

## Original Research Article

## Relationship between Serum Homocysteine and some Hematological Parameters among Steady State Sickle Cell Anemia (SCA) Individuals in North West Nigeria

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**Abstract: Background:** Sickle cell disease (SCD) is a lifelong condition, it has been recognized as a procoagulant state in which micro thrombi have influenced the evolution of many complications. Relating homocysteine levels to basic haemostatic indices will provide insight on contribution of thrombotic phenomena to ill health and wellness. **Objective:** To explore the relationship between serum homocysteine and some hematological parameters among SCA patients attending a tertiary hospital. **Methods:** This is a hospital-based comparative cross-sectional study that involves 60 participants comprising 30 patients with SCA and 30 HbAA controls. Hematological parameters were analyzed using an automated hematology analyzer, while serum homocysteine was determined using enzyme linked immunosorbent assay (ELISA) method. Data were analyzed using SPSS version 23. A p-value <0.05 was considered statistically significant. **Results:** The mean value for homocysteine in the HbSS group was  $9.72 \pm 0.96 \mu\text{mol/L}$  whilst that of the HbAA was  $6.19 \pm 0.88 \mu\text{mol/L}$ . There was significantly higher mean white blood cell (WBC) and platelets count in the subjects with p value of < 0.005 but significantly lower mean haemoglobin and haematocrit ( $P < 0.005$ ). The mean PT and APTT of controls, 13.00 and 34.50 were more prolonged than that of subjects 12.00 and 32.00 ( $p = 0.001$  and  $p < 0.0001$  respectively). **Conclusion:** The study did not find any relationship between serum homocysteine and the hematological parameters analyzed.

**Keywords:** Homocysteine, Sickle Cell Anemia, Hematological Parameters, Steady State.

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## INTRODUCTION

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide [1]. Sub-Saharan Africa has the highest prevalence as about 300,000 infants are born with the disease annually [2]. Globally, Nigeria has the highest prevalence of the disease with about 150,000 births annually [3]. Studies in US over a 26-year period revealed an 18.2% increase in mortality rate amongst sickle cell anemia (SCA) patients, with a higher mortality rate recorded amongst adults [4]. Sickle cell anemia (SCA) occur as a result of

single point mutation in the HBB gene on chromosome 11 leading to formation of an abnormal hemoglobin (HbS) [5]. The disease condition is associated with various complications affecting both hematological and biochemical parameters, which clinically present with episodes of pain and increased vulnerability to infections [6]. Studies have demonstrated that sickle cell anemia is a hypercoagulable state. [7] This is due to the findings that almost every aspect of hemostasis in the disease favors the pro-coagulant phenotype. These include but not limited to, platelets which are on the higher side, the natural anticoagulants, the pro-coagulants and the

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fibrinolytic system [7]. Homocysteine is a sulfhydryl-containing, non-proteinogenic amino acid. Raised plasma levels of homocysteine in SCA may occur due to folate and vitamin B12 deficiency that is usually consequent to chronic hemolysis [8]. It is therefore expected that in the event of hyper hemolysis which is usual in sickle cell anemia, serum homocysteine will rise. Studies have shown that elevated levels of serum homocysteine contribute tremendously to the hypercoagulable state of sickle cell anemia [9]. This is because homocysteine has been associated with the development of cardiovascular and peripheral arterial disease [10]. Additionally, homocysteine has also been suggested as a potential hemolytic toxin with some pro-oxidant features [11]. Considering this prothrombotic potential of homocysteine coupled with abnormal hematologic parameters like increased platelets, white cells and altered hemostatic indices, sickle cell anemia individual are therefore prone to several SCD-related complications such as stroke [12].

## MATERIALS AND METHODS

### Study Area and Population

The study was carried out in the Hematology Department, Ahmadu Bello University Teaching Hospital Zaria, Kaduna State, north west Nigeria. Sixty (60) participants consisting of 30 individuals with SCA patients in steady state (study group) and thirty (30) apparently healthy HbAA controls were enrolled into the study.

**Study Period:** The research was conducted with in the period of five months, from January 2023 to May 2023.

### Study Design

The research was cross sectional comprising of two arms. The first arm consisted of HbSS patients in steady state (study group). The second arm consisted of apparently healthy individuals with HbAA phenotype.

### Inclusion Criteria

- Age between 18-60 years
- Individuals who gave their written consent
- Individuals diagnosed as HbSS through cellulose acetate electrophoresis at pH 8.6
- HbAA controls (confirmed by cellulose acetate electrophoresis at pH 8.6).

### Exclusion Criteria

- Confirmed Hb phenotypes AS, AC and CC individuals
- Individuals with history of acute or chronic illness like fever and hypertension
- SCA patients in crisis
- Individuals who refused to grant a written consent

### Participant's Informed Consent

Written informed consent was obtained from the literate participants while the non-literate participants thumb printed the consent form after a detailed explanation of the nature and benefits of the study. The participatory need of the non-English speaking participants was addressed by the use of an interpreter.

### Sample Collection

Under aseptic conditions, 10 ml of venous blood sample was collected with the use of disposable syringe from each participant after informed consent. The antecubital vein was mostly used for the intravenous access. 2 ml was dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulated containers and was used for full blood count using the automated Sysmex autoanalyzer machine. The remaining blood in the EDTA bottle was used for hemoglobin electrophoresis test to confirm the Hb phenotypes of all the participants.

4.5mls was dispensed into buffered sodium citrate sample bottle, mixed gently, then spurned and obtained plasma and used for PT and APTT manually, using PT and APTT reagent.

3.5mls was dispensed in plain bottle and allowed to stand for 1-2hours, then spurned and obtained the serum, stored at -20°C and subsequently used for serum homocysteine determination using standard ELISA method.

### Ethical Consideration

Ethical clearance for the research was obtained from the Health Research Ethics Committee (HREC) of ABUTH Zaria prior to the commencement of the study and conducted in accordance with the Declaration of Helsinki.

### Statistical Analysis

Data analysis was done using the Statistical Package for Social Sciences (SPSS) version 23.0 which is a software package used for the analysis of statistical data. The results obtained were presented using tables and figures. Continuous variables were presented as means and standard deviation (SD), or median with interquartile range (IQR) where appropriate, while categorical variables were presented as percentages. Comparison of means was carried out using the student's t-test.

## RESULTS

Table 1 shows the socio demographic characteristics of subjects and controls. The median and interquartile ages for the subjects and controls were 24(15) and 27(12) years respectively, with no significant difference. There was a significant sex difference between the subjects and controls. Subjects were

predominantly females (80%) while majority of control participants were males (60%). There was no ethnic difference between the subjects and the controls as Hausa was the predominant ethnic group among the subjects (80%) and controls (73.3%). The majority of the subjects

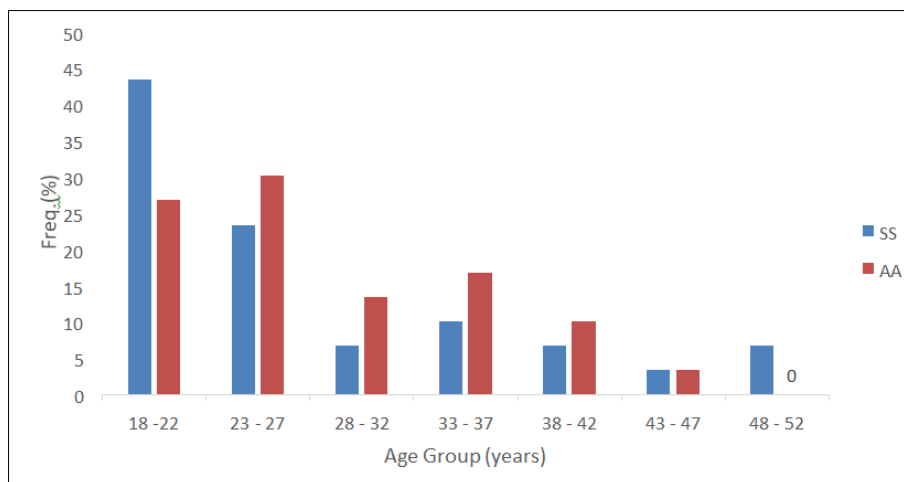
were students and traders while most of the controls were students.

Figure 1 shows the age distribution of subjects and controls.

**Table 1: Sociodemographic characteristics of subjects and controls**

Variables	SS, n=30	AA, n= 30
	Freq. (%)	Freq. (%)
<b>Age (years)</b>	24.0(15.0)*	27.0(12.0)*
<b>Min., Max. (age range)</b>	18, 50	20, 47
<b>Sex</b>		
Male	6(20.0)	18(60.0)
Female	24(80.0)	12(40.0)
<b>Marital Status</b>		
Single	20(66.7)	23(76.7)
Married	10(33.3)	7(23.3)
<b>Ethnic Group</b>		
Hausa	24(80.0)	22(73.3)
Fulani	0	3(10.0)
Yoruba	4(13.3)	3(10.0)
Nupe	2(6.7)	2(6.7)
<b>Occupational Status</b>		
Student	12(40.0)	17(56.7)
Trader	12(40.0)	5(16.7)
civil servant	3(10.0)	8(26.7)
house wife	3(10.0)	0

\*Median (Interquartile range)



**Figure 1: Age distribution of subjects and controls**

Table 2 shows the mean value of hemoglobin (Hb), hematocrit (HCT), white blood cell (WBC) and Platelet (PLT) count for subjects and controls.

The mean Hb and HCT were significantly lower in the subjects when compared with controls ( $P < 0.001$ ).

The mean  $\pm$ SD WBC count was significantly higher in the subjects ( $12.93 \pm 3.14 \times 10^9/L$ ) when compared with controls ( $5.57 \pm 1.73 \times 10^9/L$ ); ( $P < 0.001$ ).

There was also a significantly higher mean platelet count in the subjects when compared with controls; ( $P < 0.001$ ).

The mean  $\pm$ SD reticulocyte was significantly higher in the subjects when compared with the controls; ( $P < 0.001$ ).

**Table 2: The mean hematological parameters of subjects and controls**

Haematological Parameters	SS	AA	t-test	P-value
	Mean ±SD	Mean ±SD		
Hb (g/dL)	8.37 ±1.77	16.03 ±2.71	-12.981	<0.0001
HCT (%)	23.57 ±5.60	41.93 ±5.36	-12.971	<0.0001
WBC (x10 <sup>9</sup> /L)	12.93 ±3.14	5.57 ±1.73	11.240	<0.0001
MCV (fl)	86.73 ±10.19	82.60 ±8.11	1.735	0.088
MCH (pg)	31.16 ±4.68	31.89 ±3.88	-0.658	0.513
MCHC (g/dL)	35.05(1.57)	41.55(10.35)	550.000	0.139* $\alpha$
Reticulocyte (%)	8.11 ±3.16	2.00 ±0.46	10.499	<0.0001
PLT (x10 <sup>9</sup> /L)	406.33 ±139.48	268.00 ±85.18	4.636	<0.0001
PT (s)	12.00(1.25)	13.00(2.00)	659.000	0.001 $\alpha$
APTT(S)	32.00(4.00)	34.50(3.25)	698.000	<0.0001* $\alpha$

$\alpha$ -Independent Sample t-test, \*Mann-Whitney test, Median (Interquartile Range)

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

**Table 3: Mean serum homocysteine levels of subjects and controls**

Homocysteine (µmol/L)	Min., Max.	Mean ±SD	t-test	P-value
HbSS	7.01, 11.39	9.72 ±0.96	14.872	<0.0001
HbAA	4.06, 7.58	6.19 ±0.88		

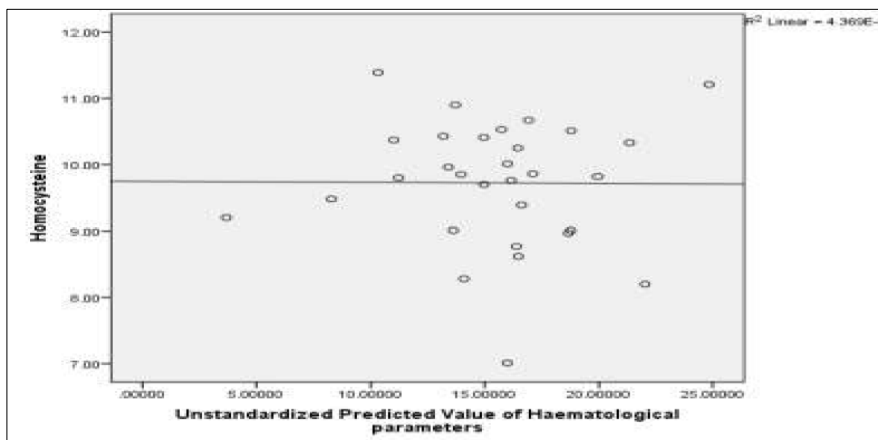
Independent Sample t-test

**Table 4: Relationship between serum homocysteine levels and hematological parameters**

	Homocysteine (µmol/L)		P-value
	B	F(df)	
Hb (g/dL)	-0.103	0.551(10,19)	0.873
HCT (%)	0.077		
MCV (fl)	-0.022		
MCH (pg)	-0.001		
MCHC (g/dL)	-0.133		
Reticulocyte (%)	0.026		
WBC (x10 <sup>9</sup> /L)	0.067		
PLT (X10 <sup>9</sup> /L)	0.001		
PT (s)	-0.033		
APTT (s)	-0.043		
(Constant)	15.799		

Multiple linear regression

B =Unstandardized Coefficients, R = 0.454, R<sup>2</sup> = 0.206



**Figure 2: Correlation between serum homocysteine and haematological parameters (Multiple scatter plot)**

## DISCUSSIONS

This is a cross sectional study of individuals with HbSS in steady state visiting the Hematology Department of Ahmadu Bello University Teaching Hospital Zaria, Kaduna State Nigeria. 60 participants were enrolled, 30 HbSS and 30 healthy controls. The median age of the subjects was similar to that obtained by Ebele *et al.*, [13], and Ugwu *et al.*, [14], who performed their studies in Lagos and Ebonyi States respectively. This indicates that the subjects were older than the presumed age (paediatric age group) of death. This may be due to introduction of public health measures such as penicillin prophylaxis, vaccinations and hydroxyurea that contributed to an impressive reduction in SCA-related childhood mortality [15].

This study did not find any relationship between serum homocysteine and haematological parameters (Table 4). This contrast with the study done by Jin *et al.*, [16], who reported positive relationship between serum homocysteine and white blood cell count. This is not unexpected as increased serum homocysteine has been implicated in the induction of inflammatory determinants including expression of adhesion molecules, leukocyte adhesion, endothelial dysfunction, oxidative stress and reduced nitric oxide bioavailability [17]. High levels are also detectable in various inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis and psoriasis [18]. A positive correlation between serum homocysteine levels and platelet, with a negative correlation between serum homocysteine levels and haemoglobin were reported in a study conducted by Yassin *et al.*, [19]. The positive correlation with platelet could be attributed to homocysteine ability to stimulate platelet aggregation and enhancement of collagen type 1 induced platelet aggregation. It could also be partly due to reactive thrombocytosis.

## CONCLUSION

The study did not find any relationship between serum homocysteine and the hematological parameters analyzed. Further studies incorporating hematological parameters of sickle cell anemia patients in crisis is recommended.

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**Conflicts of Interest:** There are no conflicts of interest.

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