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ORIGINAL ARTICLE

Association of BCL11A and HBS1L-MYB Polymorphisms in Sickle Cell Anaemia Subjects of Different Age Groups in Nigeria

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Abstract

Background

Haematological features and clinical severity of Sickle Cell Anaemia (SCA) are influenced by age, gender, genetic and community factors. *BCL11A* is cytogenetically located on short arm of chromosome 2 at position 16.1 while it is located at base pair 60,451,167 to 60,553,498 on chromosome 2 at the molecular level. Polymorphisms in the *HBS1L-MYB* Intergenic region were associated with F-cell levels and accounted for 19.4% of the F-cell variance in normal Europeans.

Objective

This study highlights possible effects of age in Haemoglobin F Induction through possible association of *BCL11A* and *HBS1L-MYB* genes polymorphism in SCA Subjects population in Oshogbo metropolis.

Materials and Methods

Thirty – Six SCA subjects were recruited with ages ranging between 1 and 40years divided into five groups of eight subjects each except age ranges 21 to 25 and 25 to 40 years which were six subjects each, while ten haemoglobin A subjects served as control for all age ranges. Red Cell indices and gene analysis such as HCT, RBC Count, Haemoglobin concentration, Hb F level, *BCL11A* and *HBS1L-MYB* genes were studied using standard methods.

Results

Results shows no statistically significant difference in fetal haemoglobin between control and subjects (P>0.05). There was a statistical decrease in haemoglobin level in the test subjects compared to controls. Also, the *HBS1L-MYB* protein expression was not significantly different in Haemoglobin A subjects and the SCA

subjects, but there was a statistically significant decrease in *BCL11A* gene expression in SCA Subjects compared to Haemoglobin A Subjects (P<0.05). A statistically significant decrease in *BCL11A* gene expression was obtained in ages 16 to 20 and 21 to 40years age groups (p<0.05). There was a positive correlation between Fetal Haemoglobin and haematocrit and haemoglobin levels (r=0.04341, p=0.2227); (r=0.01705, p=0.4479), respectively. The correlation between Fetal haemoglobin and *BCL11A* and *HBS1L-MYB* showed statistically significant negative correlations (r= - 0.1220, p=0.0368), r= - 0.1260, p=0.0336 respectively).

Conclusion

Conclusively, *BCL11A* and *HBS1L-MYB* genes promote induction of fetal haemoglobin in SCA subjects compared with controls recruited for this study.

Keywords: *BCL11A*, *HBS1L-MYB*, Sickle Cell Anaemia, Age, Osogbo Metropolis.

Introduction

Sickle Cell Disease (SCD) is a structural haemoglobinopathy associated with hereditary haemolytic anaemia with its clinical phenotype resulting from several genotypes (1,2). It is the first molecular disease described associated with a mutated protein (3), resulting from inheritance of two abnormal allelomorphic genes that control the formation of beta globin chain. This results in the production of abnormal less soluble haemoglobin S (Hb S), which tends to polymerize and deform red blood cells into the characteristic sickle shape (4).

A strong correlation has been established between the clinical severity and the level of fetal haemoglobin (HbF) in SCD (5). The expression of *HBG1*(142200) and *HBG2* (14250) genes, critical for the synthesis of HbF, is dramatically reduced shortly before birth and remains as such after birth (6). Rarely, the level of HbF may remain significantly elevated a condition known as Hereditary Persistence of Fetal Haemoglobin (HPFH) (7). Inhibition of sickling by high levels of HbF has been documented. This is as a result of ability of HbF to decrease the polymerization of deoxygenated Hb S (8). Interestingly, HPFH shows to be beneficial in SCD (7). Up to three quantitative traits loci has been associated with HPFH. These are - the combined *Xmnl-158* and *OR51B516* locus on chromosome 11p15.4 (9), the *HBS1l-MYB* intergenic polymorphism (HMIP) locus on chromosome 6q23.3 (10) and the *BCL11A* locus on chromosome 2q16.1 (11).

The fetal to adult haemoglobin switch and silencing of fetal haemoglobin have been an area of long-standing interest given the fact that clinical induction of Hb F production holds tremendous promise in ameliorating the clinical symptoms of SCA and beta thalassemia as stated in previous reports (12,13,14). Successful induction of Hb F depends on the physiological condition of SCA individual. This study is therefore designed to determine the possible effects of age on Hb F induction through

association of *BCL11A* and *HBS1L-MYB* genes polymorphisms in SCA Subjects population in Osogbo metropolis.

Materials and Methods

Study design: This hospital based, cross-sectional descriptive study was carried out in Osogbo, the capital of Osun State in the South-western part of Nigeria.

Subjects: A total of Thirty-six (36) steady-state SCD patients attending Haematology and Paediatrics clinics who met the inclusion criteria were recruited for the study. Additionally, 10 Hb AA individuals were also recruited to serve as controls.

Ethical Consideration: Ethical approval was obtained from the Ethical Committee of LAUTECH Teaching Hospital, Osogbo. Written informed consent/assent was also obtained as appropriate from individuals prior to their enrolment into the study. Confidentiality was assured for every subject and their results.

Conduct of the study: These participants were categorised, using Stratified Random Sampling with age as the characteristic of interest as follows: 1-5yrs, 6-10yrs, 11-15yrs, 16-20yrs and 21-40yrs, eight samples per age group of test categories one to three, six samples each were used for categories four and five while two samples per category for control were studied.

Inclusion criteria:

- Sickle cell disease subjects with Haemoglobin genotype SS as well as Hb AA controls.
- Consenting participants must be greater than 18 yrs.
- Assenting participants must be greater than 6yrs and less than 18 years of age
- Minor for which parent's consent has been sought

Exclusion criteria:

- Patient that had been transfused with blood or hospitalized in the past 6 weeks prior to the time of sample collection.
- Patient currently treated with hydroxyurea.

Sample Collection

Three millilitres of venous blood was collected from each subject through aseptic venepuncture and dispensed as follows. Two millilitres was dispensed into an EDTA bottle while the remaining 1mL was dispensed into the DNA/RNA Shield Fluid bottle without mixing.

Haematological Parameters

Complete Blood Count: The hemogram of all samples were determined by automation method using Sysmex KN21X 3- parts differential Auto Haematology Analyzer through the Coulter Principle of Electrical Impedance while estimation of Hb F was carried out using the Modified Betke's Method (15) based on the principle of alkaline denaturation test.

Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) method for *BCL11A* and *HBS1L-MYB* gene analysis

DNA was extracted from blood using SDS-proteinase K as described by Francesco *et. al.*, (12) with the kit Zymo-Spin Technology using Quick-DNA^{Tm} Miniprep Plus kit (Catalog Nos D4068 and D4069) while the DNA yield was done spectrophotometrically.

Polymerase Chain Reaction (PCR) and Restricted Fragment Length Polymorphism were carried out according to Gaurab (17).

Data Analysis

Data were collated using Microsoft Word and Excel package, while statistical analysis was carried out using GraphPad Prism 8.0. Results were presented in tables and figures – bar charts and correlation graphs. Student's t-test and ANOVA were used to compare group differences. P value less than 0.05 was taken to be statistically significant.

RESULTS

Results are presented in tables and figures as shown below:

Table 1: Distribution of Age Groups of Sickle Cell Anaemia Subjects Recruited for the Research

Age Range (Years)	Frequency	Percentage (%)	Mean + SE (12.39±1.31)
1 – 5	8	22	
6 – 10	8	22	
11 – 15	8	22	12.61
16 – 20	6	22	
21 – 40	6	22	
Total	**36**	100	

Table 2: Distribution of Age Groups of Haemoglobin A Controls Recruited for the Research

Age Range (Years)	Frequency	Percentage (%)	Mean + SE (15.1±3.41)
1 – 5	2	20	
6 – 10	2	20	
11 – 15	2	20	15.1
16 – 20	2	20	
21 – 40	2	20	
Total	10	100	

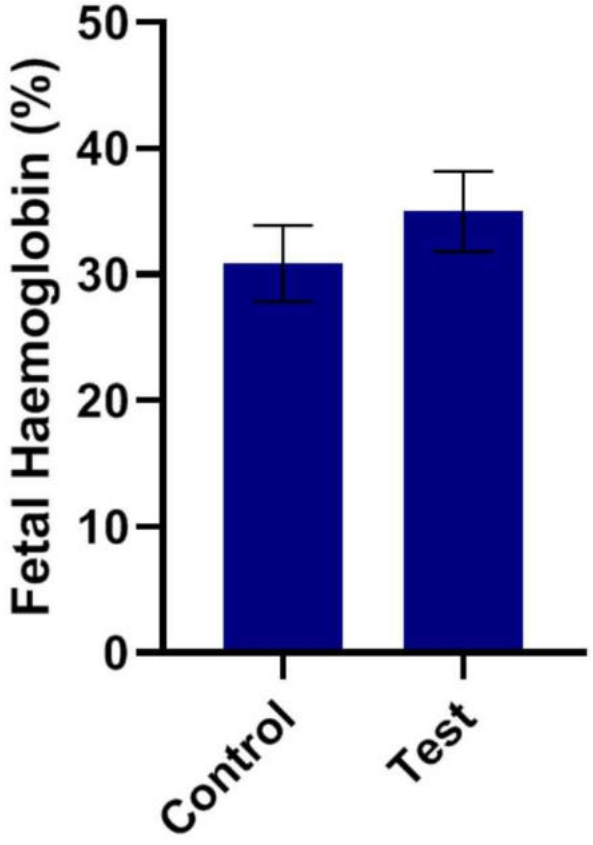

Figure 1: The percentage fetal haemoglobin in Haemoglobin A Subject (Control) and Sickle Cell Anaemia Subjects (test group). The result shows no statistically significant difference in fetal haemoglobin between control and test subjects ($p>0.05$).

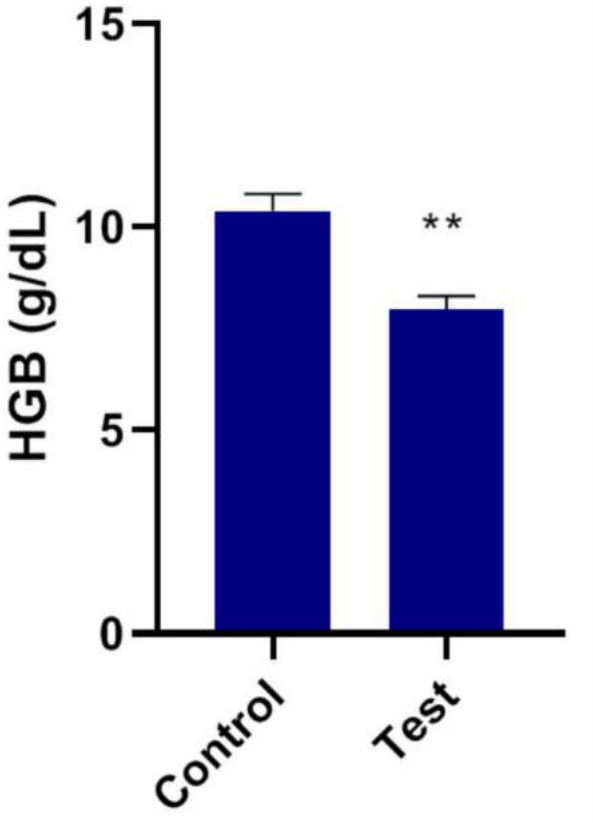

Figure 2: The haemoglobin concentration in haemoglobin A Subject (Control) and Sickle Cell Anaemia Subjects (test group).

A statistically significant decrease haemoglobin concentration in the test subjects was obtained when compared with control ($p<0.05$).

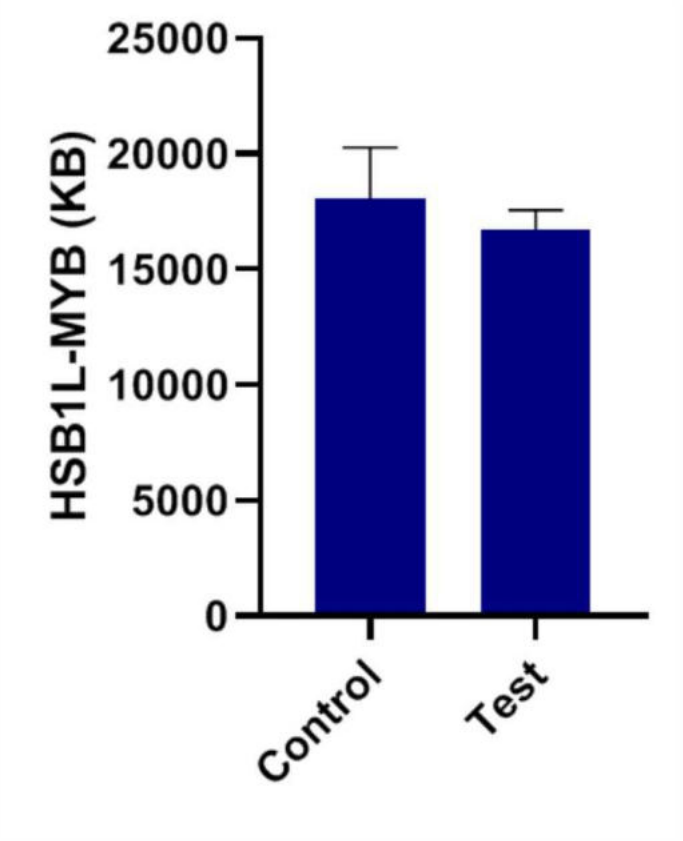

Figure 3: The *HBS1L-MYB* gene expression in haemoglobin A Subject (Control) and Sickle Cell Anaemia Subjects (test group).

The *HSB1L-MYB* gene expression is higher in Haemoglobin A subjects compared with Sickle Cell Anaemia Subjects, though the difference is not significant ($p>0.05$)

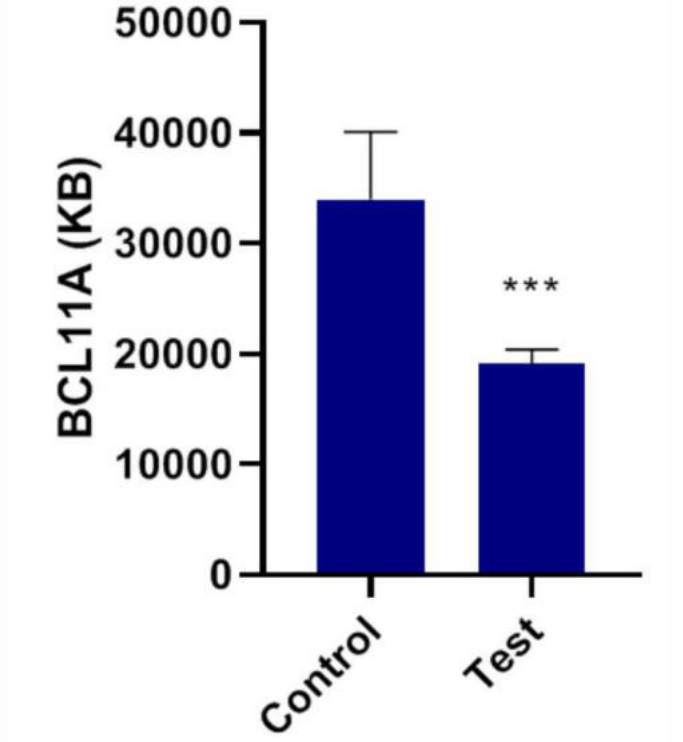

Figure 4: The *BCL11A* gene expression in haemoglobin A Subject (Control) and Sickle Cell Anaemia Subjects (test group).

There was a statistically significant decrease in *BCL11A* gene expression in Sickle Cell Anaemia subjects compared with Hamoglobin A subjects ($p<0.05$).

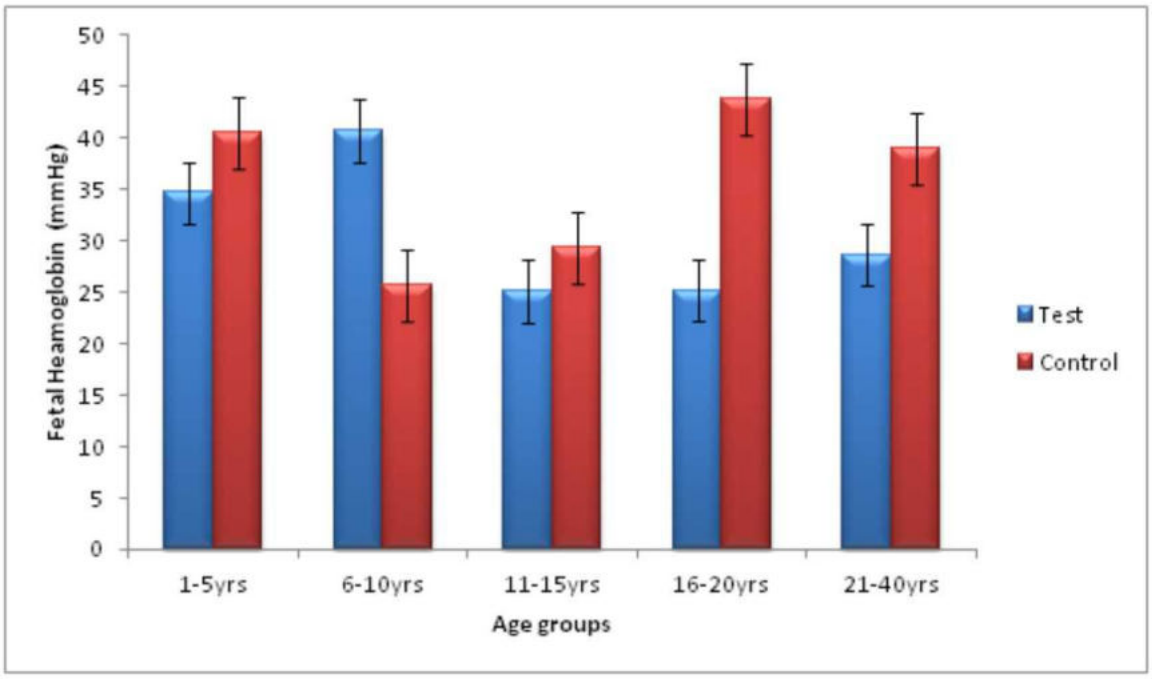

Figure 5: The fetal haemoglobin level among different age groups of test and control subjects. The result shows no significant difference in fetal haemoglobin concentration among different age groups (1 -5, 6 to 10, 11 to 15, 16 to 20, 21 to 40) compared to control ($p>0.05$).

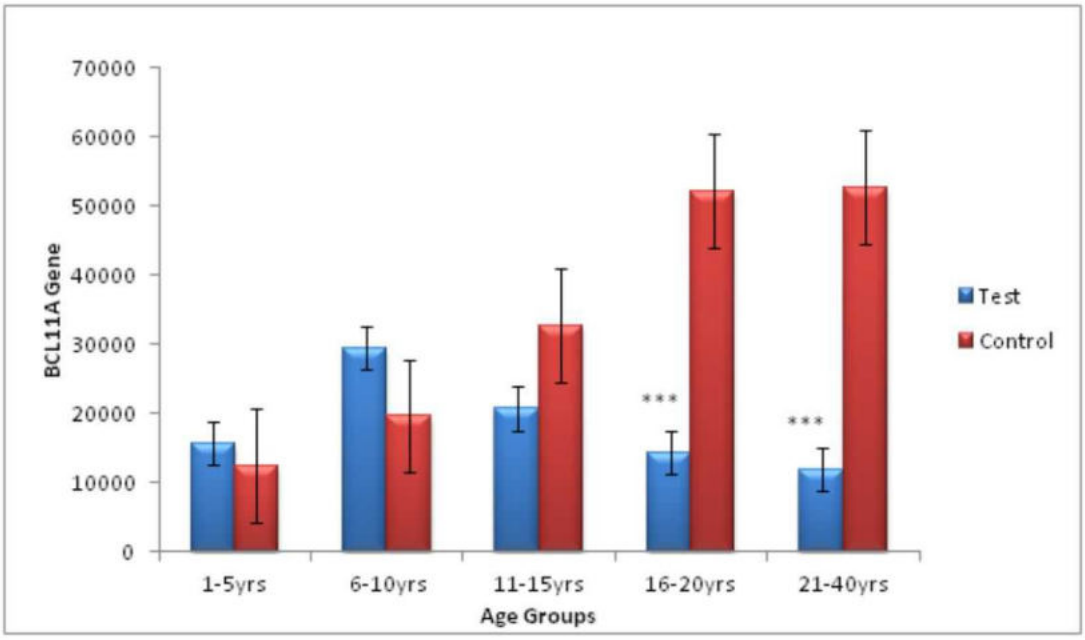

Figure 6: The *BCL11A* gene expression among different age groups of tests and control subjects. The result shows a statistically significant decrease in *BCL11A* gene expression in 16 to 20 and 21 to 40 age groups but, no statistically significant difference in 1 to 5, 6 to 10 and 11 to 15 age groups compared to control ($p<0.05$).

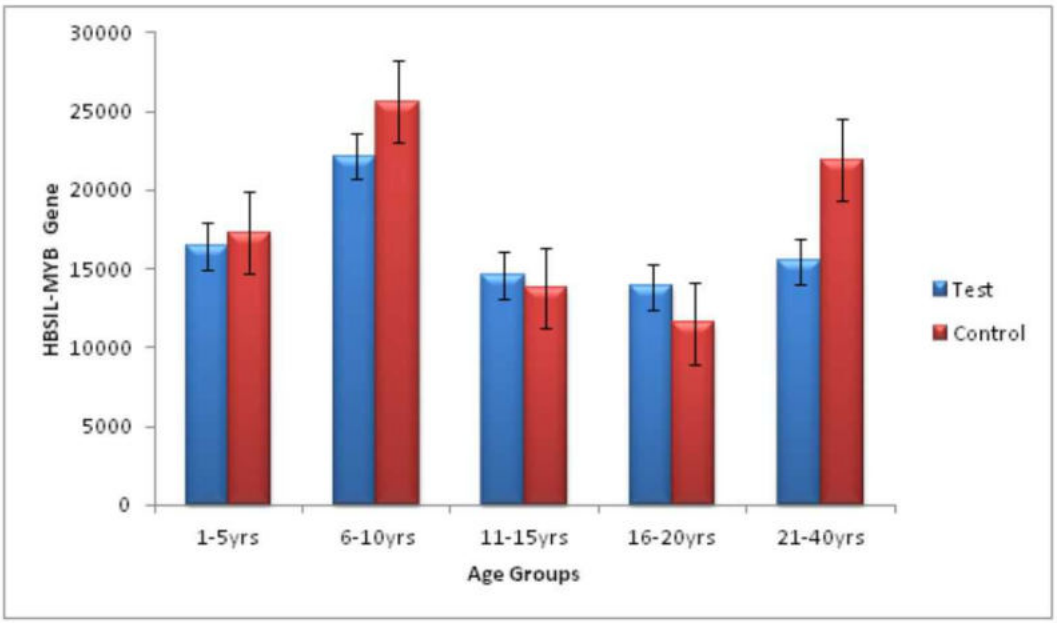

Figure 7: The *HBS1L-MYB* gene expression among different age groups of test and control subjects. Result show no significant difference in *HBS1L-MYB* gene expression among different age groups of SCA subjects compared to control subjects (p>0.05).

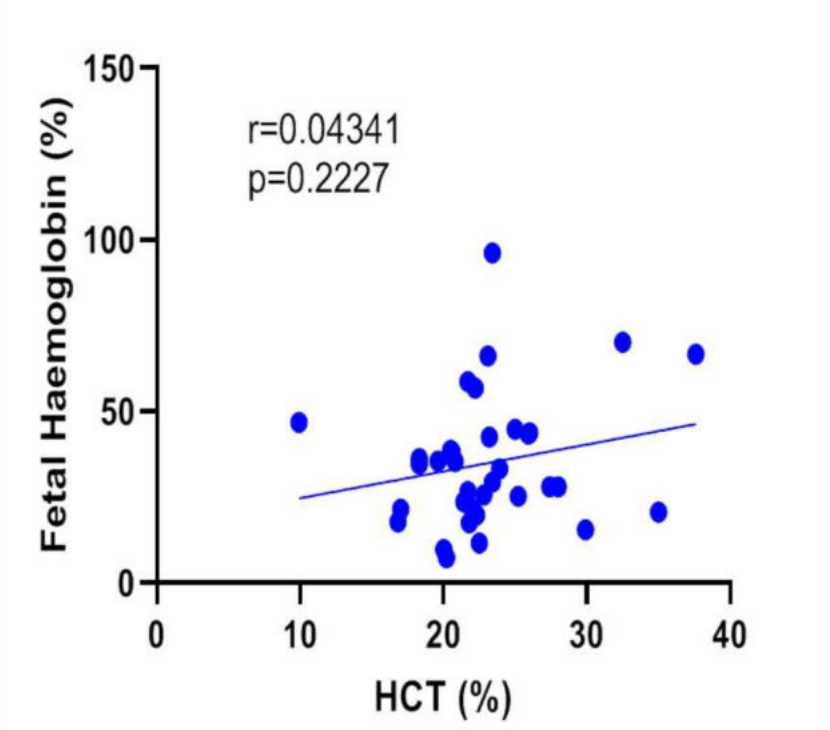

Figure 8: The correlation between fetal haemoglobin and haematocrit value. The result shows a positive correlation between fetal haemoglobin and haematocrit but was not statistically significant (r=0.4341, p=0.2227).

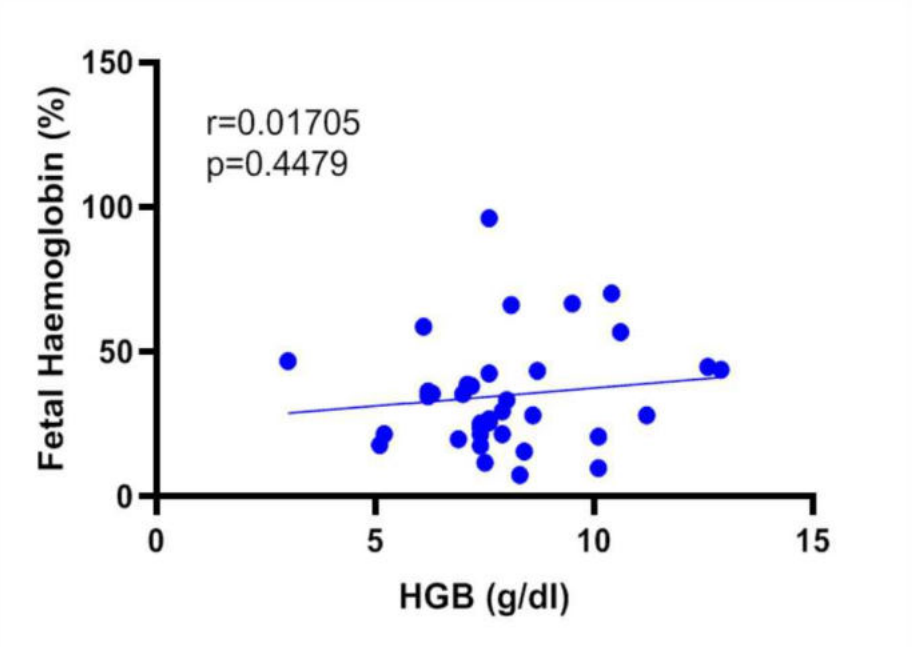

Figure 9: The correlation between fetal haemoglobin and haemoglobin concentration. Fetal haemoglobin and Haemoglobin concentration results show a positive correlation but was not statistically significant (r=0.01705, p=0.4479).

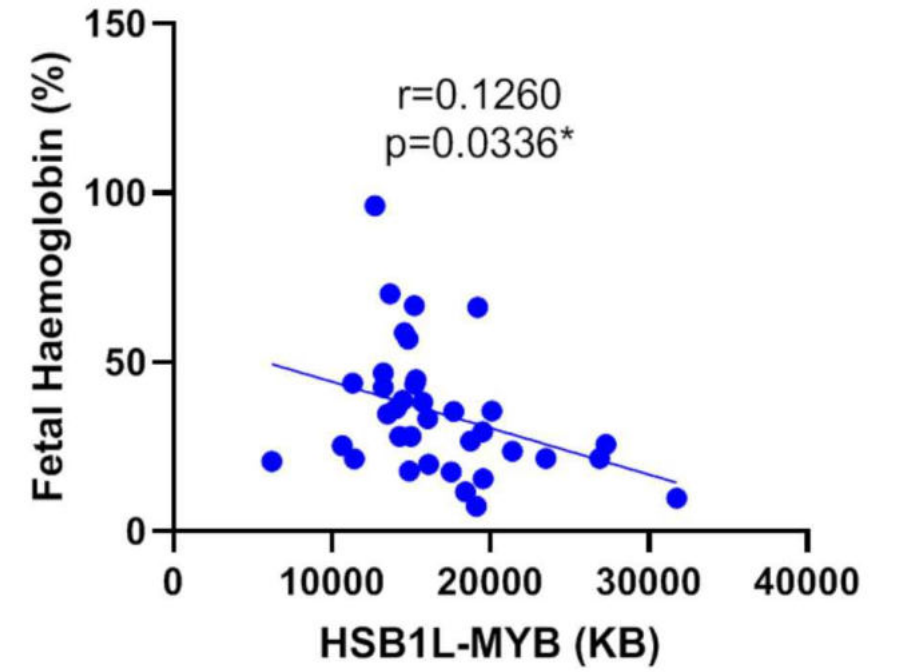

Figure 10: The correlation between fetal haemoglobin and *HSB1L-MYB* gene expression
Result shows a statistically significant negative correlation between *HSB1L-MYB* gene expression and Fetal haemoglobin.

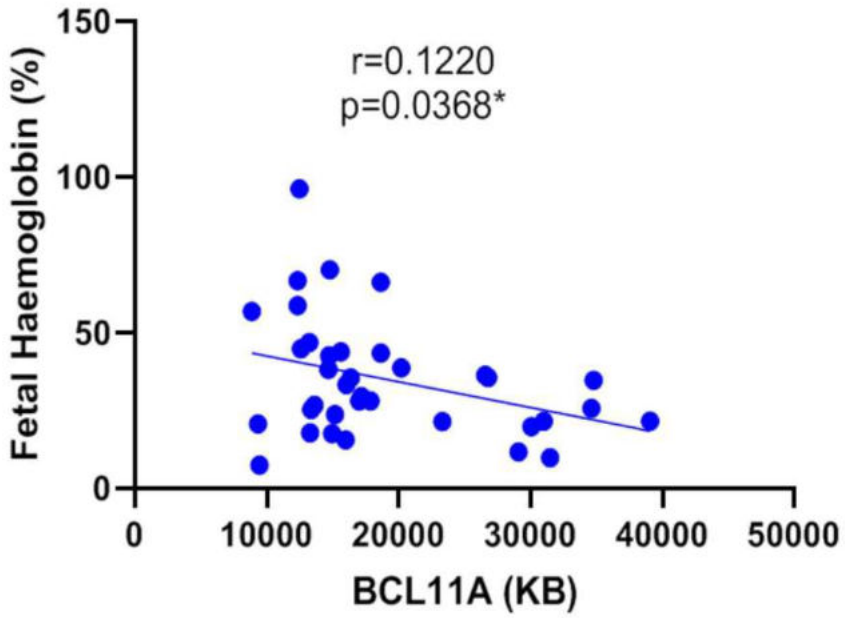

Figure 11: The correlation between fetal haemoglobin and *BCL11A* gene expression.
A statistically significant negative correlation was obtained between fetal haemoglobin and *BCL11A* gene expression (r= - 0.1220, p=0.0368).

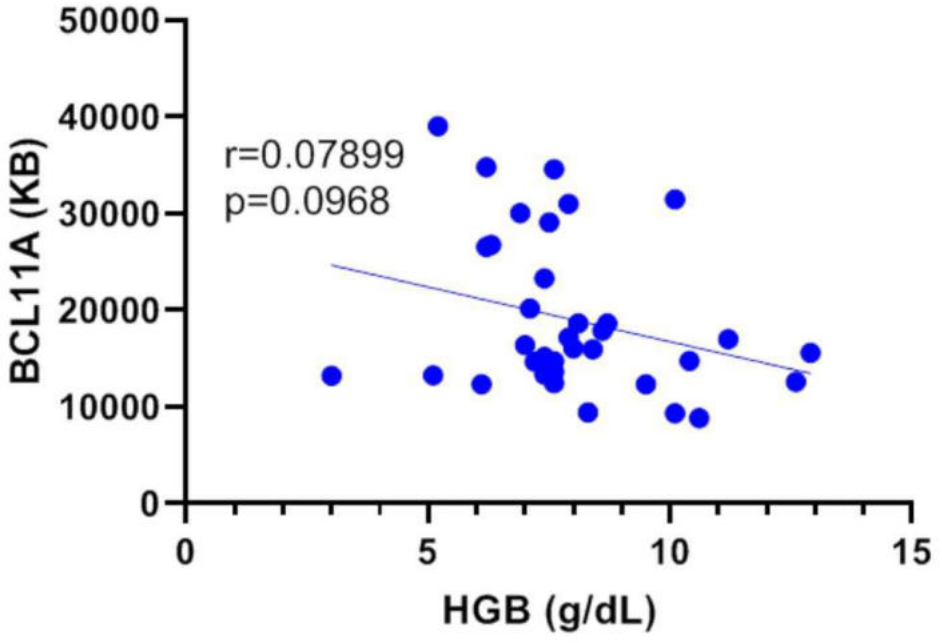

Figure 12: The correlation between *BCL11A* gene and haemoglobin concentration
The result shows a negative correlation between *BCL11A* gene expression and haemoglobin concentration but was not statistically significant (r= - 0.07899, p=0.0968).

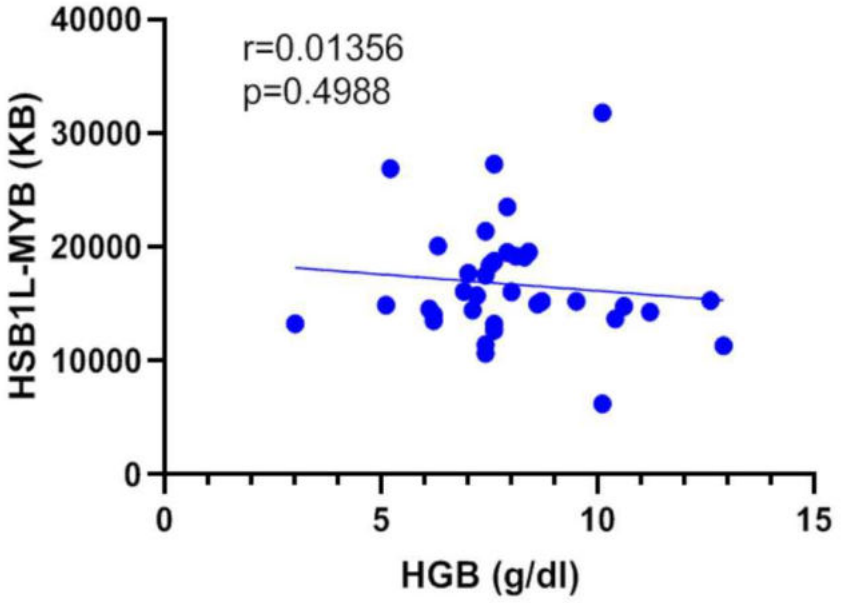

Figure 13: The correlation between *HSB1L-MYB* gene expression and haemoglobin concentration. Result shows a negative correlation between *HSB1L-MYB* gene expression and haemoglobin concentration but was not statistically significant (r= - 0.01356, p= 0.4988).

DISCUSSION

Haematological features and clinical severity of SCD are influenced by age, gender, genetic, and environmental factors. The presence of α-thalassaemia, variation in Hb F level, and the specific haplotype background that is linked to the β globin gene play an important role in the severity of disease (18). This study highlights possible effects of age on Hb F induction through possible association of *BCL11A* and *HBS1L-MYB* genes polymorphism in SCA subject population in Osogbo metropolis.

In this study, results show no statistically significant difference in fetal haemoglobin between control and test subjects (p>0.05). This outcome is at variance with a similar study conducted by Uko *et al.*, (19) who reported a higher mean Hb F value in Hb SS subjects (3.05±1.61%) compared to Hb AA controls (0.20±0.25). In another study conducted by Adekile *et al.*, (20), they showed that complications rarely occurred before 5 years of age when the haemoglobin F level was approximately 30%, but with increasing age and decline in haemoglobin F level, clinical complications begin to occur. Hb F may therefore have a beneficial effect on the prognosis of sickle cell disease and use of therapeutic approaches involving combination of Hb F-inducing agents in the management of patients with SCD (21).

The *HSB1L-MYB* gene expression was relatively stable in Haemoglobin A and sickle cell subjects (p>0.05). This outcome is contrary to the report of Farrell *et al.*, (22) who stated that *HBS1L-MYB* intergenic polymorphism is associated with Hb F among sickle cell anaemia patients of African descent, although much less significantly compared with Europeans or Chinese because of their much lower minor

allele frequencies. It could be that there are other HMIP variants associated with Hb F level among people of African descent that are not tracked well by SNP *rs9399137* (23). In another study, only *HBS1L* expression was correlated with elevated Hb F levels (24). However, higher level of *HSB1L-MYB* gene in haemoglobin A subjects when compared with control could be as a result of other genetic makeup and environmental factor. The relatively stable HBF in the controls and subjects in this study apparently has a direct link to the unchanged expression of the gene among the groups studied.

The *BCL11A* rs1427407 variant is commonly found in SCD patients (25). This study revealed a statistically significant decrease in *BCL11A* gene expression in sickle cell anaemia subjects compared to hamoglobin A subjects ($p<0.05$). The outcome in this study is contrary to Hassan *et al.*, (26) who reported that SCD patients had plasma *BCL11A* levels that were significantly higher than the control subjects ($P<0.05$).

Furthermore, the SCD patients had a higher frequency of the GG and GT genotypes at *BCL11A* rs1427407. Moreover, the *BCL11A* rs1427407 GG variant increased the risk of developing SCD. Some studies have also indicated that *BCL11A* can be used to predict overall and disease-free survivals (27, 28). The down regulation of this gene as seen in this study could be associated with the stable states of the subjects studied. Lower expression of *BCL11A* has been associated with a better efficiency in the switch of embryonic haemoglobin to fetal haemoglobin (11). Also, Funnell *et al.*, (29) stated that the increase in the expression of the *BCL11A* gene will lead to lower levels of Hb F. The *BCL11A* genotype most associated with Hb F expression correlates with reduced *BCL11A* expression since it acts as a repressor and acts directly on the HBB cluster, participating in Hb switching at different development stages (30). This study has demonstrated a direct agreement with these observations.

Furthermore, this study demonstrated a significant negative correlation between *Hb F* and *BCL11A* gene expression ($r=0.1220$) expression as well as that of *HSB1L-MYB* ($r=0.1260$).

In a similar study conducted by Thein *et al.*, (10) only *HBS1L* expression was correlated with elevated Hb F levels. Nevertheless, the expression profile of *MYB* and *HBS1L* in adults with non-gene deletion HPFH was downregulated. Overexpression of *MYB* in K562 cells inhibited *HBG* expression (27). Low levels of *MYB* were associated with reduced cell expansion and accelerated erythroid differentiation, suggesting that variation in the intrinsic levels of *MYB* might affect Hb F by its effect on the cell cycle. Overexpression of microRNA-15a and -16-1 down-regulated *MYB* in $CD34^{+}$ erythroid progenitors and increased Hb F (24). There were limited studies on Association of *BCL11A* and *HBS1L-MYB* Polymorphism in Sickle Cell Anaemia Subjects with different age groups as the characteristic of interest hence, limited previous studies were available to support results with age ranges.

The *BCL11A* rs1427407 variant is commonly found in SCD patients (25). The *BCL11A* gene was first detected in B-cell chronic lymphocytic leukemia and is a proto-oncogene for malignant hematological diseases (32). In a study conducted by Hassan *et al.*, (26), SCD patients had plasma *BCL11A* levels that were significantly higher than the control subjects (100.13 µg/L versus 70.88 µg/L, $P<0.05$). This study showed a statistically significant decrease in *BCL11A* gene expression in 16 to 20 and 21 to 25 age groups but, no statistically significant difference in other age groups.

Fetal hemoglobin (Hb F) is the major genetic modulator of the haematologic and clinical features in health and disease state. Hb F is the predominant hemoglobin from early gestation until 1 to 2 months post-natal when adult Hb A predominates (6). In this study, there was a positive correlation between fetal haemoglobin and haematocrit value (r=0.04341, p=0.2227) and also between fetal haemoglobin and haemoglobin concentration ((r=0.01705, p=0.4479). The positive correlation between Hb F and haemoglobin, and Hb F and haematocrit percentage could be as a result of erythroid precursors of normal adults expressing haemoglobin concentration at a level. According to Stamatoyannopoulos (13), a stochastic model posits that the increase in Hb F is as a result of recruitment of erythroid progenitor cells that prematurely undergo terminal differentiation and are committed to producing γ-globin and the fetal haemoglobin level among different age groups of test and control subjects.

This study equally showed no statistically significant difference in fetal haemoglobin concentration in different age groups (1 to 5, 6 to 10, 11to 15, 16 to 20, 21 to 40) compared to controls (p> 0.05 respectively). This is at variance with Olaniyi *et al.,* (33) which posited that there was a statistically significant difference in the mean level of Hb Fin (5.16+/- 4.04) patients compare to control (1.04+/- 0.44). In another study, Tshilolo *et al.* (34) submitted that Congolese SCA patients displayed low level Hb F and F-cells that contribute to the severity of SCA. It could be that there are other HMIP variants associated with Hb F level among people of African descent that are not tracked well by SNP rs9399137 (23).

This study shows a negative correlation between *BCL11A* gene expression and haemoglobin concentration but was not statistically significant (r= - 0.07899, p=0.0968). In a similar study conducted by Sedgewick *et. al.*, (35), *BCL11A* correlates most strongly with Hb F expression from the action of SNPs located within a region of about 14 kb in intron 2 of the gene. The *BCL11A* genotype most associated with Hb F expression correlates with reduced *BCL11A* expression since it acts as a repressor and acts directly on the HBB cluster, participating in Hb switching at different developmental stages (30).

The sequences of *HBS1L* and *MYB* and other genes are uninformative regarding Hb F regulation (36). This study shows a negative correlation between *HSB1L-MYB* gene expression and haemoglobin concentration but was not statistically significant (r= - 0.01356, p=0.4988). Results show no statistically significant difference in *HBS1L-MYB* gene expression in the different age groups (1 to 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25) compared to control (p>0.05).

Conclusion

Expression of *BCL11A* and *HBSIL-MYB* genes have established roles in regulation of Haemoglobin F among SCA and control subjects in Osogbo, Southwest Nigeria. As age advances, the *BCL11A* expression reduces from age 16 upward. The *HBS1L-MYB* gene remain with attendant influence on Hb F to the age of 40 in the studied population. Knocking down of *BCL11A* protein synthesis poses a promising role in induction of fetal haemoglobin and reduces sickle haemoglobin production with pancellular distribution of fetal haemoglobin, which in SCD is sufficient to mitigate hemolysis and significantly reduce vaso-occlusive crisis.

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