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

Dangana A1, Emelieke FO2, Oluwatayo BO3, Haruna AS4, Sdiris AN5 and Jessy TM6

1Department of Medical Laboratory Services, University of Abuja Teaching Hospital, Gwagwalada, Nigeria
2Haematology Unit, Ambrose Ali University, Edo State, Nigeria
3Department of Haematology Federal College of Veterinary and Medical Laboratory Technology, Plateau State, Nigeria
4Department of Family Medicine, University of Abuja Teaching Hospital, Gwagwalada, Nigeria
5Department of Medical Microbiology and Parasitology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
6Department of Medical Laboratory Science, University of Maiduguri, Nigeria

Corresponding author: Dangana A, Department of Medical Laboratory Services, University of Abuja Teaching Hospital, Gwagwalada, Nigeria, Tel: 08095434437; Fax: 234 98 821 382; E-mail: isalemit@yahoo.co.uk

Received date: April 26, 2017; Accepted date: June 2, 2017; Published date: June 7, 2017

Copyright: © 2017 Dangana A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 ± 0.4, 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

Foetal hemoglobin (HbF, α 2y2) is the predominant hemoglobin in fetal life. The globin chains of HbF are coded by y-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 foetal Hemoglobin (HbF) constitutes less than 1% of the total Hemoglobin (Hb) by the end of the first year of life [1] 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].

Feotial hemoglobin differs from the adult form of the protein in its affinity for oxygen. Production of foetal 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, foetal 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 allelomorphic 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 to a 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 antipolymerization 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/wh 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 anemia 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

\[ N = r + 1 + \left(1 - P^2\right) \left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 / \left(P_1 - P_2\right)^2 \]

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-P2 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

\[ P_{\text{case}}^{\text{exp}} = \text{OR} \times \text{P}_{\text{control}} \]

\[ = P_{\text{CONTROL}} \times (OR-1) + 1 \Rightarrow 2.0(0.005) = 0.0094 \]

0.65(2.1)+1

\[ N = (1 + 1) \times (0.0094)(1-0.0094)(0.84 + 1.96)^2 / (0.065 - 0.005)^2 \]

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 determined 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).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HbF (%)</th>
<th>HbA1 (%)</th>
<th>HbA2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD (n=75)</td>
<td>6.5 ± 0.8</td>
<td>87.3 ± 0.4</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Control (n=71)</td>
<td>0.5 ± 0.04</td>
<td>3.2 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Value (mean ± SEM)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD (n=75)</td>
<td>6.5 ± 0.8</td>
<td>0.5 ± 0.04</td>
</tr>
<tr>
<td>Control (n=71)</td>
<td>0.5 ± 0.04</td>
<td>3.2 ± 0.1</td>
</tr>
</tbody>
</table>

**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 ± 0.6253, 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).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Crisis/month (mean ± SEM)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 (n=46)</td>
<td>6.0554 ± 0.97666</td>
<td>7.0907 ± 1.39370</td>
</tr>
<tr>
<td>≥ 2 (n=29)</td>
<td>5.7345 ± 0.23937</td>
<td></td>
</tr>
</tbody>
</table>

**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 foetal 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 foetal 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 foetal 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 foetal 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 foetal 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 foetal 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 foetal 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).

Looking at the frequency of sickle cell crisis among the patients, those who have sickle cell crisis<2 times per month have lower foetal 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 foetal 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±0.9748 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 ± 0.23897, this implies that the lesser the number of sickle cell crisis the lower the foetal haemoglobin and the higher the number of sickle cell crisis the higher the foetal haemoglobin genes expression, this is because foetal 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 foetal haemoglobin is destroy early and once the foetal 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 haemolysed 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 foetal 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 foetal haemoglobin did not interact with Hbs as such the high level of foetal haemoglobin with the persistent y-globin in the foetal 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.

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.

**Reference**