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Foetal Haemoglobin Gene Expression in Patients with Sickle Cell Disease in North Central Nigeria

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Abstract

Foetal haemoglobin plays a dominant role in ameliorating morbidity and mortality of sickle cell disease. Individual's variation in foetal haemoglobin (*HbF*) expression is a known and potentially heritable modifier of sickle cell disease severity. We evaluated the distribution pattern of foetal haemoglobin in SCD (n=146), 75 were homozygous SS as test subjects and 71 homozygous AA individuals as control subject. The homozygous SS showed *HbF* levels of 6.5 with a standard error of mean 6.5 ± 0.8, *HbA1* Showed a levels of 2.6 with a standard error of mean 2.6 ± 0.3 and *HbA2* showed 4.9 with a standard error of mean 4.9 ± 0.1 respectively. The control subjects showed *HbF* levels of 0.5 with a standard error of mean 0.5 ± 0.04, *HbA1* showed a levels of 87.3 with a standard error of mean 87.3 ± 0.4 and *HbA2* 3.2 with a standard error of mean 3.2 ± 0.1 while the total haemoglobin concentration showed a levels of 6.5 with a standard error of mean 6.5 ± 0.15652 and that of control subject showed a total Hb concentrations 12.32 with a standard error of mean 12.32 ± 0.12548 respectively.

The total haemoglobin concentration of Sickle cell patients was significantly lower than that of non-sickle cell patients. There exists a positive correlation of haemoglobin concentration (g/dl) with *HbS* genes expression. Our study also show that the lesser the number of crisis the lower the fetal haemoglobin and the higher the number of crisis the higher the fetal haemoglobin genes expression. It is recommended that estimation of *HbF*, *HbA1* and *HbA2* levels be carried out in conjunction with hemoglobin electrophoresis in the diagnosis, clinical management and in the determination of the clinical course of sickle cell disease.

Keywords: Sickle cell; Fetal haemoglobin; Hemoglobin disorders; Beta thalassemia

Introduction

Feotal hemoglobin (*HbF*, α 2γ2) is the predominant hemoglobin in fetal life. The globin chains of *HbF* are coded by γ-gene of β-globin clusters on chromosome 11 in humans. After birth *HbF* is gradually replaced by adult hemoglobin (*HbA*, α 2β2) due to the switch from γ to β-globin gene expression. In normal subjects feotal Hemoglobin (*HbF*) constitutes less than 1% of the total Hemoglobin (Hb) by the end of the first year of life [The synthesis of *HbF* is restricted to a subpopulation of red cells, known as F-cells (FC) and the *HbF* levels are directly correlated to the number of FC4].

DNA mutation may lead to a persistent expression in γ-globin gene down-regulation. *HbF* levels which are regulated by multiple genes with influence of an environmental component play a dominant role in ameliorating morbidity and mortality of the principal congenital hemoglobin disorders such as sickle cell disease (SCD) [5]. The high concentration of *HbF* is a well characterized diagnostic feature and correlates with reducing morbidity and mortality in patients with these blood disorders [6].

Feotal hemoglobin differs from the adult form of the protein in its affinity for oxygen. Production of feotal hemoglobin begins about two months into gestation and helps deliver oxygen from the mother's bloodstream to the developing fetus. By about 3-6 months after birth, feotal hemoglobin is almost completely replaced by adult hemoglobin. The timing notes Orkin explain why sickle cell patients don't experience symptoms of the disease until several months after birth.

The sickle cell disease is a disorder that results from inheritance of two abnormal allelemorphic genes of the β chains of haemoglobin at least one of which is the sickle gene in which sickling of red blood cells produces prominent clinical manifestations. Red cell sickling, a sin qua non of sickle cell disease is caused by polymerization of hemoglobin tetramer as a result of replacement of glutamic acid by valine at position 6 of β-globin due to mutant sickle gene. Deoxygenation of *HbS* is crucial in causing conformational change that exposes a hydrophobic patch on the surface of β-globin chain at position 6 of the β-globin. Binding on this site to a complementary hydrophobic site on a β-subunit of another hemoglobin initiates polymerization of the hemoglobin tetramer and thus sickling of red cell containing the hemoglobin.

A variety of factors affect the pathophysiology of SCD leading multitude of clinical manifestations including intravascular hemolysis, vascular occlusion, pro-oxidant and pro-inflammatory stress, coagulopathy and altered blood rheology resulting in pain, organ damage, and a low blood count [7,8]. There is no single therapeutic modality that serves to abrogate all pathology of sickle cell disease but a better understanding of the mechanism of red cells sickling, factors that influence variability of its clinical course, the interactions of the SCDs and as well as their associated complications has led to a number of clinical interventions including induction of *HbF* production.

Elevated levels of Feotal hemoglobin (*HbF*) have been associated with lessened vaso occlusive complications and prolong survival rates of sickle cell disorder owing to its anitpolymerization property. *HbF* reduces *HbS* concentration in the same red cell, but more importantly, both *HbF* and its mixed hybrid tetramer cannot enter the deoxy sickle hemoglobin polymer phase.

This modulates the phenotypes of sickle cell disease due to variable distribution of *HbF* in sickle erythrocytes. The blood concentration of *HbF* or the number of cells with detectable *HbF* (F-cells) does not measure the amount of *HbF/F*-cell. Even patients with high *HbF* can have severe disease because *HbF* is unevenly distributed among F-cells and some cells might have insufficient concentrations to inhibit *HbS* polymerization. With mean *HbF* levels of 5%, 10%, 20% and 30% the distribution of *HbF*/F-cell can greatly vary even if the mean is constant. For example, with 20% *HbF* as few as 1% and as many as 24% of cells can have polymer-inhibiting or protective, levels of *HbF* of ~ 10 pg with lower *HbF* few or no protected cells can be present. Only when the total *HbF* concentration is near 30% is it possible for the number of protected cells to approach 70%. Rather than the total number of F-cells or the concentration of *HbF* in the hemolysate, *HbF*/F-cell and the proportion of F-cells that have enough *HbF* to thwart *HbS* polymerization is the most critical predictor of the likelihood of severe sickle cell disease. The overall aim of the study is to determine the pattern of Feotal haemoglobin expression in sickle cell anaemia patients.

Research design

The study was a case-control study which includes sickle cell patients as test subjects and non- sickle cell subjects as control subjects who are *HbAA*.

Study population

Sickle cell patients as test subjects in Abuja city and non-sickle cell patients as control subjects were confirmed.

Inclusion criteria

- All Sickle cell patients
- All apparently healthy individuals who are not Sickle cell patients
- Gender: male and female
- Age group: 1 year-15 years
- Not currently receiving therapy for an infection besides Sickle cell disease

Exclusion criteria

Patients with any form of illness beside sickle cell disease will be excluded.

Power and sample size estimation

To ensure that power will be high to detect reasonable departures from the statement of research question, we conducted a power analysis to determine that effect, using the following formula

$$N=r+1P^{*}(1-P^{*})(Z_{\beta}+Z_{\alpha/2})^{2}/(P_{1}-P_{2})^{2}$$

N=Sample size in the case group; r=ratio of control to cases; Zp=power of the study represents the desired power (typically 0.84 for 80% power); Zo/2=represents the desired level of statistical significance (typically 1.96); P1=represent the proportion exposed in the case group P2=the proportion exposed in the control group is 20%.

Pi-P_2 stands for Effect Size (the difference in proportions a measure of variability (similar to standard deviation)

Control=05% mean of *HbF* control Case=6.5 mean Hb case

^{P}caseexp=ORPcontrol EXP

=PCONTROL EXP(OR-1)+1⇒2.0(0.005)⇒0.0094

0.65(2-1)+1

$N=(1+1)(0.0094)(1-0.0094)(0.84+1.96)^{2}/1(0.\ 065-0.005)^{2}$

N=40.5 ~ 41

The minimum Number of subjects in the cases is 41.

Since the ratio of control to cases is 1, the minimum number of controls was also 41. However, a total of seventy (70) of cases and control was enrolled in to the study making a total of one hundred and forty (140) samples. A power of 0.60 gave an estimated sample size of 40.1 sample and a mean difference of 0.25 at p=0.05.

Sample selection

Five ml of blood was collected into EDTA bottle by aseptic venous puncture from Sickle cell patients in Abuja north central Nigeria and also 5 ml was also collected into EDTA bottle from the control subjects those determine to be suitable based on the selection criteria with their consents.

Sample Analysis

Methodology

HbF pattern and Hb concentration was determine by cation exchange high performance liquid chromatography(HPLC) using the VARIANT D-10™ Hemoglobin Analyzer (D-10™ Hemoglobin Testing System, Bio-Rad Laboratories, Marnes la Coquette, France) according to the procedure recommended by the manufacturer.

Principle of HPLC

This is based on the interactions of compounds in the analytes which is mobile phase) across an immobile surface (stationary phase). The compound binds at specific regions of stationary phase based on certain physical and chemical properties. These bound molecules are then eluted with a suitable buffer and the same are collected with time. These are polarity, charge, molecular weight, and present of functional group.

Result

Out of the 146 study subjects recruited for the research work 75 were homozygous sickle cell patients (SS) while 71 were homozygous (AA) individuals as control subjects. The homozygous SS showed *HbF* levels of 6.5 ± 0.8 (standard error of mean), *HbA1* shows a standard error of mean 2.6 ± 0.3 and *HbA2* showed a standard error of mean 4.9 ± 0.1 respectively. The control subjects showed a *HbF* standard error of mean of 0.5 ± 0.04, *HbA1* standard error of mean 87.3 ± 0.4 and *HbA2* standard error of mean 3.2 ± 0.1 (Table 1).

Genotype	Value (mean ± SEM)		p-valueα
	SCD (n=75)	Control (n=71)	
HbF (%)	6.5 ± 0.8	0.5 ± 0.04	<0.0001*
HbA1(%)	2.6 ± 0.3	87.3 ± 0.4	<0.0001*
HbA2 (%)	4.9 ± 0.1	3.2 ± 0.1	<0.0001*

Table 1: *HbF* Concentration and Adult Hb (*HbA1*& *HbA2*) concentration of SCD patients and their control counterparts in the studied population.

Comparisons of *HbF* and total Hb concentration of test (Table 2). The *HbF* showed a standard error of mean of 6.5 ± 0.8 and a total Hb concentration standard error of mean of 6.59 ± 0.15652, while the control subjects (AA) showed an *HbF* standard error of mean of 0.5 ± 0.04 and total Hb concentration standard error of mean of 12.32 ± 0.12548.

Genotype	Value (mean ± SEM)		p-value$^{\alpha}$
	SCD (n=75)	Control (n=71)	
HbF (%)	6.5 ± 0.8	0.5 ± 0.04	<0.0001*
Hb (mg/dl)	6.59 ± 0.15652	12.32 ± 0.12548	<0.0001*

Table 2: Comparison of *HbF* Concentration and Total Hb Concentration among SCD patients in the studied population and their control counterparts.

Whereby the No of crisis per month, those that experience crisis<2 times in a month were (n=46) while those that have crisis>2 times in a month were (n=29). The *HbF* standard error of mean of those who experience crisis<2 times in a month were 6.0554 ± 0.97666 and a total Hb concentration standard error of mean of 7.1326 ± 0.6252, while those that experience crisis>2 times in a month shows a *HbF* Standard error of mean of 7.0907 ± 1.39370 and a total Hb Concentration standard error of mean of 5.7345 ± 0.23937 respectively (Table 3).

Genotype	No. of Crisis/month (mean ± SEM)		p-value$^{\alpha}$
	<2 (n=46)	≥ 2(n=29)	
HbF (%)	6.0554 ± 0.97666	7.0907 ± 1.39370	0.546
Hb (g/dl)	7.1326 ± 0.6253	5.7345 ± 0.23937	0.289

Table 3: Comparison of *HbF* (%) and Hb concentration SCD patients with different durations of crisis.

Discussion

Sickle cell anaemia (SCA) is a monogenic disease with widely heterogeneous phenotypes. Its severity is moderated by high feotal haemoglobin levels. The mechanism responsible for *HBF* production in adults is not fully comprehended. However several studies have linked variations in *HBF* to polymorphism in β-globin cluster.

Increase levels of feotal haemoglobin are of no consequence in healthy adults but confer major clinical benefits in patients with sickle cell anaemia and beta thalassemia diseases that represent major public health problems [9].

Of the 146 study subjects examined in this study which constitutes 2 groups A & B. Group A representing SCD (n=75) shows 6.5% levels of feotal haemoglobin among the sickle cell patients with standard error of mean 6.5 ± 0.8 which is significantly higher than that of control subjects in group B which shows 0.5% of feotal haemoglobin genes expression and with a standard error of mean 0.5 ± 0.04 at a significance p-value of <0.0001 which indicates that there is persistence expression of feotal haemoglobin in the study subjects after birth. This is in agreement with the work of who state that the persistent expression of high levels of feotal haemoglobin after birth help in ameliorating the rate of frequent sickle cell crisis and subsequently mortality and morbidity of sickle cell patients. It's also agrees with the work of who state that normal individuals fetal haemoglobin constitutes less than 1% of the total haemoglobin as seen in our study that the feotal haemoglobin level is 0.5% which is less than 1% even though the mechanism responsible for the production of *HBF* in adults are not fully comprehended. However several studies have linked these variations in *HBF* to polymorphism in β-globin cluster [3].

Also our study shows lower levels of *HBA1* among the sickle cell study subjects compare to that of control in the study subjects. The sickle cell patients shows 2.6% levels of *HbA1* with a standard error of mean 2.6 ± 0.3, while that of control subjects 87.3% with standard error of mean 87.3 ± 0.4% which is significantly higher than that of group A at p<0.0001. There was also an increase in the level of *HBA2* among the group A which shows 4.9% with a standard error of mean 4.9 ± 0.1 which was also significantly higher than that of control subjects in group B which shows *HBA2* of 3.2% with a standard error of mean 3.2 ± 0.1 at a p<0.0001. *HbA1* is adult haemoglobin that is found in higher concentration among the non-sickle cell individuals compare to that of sickle cell patients. While *HbA2* have the same structure like *HbF* even though it's been considered as Beta-thalassemia by some researchers because of it tetra coil shape like *HbF*.

The total haemoglobin concentration of Group A (Sickle cell patients) was significantly lower than that of group B. Group A shows a total Concentration of 6.59 g/dl with a standard error of mean 6.59 ± 0.15652, while Group B (non-sickle cell individuals) Shows a total Haemoglobin concentration of 12.32 g/dl with a standard error of mean 12.23 ± 0.12548 which was significantly higher than that of sickle cell patients at p<0.0001. The lower levels of total haemoglobin concentration observed among the sickle cell patients is an indication that the lower haemoglobin which is the energy carrying capacity responsible for transportation of nutrients from one system to another or from one organ to another is not sufficient and that is why sickle cell patients often have low haemoglobin and subsequently result to low pack cell volume (PCV) an often gaps for oxygen during sickle cell crisis and also anaemia set in and it may lead to blood transfusion in order to keep and sustain the patients so as to enable a sufficient energy for steady transport of nutrients from one system to another.

Also our study shows that there is a positive correlation of haemoglobin concentration (g/dl) with HbS genes expression at P<0.05 which shows that there is positive r-value and a statistical significance because the lower haemoglobin concentration of *HbS* correlate with the possibility of anemic condition (Figure 1).

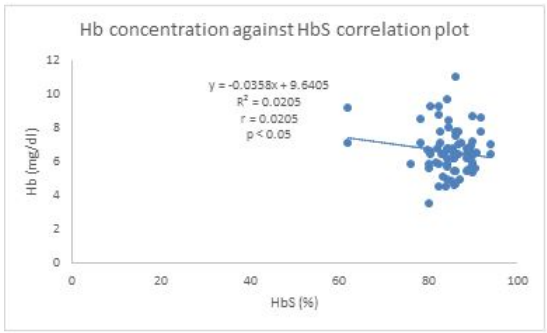

Figure 1: correlation between haemoglobin concentration (mg/dl) and *HbS* gene expression Among SCD patients in the studied population. This also shows a positive correlation with statistical significance of p<0.05 and r=0.0205.

Looking at the frequency of sickle cell crisis among the patients, those who have sickle cell crisis<2 times per month have lower feotal haemoglobin gene expression of 6.0554 ± 0.97666 compared to those that have sickle cell crisis ≥ 2 times in a month which shows a higher feotal haemoglobin concentration of 7.0907 ± 1.39370. Also the haemoglobin concentration of sickle cell patients who experience sickle cell crisis<2 times per month was significantly higher with haemoglobin concentration of 7.1326 g/dl with a standard error of mean 7.1326 ± 0.6253 than those that experience crisis<2 times per month which shows a haemoglobin concentration of 5.7345 g/dl with a standard error of mean 5.7345 ± 0.23937, this implies that the lesser the number of sickle dell crisis the lower the feotal haemoglobin and the higher the number of sickle cell crisis the higher the feotal haemoglobin genes expression, this is because feotal haemoglobin concentration favours high total haemoglobin concentration and also haemoglobin have affinity for oxygen which is needed for steady cardiopulmonary circulation and also for transportation of nutrients to the needed organs or system for proper functioning of the body system. This may also be due to the fact that after birth the feotal haemoglobin is destroy early and once the feotal haemoglobin is destroy normal sickle cell activities begin to take place, and also the lesser the frequency of crisis the higher the total haemoglobin concentration and the higher the frequency of crisis the lower the total haemoglobin concentration because more red blood cells will be haemolyse thereby destroying the red blood cells leading to low haemoglobin and subsequently anaemia set in.

There was also a positive r-value correlation between *HbF* and *HbS* genes expression and no statistical significance (Figure 2). This implies that the higher levels of circulating feotal haemoglobin in a sickle cell patient could lead to low manifestation of sickle cell symptoms because their deoxygenated erythrocytes take a longer period to sickle and will not deform extensively as those of sickle cell trait patients this agree with the work of Watson et al who state that the feotal haemoglobin did not interact with *HbS* as such the high level of feotal haemoglobin with the persistent y-globin in the feotal haemoglobin will inhibit the polymerization of *HbS* because *HbF* is a powerful modulator of the clinical and haematologic features of sickle cell anaemia. She also attributed that the high level of *HbF* were associated with a reduced rate of acute painful crisis episode.

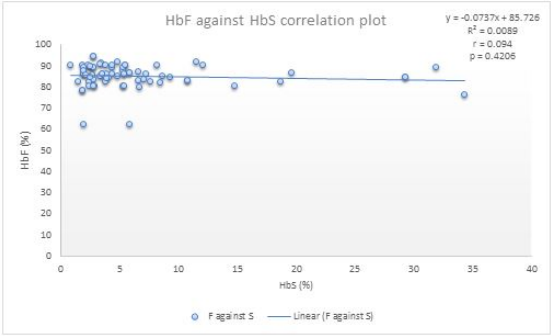

Figure 2: Correlation between *HbF* and *HbS* gene expression among SCD patient in the studied population. This shows a positive correlation but no statistical significance at p=0.4206 and r- value of 0.094.

In conclusion this study highlight that *HbF* plays an important role in gene expression and regulation, it also highlighted that *HbF* is involve in the modulation and inhibition of the polymerization of *HbS* and also that *HbF* do not interact with *HbS*. It thus improves our understanding of the physiopathology of the disease and aid to increase our ability to predict clinical severity.

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