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FREQUENCY OF FOETAL HEMOGLOBIN AND HEMOGLOBIN VALUES IN VARIOUS HEMOGLOBIN GENOTYPES IN CALABAR, NIGERIA

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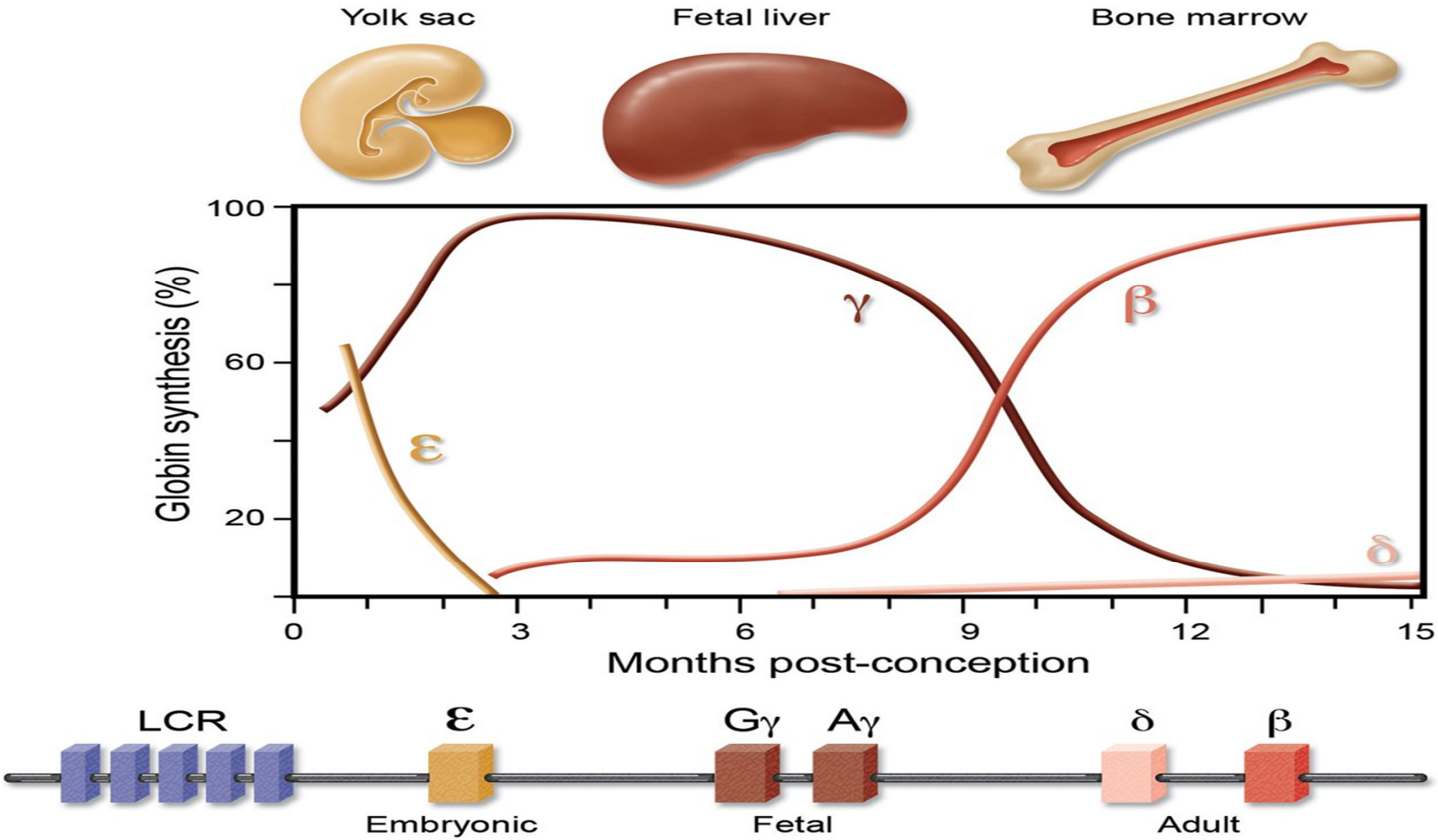
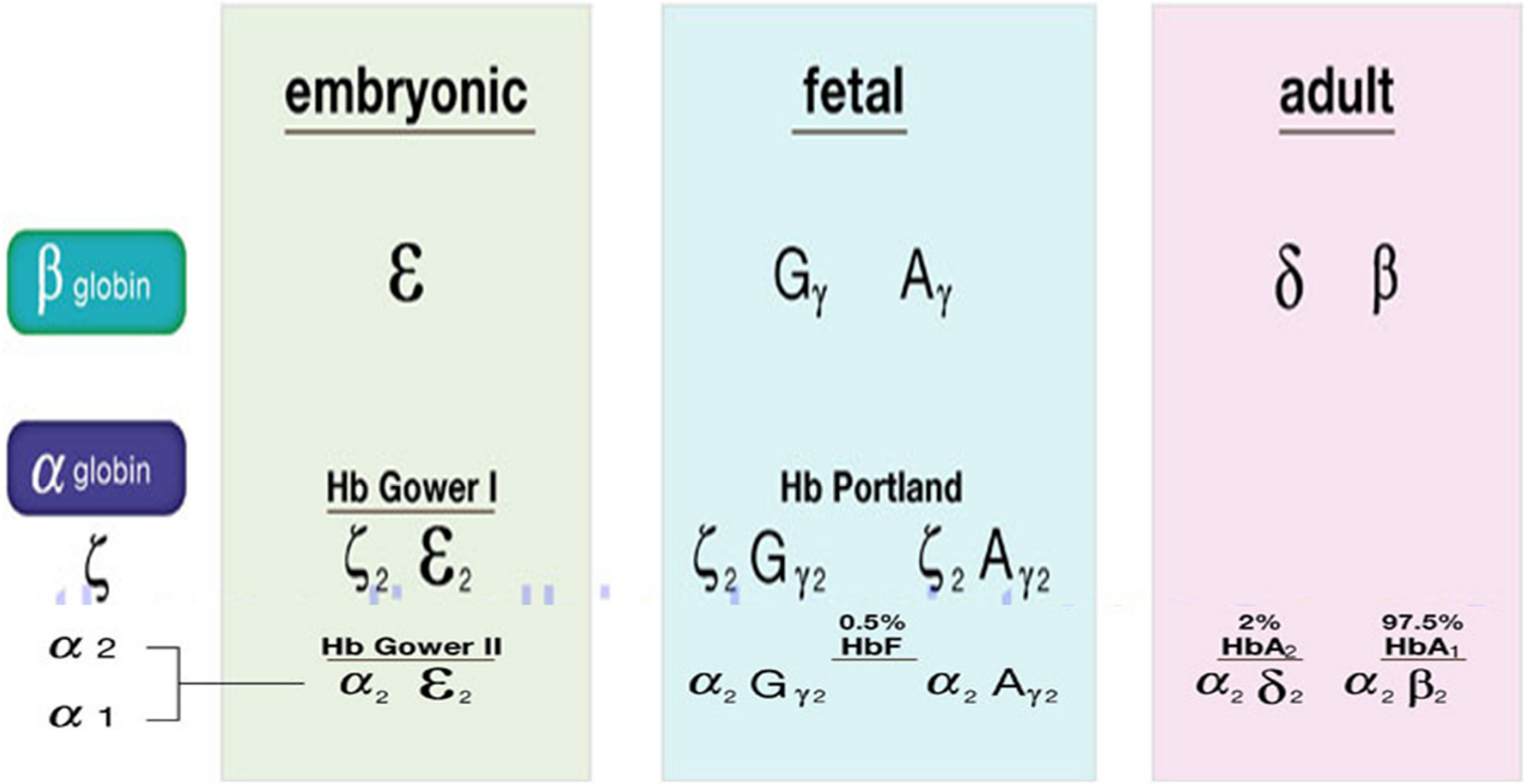
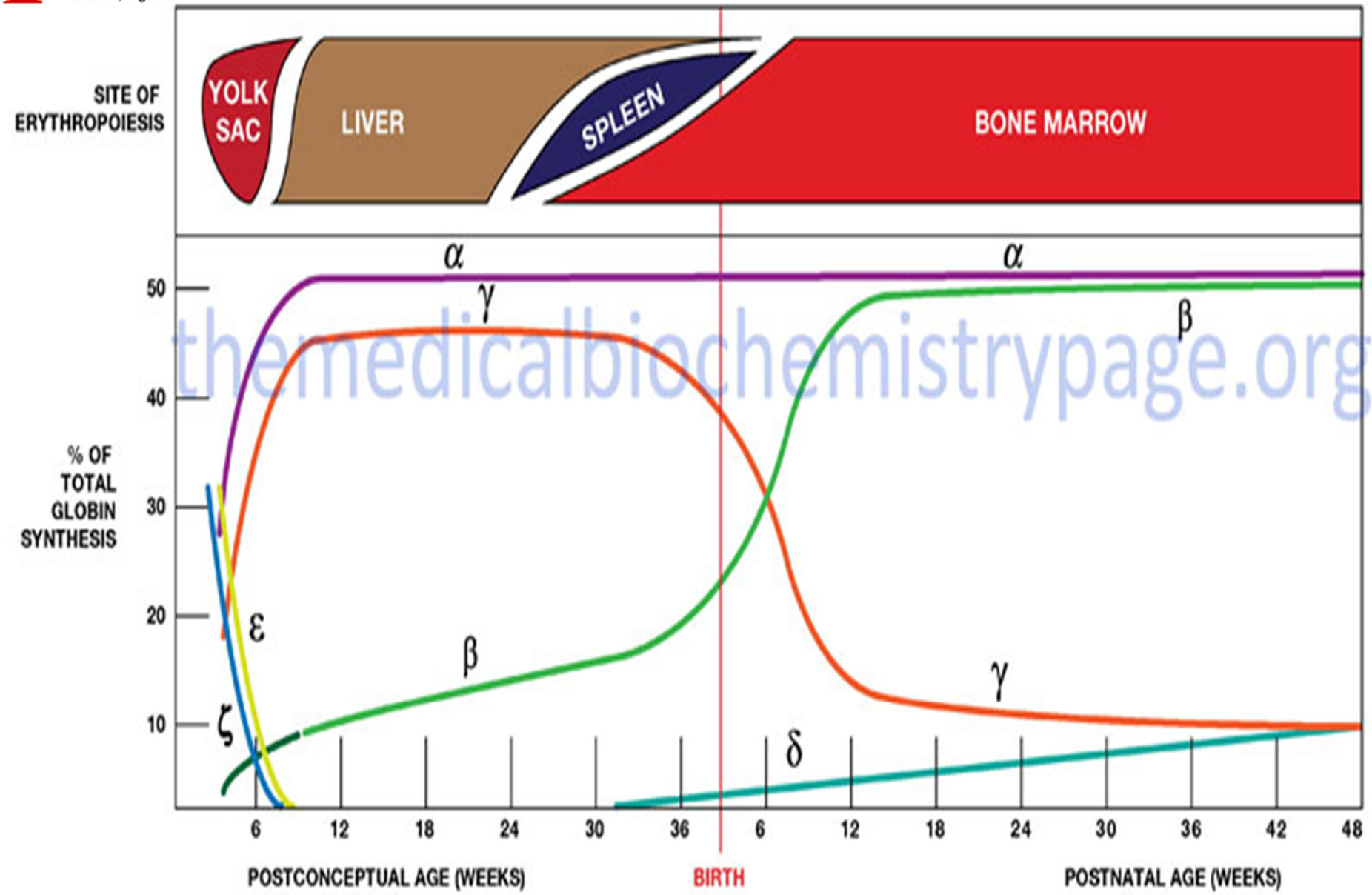
INTRODUCTION

Hb synthesis from gestation




embryonic
fetal
adult
β globin
α globin
ζ
α 2
α 1
ε
Gγ Aγ
δ β
Hb Gower I
ζ2 ε2
Hb Gower II
α2 ε2
Hb Portland
ζ2 Gγ2
ζ2 Aγ2
0.5% HbF
α2 Gγ2
α2 Aγ2
2% HbA2
α2 δ2
97.5% HbA1
α2 β2



OBJECTIVES

- In this study, we set out to determining the foetal hemoglobin (Hb) and Hemoglobin (Hb) values in various Hb-genotypes in Calabar, a south-south city in Nigeria.

- The outcome could be valuable in the prediction and management of sickle cell diseases and as well as studying the existence of hereditary foetal hemoglobin in the locality.

- Apart from predicting the extend of causes in sickle cell disease, it may provide adequate information that may lead to moderate savings in drug cost, as well as in giving hope to the patient.

- A study of this nature has not been reported in the literature for the study area.

DISORDERS WHICH HBF CAN OCCUR INTO ADULT LIFE

- Pernicious Anaemia
- Thalasaemia
- Hereditary persistence of foetal Hemoglobinaemia
- Childhood myeloid leukaemia and large variety of neoplasm

Sickle cell anemia

- SCA – Reactive Site = Sticky patches on β Chain
- Sticky patches + stick receptors on α Chain of HbS
- Binding → Long fibrous precipitate distort erythrocyte
- HbF → Sticky receptors no sticky patches
- Hb block sticky patches of HbS → No long polymer of Hb (Idowu Akinsheye *et al.,* 2011)
- HbF– Powerful modulator of clinical and hematological features of SCA
- Various concentration postulated to protect against various complication

IMPORTANT PROPERTIES OF HBF

- Higher oxygen affinity
- Resistance to acid elution
- Resistance to alkali denaturation compared to HbA
- Presence of Hb has been known to increase the minimum concentration of total Hb required for gelation

CLINICAL IMPORTANCE OF HBF

Several studies have shown that HbF does not participate in the sickling phenomenon

Resistance of HbF in sickle cell disease is associated with absence of HbA production and substantial production of γ-chain by HbF

Clinical severity of sickle cell disease may synthesis HbF in a broad cellular distribution

Edoh *et al.,* has shown that HbF is unevenly distributed throughout the red cell population in adults with sickle cell disease.

HbF at birth is same with HbA and HbS but the rate of fall in HbF after birth is shown in sickle cell disease compared to Hb-genotype A
HbF values are reported to be significantly higher in sickle cell diseases.

MATERIAL AND METHODS

Study subjects

One hundred and fifty (150) subjects were initially screened for participation in the study. These included apparently healthy school children, younger civil servant and patients that visited the sickle cell clinic of the University of Calabar Teaching Hospital, Calabar. Following Hb-electrophoresis, 108 subjects were randomly selected for final participation.

The Exclusion criteria was the age of the subjects, and Neonate whose Hb-genotypes could not be determined were also excluded. To avoid undue bias those aged over 30 years who were in the minority were not included. The age of the 108 participants ranged between 6 months- 30 years. Forty six (59.9%) of the study population were males, while females were 52(48.1%), giving a ratio of about 1:1.

The ratio of males to females among the AA and AS subjects was also about 1:1 except among the SS where males were slightly higher in number (14) to females (8), 1.7:1. (table 3)

BLOOD COLLECTION

4mls of blood withdrawn by standard venopuncture method was delivered into dipotassium salt of ethylene diamine tetra-acetic acid (EDTA) to give a final anticoagulant concentration of 2mg/ml and processed as soon as collected

DETERMINATION OF Hb-GENOTYPE

This was performed using cellulose acetate method of Decie and Lewis(1985). Known Hb-genotypes AA, AS, and SS were included as controls. In the overall analysis of the results, Hb-genotype AA subjects were taken as control

ESTIMATION OF FOETAL HAEMOGLOBIN (HbF)

The modified Betke alkali denaturation method of Betke *et al.,* (1957) was employed. HbF resist denaturation in alkaline medium than HbA. After mixing with 1.2m NaoH for 2 minutes HbF was measured in the filtrate. The absorbance was read spectrophotometrically at 540nm.

Two cord samples from neonate were collected and used as HbF control

DETERMINATION OF HAEMOGLOBIN

The method of cyanmethemoglobin technique described by Dacie and Lewis (1985) was adopted. 0.02ml of blood was added to 4ml of Drabkin's solution. The result was read spectrophotometrically at 540nm

DATA ANALYSIS

The data generated in this study were analysed using SPSS. The influence of age, sex and Hb-genotype in the HbF and Hb values of the subject was assessed

RESULTS

Table 1: Distribution of HbF and Hb values among different Hb-genotype

Hb-Genotype	Number of subjects	Mean Hb values(g/dl)	Mean HbF values (%)
AA	68 (63%)	12.79±1.19	0.195±0.25
SS	22 (20.3%)	10.63±2.05	3.059±1.61
AS	18 (16.7%)	12.69±1.18	1.072±0.98
TOTAL	108	12.04±1.48	1.44±0.93

HbSS subjects had a higher mean concentration of HbF (3.059±1.61) than AS subjects with 1.072±098.

The difference was statistically significant (P<0.02) Subjects with Hb-genotype AA had the lowest concentration of HbF.

Conversely, Hb-genotype AA subjects recorded the highest mean Hb values (12.79±1.19), followed closely by AS with 12.69±1.18g/dl.

The variation in the Hb-values of these 2-groups (AA and AS) was not statistically significant (P>0.02).

Table 2: Variation of HbF and Hb-values in different Hb-genotypes by Age

Age	HbF values (%)			Hb concentration (g/dl)		
	AA	AS	SS	AA	AS	SS
6 months – 6 years No. of subjects	0.26±0.11 (25)	1.20±0.89 (6)	2.92±0.64 (9)	12.50±1.60 (25)	11.6±1.0 (6)	10.1±0.75 (9)
7 years – 15 years No. of subjects	0.15±0.09 (25)	0.92±1.01 (9)	3.85±2.30 (6)	12.8±0.75 (25)	12.0±0.93 (9)	9.48±1.65 (6)
16 years – 30 years No. of subjects	0.16±0.44 (18)	1.27±1.16 (3)	2.50±1.10 (7)	13.2±0.08 (18)	12.57±0.48 (3)	12.3±0.40 (7)

HbF concentration decreased with increase in age though some literature have confirmed increase in HbF between the age of 7 – 15yrs and this is inline with the present study.

An inverse relationship existed between the concentration of HbF and Hb values by age.

The higher the Hb values, the lower the HbF concentration and vise versa.

The difference in Hb and HbF values was statistically significant ($P<0.02$) irrespective of genotypes

Table 3: Pattern of Hb and HbF levels in different Hb – genotypes by sex

Sex	Mean HbF values (%)	Mean Hb values (g/dl)
MALES		
AA (34)	0.142±0.11	13.06±0.79
SS (14)	3.06±1.80	10.29±2.12
AS (8)	0.90±0.82	12.73±0.53
Mean	1.367±0.91	12.02±1.15
FEMALES		
AA (34)	0.25±0.16	12.53±1.45
SS (8)	3.05±1.31	11.16±1.76
AS (10)	1.21±1.11	10.34±3.17
Mean	1.50±1.89	11.34±2.13

HbF and Hb values in males and females showed that HbAA females had a higher HbF value than the AA males.

AA males had a higher Hb concentration than AA females

DISCUSSION

Hereditary persistence of foetal hemoglobin exists in about 70 – 80% of Hb in HbSS red cells. These cells do not sickle and therefore survive longer in circulation due to the relatively high level of HbF (Idowu *et al.,* 2011.)

In this study an appreciated quantity of HbF was observed to persist into adult life with more in sickle cell disease (Table 1 & 3). The difference in HbF concentration among Hb-genotype SS in relation to other Hb-genotypes (AS, AA) was statistically significant ($P<0.02$).

The HbF concentration observed in sickle disease patients in this locality in Nigeria was far higher than the HbF concentration reported among different Hb-genotypes in other environment (Edoh D. *et al.,* 2008). They also reported that the rate of fall of foetal Hb after birth was slower in sickle cell disease than in AA and AS individuals as was observed with present study.

The persistent of HbF in sickle disease may be advantageous since the presence of critical amount of HbF in circulating erythrocyte counteract the aggregation of deoxygenated HbS, which is the molecular basis of sickling (He *et al.,* 2001)

Since HbF can be considered an effective natural antisickling agent, the reactivation of its synthesis in patients with sickle cell anemia has a desirable therapeutic goal which should be researched into, for eventual exploitation

It was observed that patients with sickle cell disease anaemia in the study locality in Nigeria were rarely in crises. This may be due to the anti-sickling effect of HbF that appears persistent in these patients (Adachi *et al.,* 2008)

The degree of persistence of HbF into adult life observed in the study area is suggestive of certain degree of persistence of HbF in this locality

The activation of HbF in baboons has been carried out using 5-azacytidine and hydroxyl urea (Hu) (Lovelle and Desimer, 2003). More research could be needed, to see whether these agents or any other can be of used in humans for suppression of β-chain and enhance production of γ-chain for increased HbF production. This may enhance the management of patients with sickle disease anaemia

HbF concentration in females generally were higher than in males of same Hb-genotype ($P<0.02$) with no variation among SS subjects of both sexes (Table 3). This observation conforms with the report of Decie and Lewis (1995) who reported a significant fall in HbF values in males of AA genotype compare to females of same Hb-genotype between the age group of 5 – 9 years and 10 – 14 years

It has been suspected that the pulsating production of gonadotrophic hormones that initiate the early production of ovaries hormones at puberty may be partly accountable for the persistent HbF in females.This hormones could suppress the production of β-chain and enhances substantial γ-production by the HbF-gene, resulting in increased production of HbF in females

The significant lower ($P<0.02$) Hb concentration seen among HbSS individuals compared with other Hb-genotype was not surprise but surprisingly there was a significant higher Hb values among HbSS males (Table 3). This may be because the female sicklers in the study area were observed to be attending sickle cell clinic more regularly than the males and probably the females might abide by medical advice given at the clinic. More so, the former are less exposed to risk factors and are not usually involved in vigorous jobs like the males. This account for the higher Hb level in females

In conclusion, our findings have established a significantly higher HbF value in HbSS individual than in HbAA and AS in Calabar, Nigeria. Also higher HbF and Hb values were seen in female sicklers compared to the males

Since HbF does not participate in the sickling process and counteract the aggregation of deoxygenated HbS, its activation could be of immense benefit to the sicklers. We therefore suggest research into the mechanism of suppressing β-chain and enhance production of γ-chain needed for significant production of HbF.

Routine estimation of HbF in sicklers is further recommended. This may be used as a management marker and could help the clinicians to predict the severity of crises

We believe that the findings in this research work and further insights of the suggestion would give certain degree of self confidence and hope to the clinicians as well as sickle cell anaemic patients

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Thank You