International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Homocysteine level among sickle cell anemia patient with vascular occlusion Sudanese population

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Abstract

Background:
Sickle cell disease (SCD) is an inherited hemoglobinopathy due to a homozygous mutation in the β-globin gene (HbSS), which gives rise to hemoglobin polymerization in a reduced oxygen environment, thereby producing sickled erythrocytes. Recurrent VOC are responsible for the development of primary vasculopathy involving peripheral, cerebral, as well as pulmonary circulations. Hyperhomocysteinemia, often combined with a deficiency of folate, is a recognized risk factor for vascular diseases.
The aim of this study was to investigate the relationship between the frequency of crises in SCD and the level of plasma homocysteine, to compare the level of Hcy in different hemoglobin genotypes, and to examine the CBC parameters.

Method:
One hundred Sickle Cell Disease patients and 100 healthy controls matched for age and sex were taken. Hemoglobin type was identified using electrophoresis, the value of the CBC was analyzed using the Mindray hematology analyzer, and the level of homocysteine was estimated using the ELISA method. Statistics were analyzed using the SPSS version 26 software.

Result:
There were no significant differences in blood parameters compared among different groups of age ($p > 0.05$). The concentration of homocysteine was found to be high in controls (HbAA) compared to patients (SCD) possessing varying hemoglobin

Conclusion
Plasma homocysteine levels were significantly lower in SCD patients compared to healthy controls, regardless of hemoglobin genotype. No association was observed between age and CBC parameters. These findings suggest that elevated homocysteine is unlikely to contribute to vaso-occlusive crises in SCD, highlighting the need for further studies to clarify its role in disease pathophysiology and vascular complications.

Key word: Electrophoresis, hemoglobinpathies

1. Introduction

A person who is homozygous for the sickle cell mutation (HbSS or SCA) or compound heterozygote for sickle hemoglobin and β-thalassemia, hemoglobin C, and certain less frequent β-globin mutations would have sickle cell disease, a multi-systemic condition. The most prevalent type of sickle cell disease is SCA. A mutation in the HBB gene (beta-globin gene) on chromosome 11p15. causes hemoglobin S by changing the 17th nucleotide from adenine to thymine (GTG→GAG), which causes valine to replace glutamic acid at the sixth position of the amino terminus of the β chain of hemoglobin [1]. SCA is also one of the most prevalent genetic conditions globally. Sub-Saharan Africa has the greatest sickle cell disease prevalence. According to recent studies, more than 230,000 impacted infants—roughly 80% of all children worldwide—are born in this area each year. With reported prevalence levels ranging from 2% to 3%, Nigeria has the highest illness prevalence worldwide [2].

Clinically, the erythrocyte shape change causes the acute vaso-occlusive crises (VOC) in the microcirculation that are characteristic of sickle cell disease (SCD); as more crises occur, patients may develop a chronic vasculopathy in the peripheral, cerebral, and pulmonary circulations, which can result in leg ulcerations, ischemic strokes (IS), and pulmonary hypertension. Additionally, compared to the general population, SCD patients are more likely to get venous thromboembolism. Intravascular hemolysis and neutrophil-platelet aggregates in the pulmonary circulation, which both result in oxidation, complement, and coagulation activation, are additional variables that contribute to the VOC [3].

A sulfur-containing amino acid called homocysteine (Hcy) is created when methionine (Met) is converted to cysteine (Cys). Increased blood levels of Hcy, or hyperhomocysteinemia (HHcy), are widely acknowledged as a separate risk factor for peripheral, cerebral, and coronary atherosclerosis. Defective Met metabolism, which can be caused by deficiencies in specific vitamin cofactors or mutations in the genes encoding the enzymes involved in Hcy metabolism, can raise Hcy levels [1]. In the presence of sufficient Met, Hcy produces cysteine through the enzyme cystathionine β-synthase. However, in the event of Met deficiency, Hcy can be re-methylated to salvage Met through the enzyme N5, N10-methylenetetrahydrofolate reductase. Although Hcy is not directly involved in protein synthesis, its specific function in folate metabolism and choline catabolism is crucial for regulating Met availability and function[4].

A known risk factor for a number of conditions, such as arteriosclerosis, venous thrombosis, cardiovascular disease, and stroke, is a high plasma concentration of Hcy [4]. Plasma total homocysteine (tHcy) levels in SCD patients have drawn some attention since Hcy is a significant vascular risk factor that may contribute to the ischemic phenomena of SCD. Nevertheless, the majority of SCD research has focused on children, who are thought to be less susceptible to hyperhomocysteinemia than adults. There has been some interest in plasma total tHcy since homocysteine may contribute to the ischemic phenomena seen in HbSS. The plasma concentration of tHcy in HbSS sufferers was shown to be almost 1.5 times greater than in healthy controls in a prior investigation. Furthermore, despite having greater plasma folate and vitamin B12 concentrations that are comparable to those seen in controls, SCD patients had higher plasma tHcy concentrations [5].

2. Material and method

This study was conducted as case control one, concerning about 100 Sickle cell disease (SCD) and sickle cell anemia (SCA) patients, who recruited from Suleiman Salih Fedial Hospital-Khartoum, and other 100 healthy participants, they were undergoing blood collection for complete blood count (CBC) via Mindray hematological device 8000-China, Hb type confirmation via electrophoresis and homocysteine levels, which conducted via Enzyme linked sorbent assay (ELISA).

3. Results

Total of 200 sample of Sudanese were involved in this study, 100 of them were SCD patients a confirmed diagnosis according to clinical and laboratory findings as case group, while 100 health Sudanese were involved as control group, the age matched between two group. Gender distribution among case group and healthy subjects 55% and 57% respectively and 45% and 43% for females as well, as in figure 1.

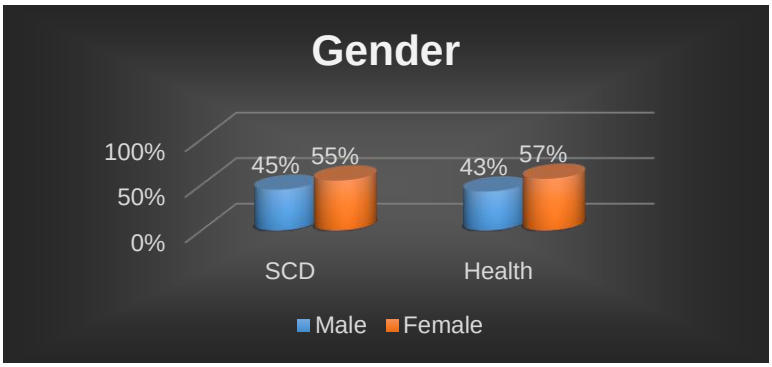

Figure 1: Distribution of the studied Sudanese according to Gender.

Distribution of Hemoglobin Differences in the SCD Group, showed the distribution of hemoglobin variants among individuals with SCD. The most common hemoglobin type observed was AS (47%), followed by SC (29%), and SS (24%) as figure 2.

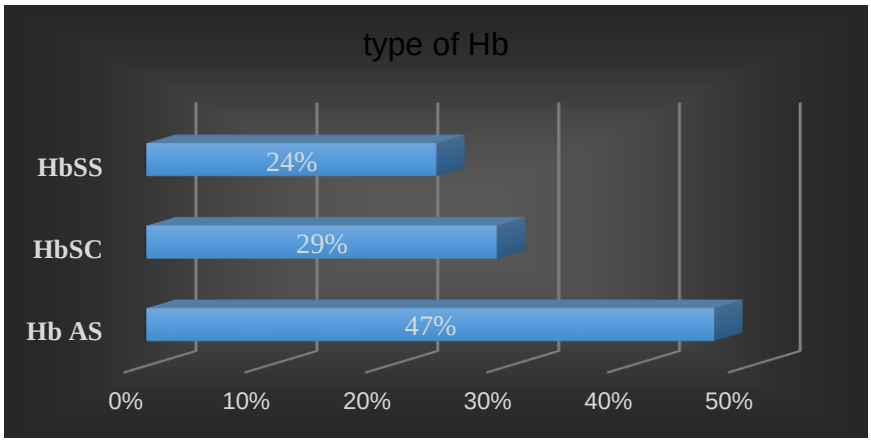

Figure 2: types of hemoglobin among case group

The comparison of hematological parameters and plasma homocysteine between SCD and healthy groups. SCD patients showed significantly lower values of RBC count, Hb, PCV, and MCHC compared to healthy subjects ($p < 0.001$). Conversely, WBC count, MCV, MCH, neutrophil count, platelet counts were significantly higher in SCD patients ($p < 0.001$), but and homocysteine levels were lower among SCD than control group with significant difference (p value <0.05). No significant differences were observed in lymphocyte count, or MCHC values between the groups ($p > 0.05$) as in table 2.

Table 2: Comparison of CBC Parameters and Homocysteine in SCD group with control Group

P Value	Control (Mean ± SD)	SCD (Mean ± SD)	Parameters
< 0.001	6.9 ± 1.9	8.6± 3.6	**WBC**
<0.001	4.86 ± 0.5	3.03 ± 0.7	**RBC**
<0.001	13.4 ± 1.4	8.5 ± 2.2	**Hb**
<0.001	39 ± 3	26 ± 6	**PCV**
<0.001	82 ± 4	93 ± 8	**MCV**
<0.001	27 ± 1	30 ± 2	**MCH**
0.666	33 ± 3	32 ± 1	**MCHC**
<0.001	3.7 ± 1	4.8 ± 1	**Neutrophil**
0.832	2.4 ± 0.9	2.4 ± 1	**Lymphocyte**
<0.001	264 ± 60	341 ± 120	**Platelet**
<0.001	27 ± 7	15 ± 9	**Homocysteine**

Regarding to age of patients, patients were sorted to 4 ranges of years, CBC parameters compared between the ranges, there was no significant difference for each parameter, as p value for each was>0.05 as in table 3.

Comparing homocysteine levels among patients with different types of hemoglobin and control (Hb AA) showed that it was high among control than other types of SCD patients giving high significant difference with P value<0.001 as in table 4.

Table 3: The Mean difference of CBC Parameters between Age ranges of SCD

P Value	30 - 59 Y	20 - 29 Y	13 – 19 Y	0 -12 Y	Parameters
0.994	8.7 ± 5.8	8.3 ± 3.7	8.7 ± 3.7	8.7 ± 3.5	**WBC**
0.253	2.7 ± 0.5	2.9 ± 0.8	2.7 ± 0.6	3.1 ± 0.7	**RBC**
0.378	7.7 ± 1.5	8.0 ± 2.5	8.0 ± 2.4	8.8 ± 2.2	**Hb**
0.099	24 ± 7	25 ± 7	24± 5	27 ± 6	**PCV**
0.981	93 ± 5	94 ± 8	92 ± 8	93 ± 7	**MCV**
0.229	31 ± 2	30 ± 2	30 ±2	29 ± 2	**MCH**
0.369	33 ± 1	33 ± 1	33 ± 1	32 ± 1	**MCHC**
0.962	6.3 ± 4.8	4.8 ± 3.2	4.6 ± 2.0	4.8 ± 2.8	**Neutrophil**
0.718	2 ± 1.1	2.7 ± 1.3	2.4 ± 1.3	2.3 ± 1.4	**Lymphocyte**
0.788	320 ± 185	331 ± 120	342 ± 109	345 ± 120	**Platelets**
100	**5**	**11**	**20**	**64**	**Total**

Table 4: The Mean difference of Homocysteine Level between types of Hemoglobin

P - Value	SS	SC	AS	AA	Type of HB
<0.001	15.9 ±9	15.2 ±8	15.3 ± 9	27.1 ± 7	**Homocysteine Mean ± SD**
	24	29	47	100	**Total**

Pearson's correlation of homocysteine with CBC measured parameters among SCD patients revealed that negative correlation with WBC, MCV, MCH, Neutrophil and lymphocyte and all of them brought significant differences as p value for each <0.05 and positive correlation with RBC, Hb, PCV and platelet with significant difference for each (p value <0.05), non-significant difference obtained with MCHC which has positive correlation and p value >0.05 as in table 5.

Table 5: Correlations between CBC Parameters and Homocysteine Level of SCD

Homocysteine		
P. value	**R. Value**	**Correlation variables**
0.011	-0.179	**WBC**
< 0.001	0.573	**RBC**
< 0.001	0.529	**Hb**
< 0.001	0.534	**PCV**
< 0.001	-0.431	**MCV**
< 0.001	-0.358	**MCH**
0.830	0.015	**MCHC**
< 0.001	-0.232	**Neutrophil**
0.020	-0.165	**Lymphocyte**
0.041	0.145	**Platelets**

Pearson's of age of SCD and CBC Parameters, revealed negative correlations with WBC and platelet (with significant differences), Hb, PCV with no significant differences, and positive correlation with rest of parameters and no significant differences as in table 6.

Table 6: Correlations between CBC Parameters, Homocysteine Level among SCD

Age		
P. value	**R. Value**	**Correlation variables**
0.903	-0.009	**WBC**
0.255	-0.081	**RBC**
0.201	-0.091	**Hb**
0.088	-0.121	**PCV**
0.838	0.015	**MCV**
0.117	0.111	**MCH**
0.887	0.01	**MCHC**
0.327	0.07	**Neutrophil**
0.66	0.031	**Lymphocyte**
0.637	0.034	**Ae and Platelets**
0.912	-0.008	**Homocysteine**

4. Discussion

In this study it findings out of assessment of levels of red blood cells (RBCs) and related parameters, beside white blood cell count (WBC), platelet and homocysteine among sickle cell disease patients as well as healthy participants. WBC: Significantly higher white cell counts in SCD reflects chronic inflammation, stress on bone marrow, and possible infection/inflammatory activity. RBC: Lower red cell count indicates chronic hemolytic anemia (common in SCD due to sickling and destruction of RBCs). Hb: Markedly lower hemoglobin due to hemolysis and ineffective erythropoiesis. Packed cell volume is lower, supporting anemia. MCV and MCH: Larger average cell size and more Hb per cell — this may be due to reticulocytosis (young cells larger than mature RBCs). MCHC: No significant difference: Suggests that hemoglobin concentration within cells isn't significantly changed; typical in some SCD cohorts.

-Platelets: Elevated platelets in SCD (significant difference), reflects reactive thrombocytosis often seen in anemia and chronic inflammation.

-Homocysteine was low in SCD; $P < 0.001$) Lower in SCD vs control This is an unusual finding — studies often report elevated homocysteine in SCD (discussed below).

A partial agreement obtained with outcomes of a study conducted to evaluate the hematological parameters levels in sickle cell anemia condition of patients with sickle cell anemia. A total of one

hundred and twenty-four subjects were recruited for this study which consists of 84 sickle cell anemia subjects who (48 males and 36 females) and 40 healthy (20 males and 20 females) subjects as control who matched by age and sex of the patient's groups. The outcomes indicated decrease in the level of RBC, Hb, MPV and HCT, while observed increased in the level of WBC, PLT, RDW-SD, RDW-CD, MCV, MCHC and PDW in sickle cell anemia patients compared with the control groups, however observed no significant in level of MCH in sickled patients compared with the control groups[6].

A disagreement obtained with a study conducted to assess level of homocysteine among patients with 49 adults sickle cell anemia (SCA) and 16 normotensive controls. All subjects with sickle cell disease had been prescribed folic acid 1 mg by mouth daily. The median plasma concentration of homocysteine of subjects with sickle cell disease was approximately 1.5-fold higher than that of controls (p=0.0008). There was no difference in plasma homocysteine concentrations between transfused and non-transfused sickle cell subjects [7].

Our finding indicates that plasma homocysteine levels were significantly higher in the control group with normal hemoglobin (Hb AA) compared with patients with sickle cell disease (SCD) of different hemoglobin variants. The very low P value (<0.001) confirms that this difference is highly statistically significant and unlikely to be due to chance. This suggests that SCD patients tend to have lower homocysteine levels than healthy controls. Several mechanisms may explain this observation, including: Increased erythropoiesis in SCD, which leads to higher utilization of folate and vitamin B_{12}, key cofactors in homocysteine metabolism. Chronic hemolysis and increased metabolic turnover, which may reduce circulating homocysteine levels. Possible nutritional supplementation (folic acid) routinely given to SCD patients, which can lower homocysteine concentrations. Overall, the result implies that hyper homocysteinemia is not a common biochemical feature in SCD patients, despite their increased risk of vascular complications.

An agreement with a comparative cross-sectional study conducted on 110 adults consisting of participants with SCD in vaso-occlusive crises (VOC), SCD in hyper-hemolytic crises (HHC), SCD in steady-state (SS), and healthy controls. Serum homocysteine was determined using the Enzyme-linked immunosorbent assay method. There was a statistically significant difference in mean serum homocysteine levels in participants in VOC, HHC, SS, and controls, respectively (P = 0.016). Conversely, no participant in the SS or the control group had hyper homocysteinemia [8].

Another disagreement obtained with a study conducted to evaluate the plasma Hcy level in SCA and their effect on the vaso-occlusive crisis (VOC) in SCA patients. One 120 cases of SCA (HbSS) and 50 controls with normal hemoglobin(HbAA) were studied. It was found that the plasma Hcy level is significantly higher ($p< 0.0001$) in patients with SCA (22.41 ±7.8 µmol/L) compared to controls (13.2 ± 4.4 µmol/L). Moreover, patients without FA supplementation had a significantly ($p< 0.001$) higher Hcy level (27 ± 7 µmol/L) compared to those with supplementation (17.75 ± 5.7 µmol/L) [9].

5. Conclusion

In sickle cell disease (SCD) patients, a low homocysteine level is typically not regarded as pathogenic and might be interpreted in a number of clinically significant ways:

1. Sufficient or elevated levels of folate and vitamin B12. Folate, vitamin B12, and vitamin B6 are necessary for homocysteine metabolism. To promote enhanced red blood cell turnover, many SCD patients take folic acid supplements on a regular basis. Remethylation of homocysteine to methionine is improved by effective supplementation, which lowers plasma homocysteine levels. Therefore, low homocysteine frequently indicates adequate nutritional support and metabolic regulation.
2. Decreased Risk of Vaso-occlusive and Thrombotic consequences: Endothelial dysfunction, inflammation, and thrombosis are linked to elevated homocysteine, which can exacerbate SCD consequences. A protective cardiovascular profile may be indicated by low homocysteine, which could lower the risk of stroke, pulmonary hypertension, or vaso-occlusive crises.
3. Potential Impact of Age and Renal Function: Compared toolder patients, children and young people with sickle cell disease (SCD) frequently have lower homocysteine levels. Normal or improved renal clearance, which is prevalent in early SCD due to hyper-filtration, can also lower homocysteine.

Recommendation

Upgrading the check-up routine for sickle cell disease to cover or manage complications for the disease.

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