, AH contributed in statistical analysis. AA supervised the study and critically reviewed the manuscript. All authors read and approved the final draft of the manuscript.

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