



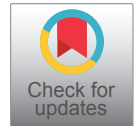
## ORIGINAL ARTICLE

## Under Reported Potentials of Low Foetal Haemoglobin Concentration in Sickle Cell Disease

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### Abstract

**Background:** The liver plays a significant role in the maintenance of health and survival of an individual. Any threat to this important organ is a threat to the existence of life. Hence its protection is vital to survival. Individuals with SCD are at high risk of suffering from liver failure due to events resulting from HbS polymerization. Current studies on Foetal haemoglobin are now focused on high HbF concentration because of the promises it has shown in ameliorating clinical severity of the disease. This feat has however diverted the attention of researchers away from studies on low foetal haemoglobin which has consequently led to the underestimation of its value in SCD. This study therefore was designed to assess the potential benefits of low foetal haemoglobin in individuals with SCD.

**Method:** Thirty-five (35) individuals with sickle cell disorder and Fifteen (15) apparently healthy individuals were enrolled in this study. Blood samples collected from the subjects were used to determine the function of the liver and foetal haemoglobin concentration of the subjects. The liver function markers including bilirubin, Aspartate transaminase (AST), and alanine transaminase (ALT) were measured using the spectrophotometric method while the foetal haemoglobin was determined by alkali denaturation method.

**Results:** The foetal haemoglobin level of the sickle cell subjects was low and correlated inversely with bilirubin, AST and AST/ALT ratio. However only bilirubin correlated significantly with HbF.

**Conclusion:** Low foetal haemoglobin concentration also ameliorates disease severity in Sickle cell disease.

### Keywords

Sickle cell disease, Foetal haemoglobin, Liver damage, Aspartate transaminase, Bilirubin, Alanine transaminase

### Abbreviations

HbF: Foetal Haemoglobin; HbS: Sickle Haemoglobin; TB: Total Bilirubin; AST: Aspartate Transaminase; ALT: Alanine Transaminase; SCD: Sickle Cell Disease

### Introduction

Sickle cell disease is a genetic disorder that affects the haemoglobin of individuals with the disorder [1]. The disorder results in the formation of an abnormal sickle shaped red blood cell when exposed to crises triggers such as low oxygen condition. The cause of the disorder is a point mutation that replaces glutamate with valine on the beta chain of the haemoglobin molecule. In the presence of crises triggers the sickled haemoglobin (HbS) polymerizes to form an abnormal shape of the red blood cell [2,3]. This condition forms the basis for all the clinical complications associated with the disease.

Liver diseases collectively known as sickle hepatopathy are a major contributor to the cause morbidity and mortality in SCD. Intrahepatic sickling of red cells, multiple blood transfusion and ischaemic events are the major causes of the disease in SCD [4,5]. Because of the vital roles played by the liver in the preservation of life, the need for its protection is paramount. Liver function test is therefore carried out to monitor the integrity of the liver and prevent its damage [6,7].

Foetal haemoglobin is the most important modulator of sickle cell crises. It has the ability to slow down HbS polymerization and avert clinical complications arising from its action [8]. It is produced by a sub-section of

red cells known as F-cells. In adults, its production is stopped with only about 1-2% synthesis being retained [9,10]. However, adults with SCD still retain the ability to synthesis a substantial amount of the haemoglobin. Studies have shown that individuals with a higher concentration of the haemoglobin do not suffer severe clinical complications of SCD [11].

## Methods

### Study area and population

Ebonyi State is one of the 9 states from south-eastern region of Nigeria. It is located on latitude 6°15'0" N& longitude 8°4'60"E. It has a land mass of about 5,935 km<sup>2</sup> and a population of over 2 million people based on 2006 census. It shares boundary with 4 states including Abia, Enugu, Benue and Cross-rivers states.

### Subjects

The subjects of this study were sickle cell individuals who attended clinic at the Alex Ekwueme Federal university teaching hospital Abakaliki (FUTHA), Ebonyi, state from March to December 2021. The subjects were comprised of 35 sickle cell individuals and 15 apparently healthy control individuals both within the age of 17 to 45 years. Clinical diagnosis of the sickle cell subjects was confirmed by haemoglobin electrophoresis.

### Ethical approval

Ethical approval for the study was obtained from the health and ethical committee of FUTHA. Samples were collected from subjects who voluntarily consented to be included in the study.

### Inclusion criteria

Criteria for inclusion in the study included individuals

that have been confirmed to be diagnosed with sickle cell disease and had not received blood transfusion three months prior to the study. Apparently healthy individuals were included based on absence of liver disease history.

### Sample collection and analysis

Four millilitres of venous blood sample was collected from each subject in an EDTA container labelled with the subject's name, age and gender. The blood was centrifuged for 5 minutes at 3000 rpm. The serum was then separated from the red cells using a Pasteur pipette into a dry plain specimen container. The serum was then used to assay for the activity of Alanine transaminase (ALT), Aspartate transaminase (AST) and Total bilirubin (TB). The red cells were washed, haemolysed and used for the determination of Foetal haemoglobin (HbF) concentration.

### Statistical analysis

The results of this study were analysed statistically using SPSS version 10. Mean and standard deviation were used to record descriptive values. Students T-test was used to compare means and Pearson correlation was used to measure association between the different parameters. A confidence interval of 95% was used and P values equal to or less than 0.05 ( $P \leq 0.05$ ) were considered statistically significant.

## Results

The concentration of the various biochemical markers in sickle cell subjects and apparently healthy control subject was studied (Table 1). The foetal haemoglobin concentration, Total bilirubin (TB), aspartate transaminase (AST) and alanine transaminase (ALT)

**Table 1:** A comparison of the HbF Concentration and liver function markers between subjects with SCD and apparently healthy control subjects.

Biochemical Markers	SCD (35) (Mean ± SD)	Control (15) (Mean ± SD)	P-value
TB (µmol/L)	35.52 ± 25.70	18.68 ± 3.76	0.001*
ALT	23.47 ± 11.69	13.27 ± 5.04	0.020*
AST	61.80 ± 11.58	28.17 ± 16.18	0.001*
HbF (%)	2.06 ± 1.39	1.42 ± 0.84	0.018*

HbF: Foetal Haemoglobin; TB: Total Bilirubin; ALT: Alanine Transaminase; AST: Aspartate Transaminase

\*Correlation is significant at 0.05 ( $P < 0.05$ ).

**Table 2:** Gender classification of HbF and Liver function markers in SCA individuals.

Biochemical Markers	Male (N = 12)	Female (N = 18)	P-Value
HbF (%)	1.72 ± 0.99	2.29 ± 1.59	0.242
Bilirubin. (µmol/L)	35.04 ± 13.75	35.84 ± 31.68	0.926
ALT	22.58 ± 9.47	24.06 ± 13.20	0.723
AST	64.06 ± 13.81	60.29 ± 9.97	0.425
AST/ALT	3.14 ± 0.99	2.99 ± 1.12	0.703

HbF: Foetal Haemoglobin; TB: Total Bilirubin; ALT: Alanine Transaminase; AST: Aspartate Transaminase; AST/ALT: De-ritis ratio

in subjects with sickle cell anaemia were significantly higher than the apparently healthy subject.

The gender classification of the biochemical markers in subjects with sickle anaemia is as presented in Table 2. There was no significant difference in the concentration of HbF, TB, AST, and ALT between the male and female subjects.

The concentration of the liver biomarkers (TB, AST and ALT) and HbF, between sickle cell subjects in crises and those in steady state is presented in (Table 3). The concentration of HbF, TB, AST and ALT in crises state subjects were not significantly different from the steady state subjects.

The relationship between foetal haemoglobin and the liver function markers was also studied (Table 4). HbF concentration correlated inversely with TB, AST and AST/ALT ratio but not ALT.

## Discussion

Liver failure is a common cause of morbidity and mortality in Sickle cell disease. Individuals with SCD are at greater risk of developing liver failure due to several factors including the effects of iron overload from repeated blood transfusion, pain medications and intrahepatic sickling. Unless the prevailing causes of the liver diseases are monitored and promptly treated, liver failure is bound to occur. Foetal haemoglobin is gaining grounds in SCD studies due to its role in ameliorating clinical outcome of the disease. Hence this study was carried out to ascertain the effect of foetal haemoglobin on some markers of liver function.

The subjects with SCD had a significantly higher concentration of TB, AST, ALT and HbF than the apparently healthy control subjects. The studies of Obi, et al. [12] and Akuyam, et al. [13] on liver diseases also reported similar findings. The increased

serum concentration of the liver markers in SCD has been attributed to the effect of the mutated, sickled haemoglobin that polymerizes under low oxygen condition. This condition results to other clinical events including haemolysis and intrahepatic sickling, which have been associated with hepatic damage.

The higher foetal haemoglobin concentration in SCD subjects compared to the control have been associated to genetic factors [14-16]. Previous studies have associated high foetal concentration with the ability to neutralize the effect of HbS polymerization and reduction in severity of the disease [17,18]. This promising potential of high foetal haemoglobin has led to little attention given to studies that could completely unlock the potentials hidden in low foetal haemoglobin concentration.

Our finding also revealed an insignificant difference in the concentration of TB, AST, ALT and HbF across gender. This finding suggest that SCD is not gender sensitive as it equally affects both genders. Our finding agrees with the study of Obi, et al. [12] and Isah, et al. [19], who also found an insignificant difference in the concentration of TB, AST, ALT across gender in SCD subjects.

A comparison of the concentration of TB, AST, ALT and HbF across the different states of the disease revealed an insignificant difference between the steady state and crises state subjects. Though the difference in concentration of TB, AST, ALT and HbF was not significant, the increased serum concentration of TB, AST and ALT in crises state subjects is an indication of an increased disease severity in crises state while the elevated foetal haemoglobin concentration in steady state subjects suggest the effect of increased foetal haemoglobin concentration in reducing disease severity. This finding agrees with studies of Tukur, et al. [20] and Omoti [21].

**Table 3:** Comparison of HbF concentration and markers of Liver function in steady state and crises state subjects in SCD.

Biochemical Markers	Steady State (N = 20)	Crises State (N = 10)	P-Value
HbF (%)	2.15 ± 1.44	1.62 ± 1.16	0.401
Bilirubin. (µmol/L)	30.95 ± 18.82	36.44 ± 27.09	0.599
ALT	22.89 ± 11.20	26.36 ± 15.05	0.554
AST	61.07 ± 11.12	65.41 ± 14.51	0.555
AST/ALT	3.08 ± 1.07	2.90 ± 1.11	0.738

HbF: Foetal Haemoglobin; TB: Total Bilirubin; ALT: Alanine Transaminase; AST: Aspartate Transaminase; AST/ALT: De-ritis ratio

**Table 4:** Correlation of HbF concentration with markers of liver function in SCD.

Biochemical Markers	Correlation coefficient (r)	P-Value
HbF	TB	-0.372
	ALT	0.128
	AST	-0.273
	AST/ALT	-0.307

HbF: Foetal Haemoglobin; TB: Total Bilirubin; ALT: Alanine Transaminase; AST: Aspartate Transaminase; AST/ALT: De-ritis ratio

\*Correlation is significant at 0.05 (P < 0.05).

The correlation between HbF concentration and the liver function markers of the SCD subjects was also studied. The HbF concentration of the subjects was low (< 10%) and correlated inversely with TB, AST and AST/ALT ratio. Previous studies have associated individuals with a higher concentration of HbF to a less severity of the disease [16,22]. However, this study observes that low HbF concentration also has an ameliorating effect on disease severity. This conclusion is evident from the decreasing concentration of liver function markers with increasing levels of foetal haemoglobin.

## Conclusion

We conclude that low HbF concentration also potentially ameliorate disease severity. However, this potential has been overshadowed by the promises recorded in use of high HbF concentration to ameliorate clinical severity of SCD. To fully understand its potential more studies on the potentials locked in low HbF concentration should be encouraged.

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## Author Contribution

Dogonzo IY and Enewor NT performed experiment while Dogonzo IY, Enewor NT and Ekoh OC participated in the analysis and interpretation of data and writing of the manuscript.

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