ORIGINAL RESEARCH ARTICLE

FOETAL HAEMOGLOBIN LEVELS IN SICKLE CELL DISEASE

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ABSTRACT

BACKGROUND

Foetal haemoglobin (HbF) is the most powerful modulator of the clinical and haematologic features in sickle cell anaemia. In sickle cell disease (SCD), an increase in HbF inhibits the polymerization of sickle haemoglobin and the resulting pathophysiology making them less prone to crises. The aim of this study is to quantify HbF levels in sickle cell disease patients.

METHODS

A total of 100 blood samples were collected from sickle cell anaemic patients who were all in steady state (apparently healthy). Fifty three (53%) were male, forty seven (47%) were females and fifty were from individuals with Hb-genotype AA who served as controls. Hb F estimation was carried out using the modified Betke method. The blood samples were transported to the laboratory where the following analysis were done. Haemoglobin genotype, haematocrit levels and HbF estimation. Other informations such as their ages, what they do for a living and also if they were on drugs gotten from the subjects. Results obtained were subjected to statistical analysis using paired t-test..

RESULTS

HbF levels in SCD was found to be significantly increased when compared with controls ($P < 0.01$). There was a weak correlation between increase in PCV ($R^2 = -0.16604$) of test subjects when compared with controls.

CONCLUSION

Foetal haemoglobin is higher in sickle cell anaemia patient than HbAA individuals and the pattern of increase does not necessarily depend on sex, age and HCT of the patient.

Keywords: *Foetal hemoglobin, sickle cell disease, crisis.*

BACKGROUND

Appreciating the role of foetal haemogloboin (HbF; alpha ($_2$) gamma (Y_2) in sickle cell disease started many years ago when it was confirmed that infants with SCD had few symptoms and that their deoxygenated erythrocytes took longer time to sickle and did not deform as extensively as did their sickle cell trait carrying mother's cells. It is the predominant form of haemoglobin produced during foetal life. This is replaced with the adult haemoglobin molecule in the postnatal life and it accounts for not more than 1.5% of the total adult haemoglobin depending on the region [1]. Several genetic factors have been reported to control HbF production [2-4]. Increased levels of HbF have been found to protect against various complications of disease such as leg ulcers, septicaemia and also have a beneficial effect on mortality [5]. Higher HbF levels were associated with a reduced rate of acute painful episodes, few leg ulcers, less osteonecrosis, less frequent acute chest syndromes and reduced disease severity. In sickle cell anaemia, F cells survive longer than non-F cells and this depends on the amount of HbF/F cells. A high correlation ($R^2 = 0.967$) is present between the number of F cells and the percentage of HbF [6]. The failure of HbF to modulate uniformly all complications of sickle cell disease might be related to the pathophysiological events that impact the likelihood of developing these complications. Many epidemiological studies suggested that disease complications most closely linked to sickle vasoocclusion and blood viscosity were robustly related to HbF concentration while complications associated with the intensity of haemolysis were less affected [7,8].

The predominant pathophysiological feature of SCD is vaso occlusion, which leads to acute and chronic complications such as painful crises, acute chest syndrome and strokes. Patients with SCD have a markedly decreased life expectancy and their quality of life is greatly compromised by their disease. Therefore the level of Foetal haemoglobin (HbF) in erythrocytes account for a large part of the clinical heterogeneity observed in patients with SCD. The pathophysiology of SCD is dependent on the polymerization of deoxy sickle haemoglobin (deoxyHbS). Increased levels of HbF retard this process. The aim of this study is to quantify HbF levels in sickle cell disease

patients.

METHODS

A total of 100 SCD patients in steady states (apparently healthy) attending the sickle cell clinic in Central Hospital, Benin City were selected randomly and recruited for the study. Most of the study population were children in their post primary education levels. The study population comprised of 53 males (53%) and 47 females (47%). Fifty (50) individuals who had normal haemoglobin (HbAA) were used as controls. Three milliliters of venous blood was collected from each patient into 1% ethylene diamine tetra acetic acid (EDTA) containers and immediately transported to the laboratory for analysis. Haemoglobin F estimation was done using the modified Betke method [9]. This method is based on the principle that foetal haemoglobin is more resistant to alkaline denaturation than any other haemoglobin. Denaturation was stopped by adding ammonium sulphate to lower the pH and precipitate the denatured haemoglobin and filtered. After filtration, the amount of unaltered haemoglobin was measured colourimetrically and the results expressed as a percentage of the total amount of haemoglobin present. The Hb genotype was determined using Hb electrophoresis where charged molecules migrate under an electric field at different rates depending on their electro- negativity [7]. While the haematocrit (HCT) levels was obtained using the microhaematocrit method where anticoagulated blood in a glass capillary tube was centrifuged in a microhaematocrit centrifuge at 15000rpm for 15 minutes to obtain constant packing of the red cells [7].

Statistical analysis was carried out using paired t test for significant difference between HbF in sickle cell condition (SS) and in normal condition (AA) and also between sexes of sickle cell patients. Correlation was done between age and haemoglobin F, HCT and HbF. The single factor analysis of variance (ANOVA) was used to test for significant difference between the age groups of sickle cell patients.

RESULTS

Most of the study population were children in their post primary education levels. They comprised 53 males (53%) and 47 females (47%) with a mean age of 16.61 years. Results showed a significant increase $P<0.05$ in HbF between sickle cell patient (SS) and normal haemoglobin (AA) as shown in table 1. Tables 2 and 3 showed the relationship of HbF among males, females and age ranges in sickle cell anaemia population and there was no significant difference.

Table 1: Foetal Haemoglobin Levels between Sickle Cell (SS) and Normal (AA) Individual (as controls)

TEST	NUMBER OF SAMPLES	X ± S.D	p value
HbF (SS)	100	7.10 ± 7.13%	
			P < 0.005 0.0000131
HbF (AA)	50	3.70 ± 1.60%	

Table 2: Foetal haemoglobin Levels between Male and Female Sickle cell Individuals

SEX	NUMBER OF SAMPLES	X ± S.D	p – value
HbF Males	53	6.31 ± 4.64%	
			P>0.05 0.259
HbF Females	47	7.96 ± 9.07%	

Table 3: Foetal haemoglobin levels between the different age ranges in sickle cell individuals

AGE RANGE	NUMBER OF SAMPLES	X ± S.D	p – value
0 – 10 years	33 (33%)	5.60 ± 4.64%	
			P>0.05 0.268
11 – 20 years	34 (34%)	8.43 ± 7.89%	
21 and above	33 (33%)	7.24 ± 8.21%	

Table 4: Percentage of Foetal haemoglobin of test and control subjects

NUMBER (%)	PERCENTAGE HbF
46 (46%)	3.26
35 (35%)	6.54
15 (15%)	12.88
4 (4%)	34.07
Controls	
40 (80%)	2.2
10 (20%)	3.4

DISCUSSION

Foetal haemoglobin genes are genetically regulated and the level of HbF and its distribution among sickle erythrocytes is highly variable. Some patients with sickle cell disease have exceptionally high levels of HbF while others do not. Many epidermiological studies suggested that disease

complications most closely linked to sickle vaso occlusion and blood viscosity were robustly related to HbF concentration while complications associated with the intensity of haemolysis were less affected [9]. The mean HbF levels of 7.10% observed in this study was found to be significantly increased (P<0.005) when compared to the controls which had a mean HbF levels of 3.70%. This agrees with the work of Idowu et al [10] who recorded that in African Americans with sickle cell anaemia, 2% to 8% of their erythrocytes were F cells compared with 2.8 ± 1.6% in normal African Americans. They also found that sickle cell trait carriers had a mean HbF of 1.4%.

Haemoglobin F value seen in normal individual is usually less than 1% of the total haemoglobin, this is contrary to the fact that in majority of sickle cell individuals there is a high level of foetal haemoglobin which ranges from 5% to 8% [6]. This agrees with the findings in this work. The distribution of HbF levels were 46% of the test subjects had a mean HbF level of 3.26%, 35% had 6.5%, 15% had 12.88% while the remaining 4% had HbF levels of 34.07%. The controls (Hb genotype AA individuals), 80% had a mean HbF levels of 2.2% and the remaining 20% had 3.4%. The difference in the HbF rise in sickle cell individuals is due to rise in circulatory F cell[6] and the difference in F cell survival.[8] The HbF in mature sickle red blood cells is much higher than the corresponding value in newly released reticulocytes. This might be related to the premature destruction of erythrocytes that do not contain HbF, even though the total HbF concentration is high.

The increase in HbF in sickle cell individuals is due to the presence of F cells which is seen to contain both foetal haemoglobin and sickle cell haemoglobin, however the amount of HbF in each F cell undoubtedly varies to some extent. It has been found that a high correlation was presented between the number of F cells and the percentage of HbF [6]. With a conscious effort to know the pattern of expression of F cell with sex and age, a comparison was done between sickle cell male and female of different age range, it was shown that there was no significant difference (P>0.05) between age and sex as it affects HbF production. This may be attributed to the fact that HbF production may be induced by neither the age nor the sex of the individual. It has been shown that certain drugs such as hydroxyurea and butyrates induces HbF production and is usually administered to SCD patient as part of their routine therapy [11-17]. This may have also accounted to the increase in HbF levels in SCD recorded in this work as all the subjects recruited in this research had at one time or the other been on these drugs even though none were on these drugs as at the point of the sample collection. Also the increase in HbF levels in the control subjects may be attributed to the geographical location and the diet in this region.

There was a weak correlation (R^2 = - 0.16604) in haematocrit (HCT) values between controls and test subjects which implies that an increase in HCT values may not necessarily amount to an increase in HbF production although HbF red cells have been seen to carry and distribute more oxygen from red cells to tissues.

CONCLUSION

In conclusion, this study shows that foetal haemoglobin levels is higher in sickle cell individuals when compared to the controls and such increase are not dependent on age, sex, or HCT levels of the individuals

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