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Research Article
Fetal Haemoglobin Level in Sickle Cell Anaemia Subjects Attending University of Calabar Teaching Hospital, Cross River State, Nigeria

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Abstract

Background and Objective: Fetal haemoglobin is the major genetic modulator of the haematologic and clinical features of sickle cell anaemia, an effect mediated by its exclusion from the sickle haemoglobin polymer. Fetal haemoglobin genes are genetically regulated and the level of fetal haemoglobin and its distribution among sickle erythrocytes is highly variable. This study was carried out to estimate the levels of fetal haemoglobin in sickle cell subjects attending University of Calabar Teaching Hospital, Calabar. The percentage of fetal haemoglobin was measured in 30 subjects with diagnosis of sickle cell disease in the University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria as well as in 30 haemoglobin AA and 30 haemoglobin AS individuals who were enrolled as controls subjects. **Materials and Methods:** Haemoglobin electrophoresis was done using the cellulose acetate electrophoresis at pH 8.6 while fetal haemoglobin was estimated using modified Betke method. **Results:** The result showed the mean fetal haemoglobin among the SS subjects (1.41±0.98 %) to be significantly higher than that of AA (0.96±0.71%) and AS (1.17±0.780%) subjects, respectively ($p = 0.04$). The result of this study has also shown a significant ($p = 0.01$) higher level of fetal haemoglobin in female sicklers (1.51±0.74%) when compared to male counterparts (1.29±1.14%). **Conclusion:** The results also showed a higher level of fetal haemoglobin in higher age group when compared to lower age group. The study has shown that the Haemoglobin F level is lower than that found in other parts of the country and world.

Key words: Fetal haemoglobin, sickle cell, sickle cell anaemia, haemoglobin AA, haemoglobin AS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Haemoglobin is a red globular protein, which has a molecular weight of 68,000 and comprise almost one-third of the weight of a red cell[1]. It is a chromoprotein consisting of a globin molecule attached to 4 red coloured haem molecules. The haemoglobin is composed of haem and globin. Its function is to carry oxygen from the lungs to the tissues and carry carbon dioxide from the tissues to the lungs. Each red cell contains approximately 640 million haemoglobin molecules. About 65% of the haemoglobin is synthesized in the erythroblast and 35% at the reticulocyte stage[2].

Fetal haemoglobin (also known as Hb F $\alpha_2\gamma_2$) is the main oxygen transport protein in the human fetus during the last seven months of development in the uterus and persists in the newborn until roughly 6 months old. Functionally, fetal haemoglobin differs most from adult haemoglobin in that it is able to bind oxygen with greater affinity than the adult form, giving the developing fetus better access to oxygen from the mother's bloodstream. In newborns, fetal haemoglobin is nearly completely replaced by adult haemoglobin by approximately 6 months postnatally, except in a few thalassaemia cases in which there may be a delay in cessation of fetal haemoglobin production until 3-5 years of age. In adults, fetal haemoglobin production can be reactivated pharmacologically, which is useful in the treatment of diseases such as sickle cell disease[3].

Fetal haemoglobin's affinity for oxygen is substantially greater than that of adult haemoglobin. Notably, the P_{50} value for fetal haemoglobin is lower than adult haemoglobin (that is, the partial pressure of oxygen at which the portion is 50% saturated; lower values indicate greater affinity). The P_{50} of fetal haemoglobin is roughly 19 mmHg whereas adult haemoglobin is approximately 26.8 mmHg as a result, the "oxygen saturation curve", which plots percentage saturation vs. pO_2, is left-shifted for haemoglobin F as compared to adult haemoglobin. This greater affinity for oxygen is explained by the fetal haemoglobin's interaction with 2,3-bisphosphoglycerate (2,3 BPG or 2,3 DPG)[4].

Sickle cell disease (SCD) also known as sickle cell anaemia (SCA), is a group of genetically passed down blood disorders. It results in an abnormality in the oxygen-carrying protein haemoglobin found in the red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5-6 months of age. A number of health problems may develop, such as attacks of pain (sickle cell crises), anaemia, bacterial infections and stroke[5].

When production of fetal haemoglobin is switched off after birth, normal children begin producing adult haemoglobin (Hb A). Children with sickle cell disease instead begin producing a defective form of haemoglobin called haemoglobin S (HbS). Under decrease oxygen tension, HbS is much less soluble than haemoglobin A. Thus forcing the red cells into a rigid sickle shaped cell resulting in so called crises which is characterized by severe abnormal, bone and joint pain, local thrombus formation leading to the formation of infarcts. If fetal haemoglobin remains the predominant form of haemoglobin after birth, the number of painful episodes decreases in patients with sickle cell disease[6]. Hydroxyurea promotes the production of haemoglobin F and can thus be used to treat sickle cell disease[7]. Since fetal haemoglobin confer protection to sickle cell anaemic patients, there is need to estimate the level of fetal haemoglobin in sickle cell individuals in this locality. Secondly, there is a paucity of data on the level of fetal haemoglobin among sickle cell patients in this area. This study will help to generate evidence-based data to help optimize the care offered to people living with sickle cell anaemia in this vicinity and to help ameliorate the complication and mortality associated with sickle cell anaemia.

MATERIALS AND METHODS

Study area and design: This was a case control, cross-sectional study carried out in Haematology Clinic of the University of Calabar Teaching Hospital Calabar, Cross River State, Nigeria.

Subject selection: Thirty subjects with diagnosis of sickle cell anaemia attending the Haematology Clinic of the University of Calabar Teaching Hospital, Cross River State, Nigeria were recruited as subjects in the study. Thirty haemoglobin AA and 30 haemoglobin AS subjects who were residing within Calabar metropolis were enrolled as controls. A simple random technique was used for the collection of sample and questionnaire used to record necessary information.

Ethical clearance: Ethical clearance for this study was sought and obtained from the University of Calabar Teaching Hospital Ethical Committee. The purpose and nature of the study were explained to the participants and their informed consent was obtained.

Sample collection and processing: Three milliliters of venous blood was collected by clean venipuncture from each subject via the antecubital vein using a plastic syringe with

minimum stasis into an EDTA sample bottles. The red cells were then separated and washed three times in isotonic saline (0.15 mol L^{-1}) and a lysate is prepared by lysing 1 volume of washed packed red cells in 4 volume of lysing reagent (3.8 g EDTA, tetra sodium salt, 0.7 g potassium cyanide and water to 1 L). Haemoglobin electrophoresis was done using the cellulose acetate electrophoresis at pH 8.6.

The modified Betke method was used for the quantification of fetal haemoglobin[8].

Principle: To measure the percentage of Hb F in a mixture of haemoglobins, sodium hydroxide was added to a lysate and after a set time denaturation was stopped by adding saturated ammonium sulphate. The ammonium sulphate lowered the pH and precipitates the denatured haemoglobin. After filtration, the quantity of undenatured (unprecipitated) haemoglobin was measured. The preparation of alkali-resistant (fetal) haemoglobin was then calculated as a percentage of the total amount of haemoglobin present.

Method: About 0.25 mL of haemolysate was added to 4.75 mL cyanide solution to make a solution of haemoglobin cyanide (HiCN).

- About 2.8 mL of HiCN solution was transferred to another glass test tube and was allowed to equilibrate at 20°C
- Rapidly, 0.2 mL of 1.2 mol L^{-1} of NaOH was added, mixed and allowed to stand for 2 min
- After exactly 2 min, 2 mL saturated ammonium sulphate solution was added and mixed on a vortex mixer. It was then allowed to stand for 5-10 min at 20°C. It was then filtered through a What man No. 42 filter paper using a clean test tube to collect the filtrate each time. This filtrate contains the alkali-resistant haemoglobin. Also, total haemoglobin was measured by transferring 0.4 mL of the haemoglobin cyanide solution from step 1 into another tube and 13.9 mL of water was added. Absorbance of alkali-resistant haemoglobin and total haemoglobin was then read at 413 nm wavelength against a water blank. Finally, the alkali (%) resistant haemoglobin was calculated as follows:

$$\text{Alkali (\%)} - \text{Resistant haemoglobin} = \frac{A^{413}\ \text{alkali} - \text{resistant Hb}}{A^{413}\ \text{total Hb} \times 20} \times 100$$

Statistical analysis: Data obtained from this study were presented using tables, the level of significance was analyzed using student t-test and ANOVA.

RESULTS

The Table 1 showed the fetal haemoglobin level of Haemoglobin AA, Haemoglobin AS and Haemoglobin SS individuals. The mean level of Hb F was found to be 1.41±0.98 in SS subjects,1.17±0.78 in AS individuals and 0.96±0.71 in AA individuals, respectively. There was a significance difference between the Hb F level of sickle cell subjects and control subjects (p = 0.04).

The Table 2 showed the fetal haemoglobin level of male sickle cell subjects and female sickle cell subjects attending University of Calabar Teaching Hospital, Calabar. The mean Hb F for male sickle cell subjects was 1.29±1.14 and that of female sickle cell subjects was 1.51±0.74, respectively. There was a significance difference in fetal haemoglobin level of male and female sickle cell subjects (p = 0.01).

The Table 3 showed fetal haemoglobin level based on ages of sickle cell subjects and control subjects. The mean Hb F level for sickle cell subjects between the ages of 5-15 years was found to be 1.35±1.29 and between 16-30 years was found to be 1.44±0.82, respectively. The mean Hb F level for haemoglobin AA individuals between the ages of 5-15 years was found to 0.71±0.79 and between the ages of 16-30 years, it was found to be 1.04±0.68, respectively. The mean Hb F level for haemoglobin AS individuals between the ages of 5-15 years was found to be 0.81±0.66 and between 16-30 years, it was found to be 1.23±0.78, respectively. There was no significant difference in fetal haemoglobin level when compared according to their ages.

Table 1: Fetal haemoglobin level of HbSS, HbAA and HbAS Individuals

Parameters	Sickle cell subjects (SS) (n = 30)	Control subjects (n = 30) AA	AS	p-value
Hb F (%)	1.41±0.98*	0.96±0.71*	1.17±0.78	0.04

Attending University of Calabar teaching hospital calabar

Table 2: Fetal haemoglobin level of male sickle cell subjects and female sickle cell subjects attending University of Calabar teaching hospital calabar

Parameters	Male sickle cell subjects (n = 17)	Female sickle cell subjects (n = 13)	p-value
Hb F (%)	1.29±1.14	1.51±0.74	0.01

Table 3: Fetal haemoglobin level based on ages of sickle cell subjects and control subjects attending University of Calabar teaching hospital calabar

Parameters	5-15 year	16–30 year	p-value
SS	1.35±1.29	1.44±0.82	0.40
AS	0.81±0.66	1.23±0.78	0.10
AA	0.71±0.79	1.04±0.68	0.15

DISCUSSION

Fetal haemoglobin, a heritable traits in adults accounting for substantial phenotypic diversity of sickle cell disease was estimated in both controls (Hb AA and Hb AS individuals) and known Hb SS subjects in steady state for comparison.

This study showed that the mean Haemoglobin F level measured by alkaline denaturation method that was the modified Betke method in 30 sickle cell subjects was found to be 1.41±0.98%. This lowered than the result obtained by Wrightone and Huisman[9] who recorded the mean fetal haemoglobin of individual of African origin to be 6.6%. The result of this study also slightly differed from that of Omoti[10] and Isah[11] who recorded mean haemoglobin F level of 2.17±1.81 and 2.99±5.16 among haemoglobin SS subjects, respectively. The difference in the result may be attributed to genetic factors; it has been demonstrated that there is strong genetic component controlling the number of haemoglobin F containing cells (F cells) in individuals[12]. This difference may also be due to the inhibitory effects of haemoglobin F on the polymerization of Hb S molecules[13] resulting in a reduced likely hood of cells containing large amount of haemoglobin F undergoing sickling.

This study also showed statistically significant increased fetal haemoglobin level in sickle cell subjects when compared to haemoglobin AA individuals. A higher level of fetal haemoglobin level was also observed in haemoglobin SS subjects when compared to haemoglobin AS subjects. This agreed with the work of Uko *et al.*[14] who also obtained a high level of fetal haemoglobin among haemoglobin SS subjects (2.01±1.94%) followed by haemoglobin AS subjects (0.85±0.54%) and haemoglobin AA individuals (0.69±0.46%).

The result of this study which showed increased level of fetal haemoglobin in haemoglobin SS subjects when compared to Hb AA and Hb AS subjects was in accordance with the work of Steinberg[15] which showed that increases in fetal haemoglobin levels have been noted in individuals with sickle cell anaemia which were caused by mutation affecting the HBB gene and inherited as Mendelian recessive gene and also that individual with sickle cell anaemia have fetal haemoglobin levels ranging[14] from 1-10%. Haemoglobin F level may also be elevated in these subjects as a result of genetic abnormalities of haemoglobin production or because of haematopoietic stress[16].

A significant higher level of fetal haemoglobin was also observed in female haemoglobin SS individuals compared to male counterparts in this study. The mean haemoglobin F level in male and female sickle cell subjects was found to be 1.29±1.14 and 1.51±0.74%, respectively. This agreed with the work of Mason *et al.*[17] who also recorded a higher level of fetal haemoglobin in females (2.41±0.82) than in males (1.56±0.71) but disagrees with the work of Isah *et al.*[11]. This difference may be attributed to the hormonal effects of puberty. It has also been reported that after the age of 10, that fetal haemoglobin level were consistently higher in females than males[17].

The result of this study also showed an increased level of fetal haemoglobin in higher age group than lower age group and this disagreed with the work of Maude *et al.*[18] who recorded decreased mean haemoglobin F level in lower age group than higher age group. But this work was in accordance with the work of Revista who reported that fetal haemoglobin levels may be increased in adults in certain conditions.

CONCLUSION AND FUTURE RECOMMENDATIONS

This study has shown significantly higher level of fetal haemoglobin in sickle cell subjects when compared to haemoglobin AA and haemoglobin AS individuals and higher level of fetal haemoglobin in female subjects when compared to male counterparts. Therefore there is need for government and NGOs to support policies that will help to improve access to Haemoglobin F inducing agents, specifically hydroxyurea in order to reduce the morbidity and mortality among Haemoglobin SS patients. This work also recommend haemoglobin F quantification as part of routine diagnostic test in the management of sickle cell anaemia.

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