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Levels of Estrogen, Progesterone and Follicle Stimulating Hormone among Nigerian Females Living with Sickle Cell Anaemia

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ECO and POM designed the work, wrote and reviewed the manuscript. Author HOO collected data, did the experiments and wrote the manuscript. Author NIU wrote the manuscript. Author CO performed statistical analysis. Author CGO reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell Anaemia (SCA) is an inherited disorder characterized by abnormality in sexual development and reduced fertility which may be due to derangement in the level of sex hormones. This study aimed to evaluate the levels of estrogen, progesterone and follicle stimulating hormone (FSH) among adult females with SCA (HbSS).
Methods: A cross-sectional study of adult female patients with HbSS as well as individuals with sickle cell trait (HbAS) and normal adult haemoglobin (HbAA) was done. Hemoglobin phenotype was determined using electrophoretic method. Estrogen, progesterone and FSH levels were assayed using enzyme-linked immunosorbent assay (ELISA) technique, full blood count was

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determined using Hematology autoanalyzer while disease severity was evaluated using an objective scoring system.

Results: Seventy females with mean age of 23.8 ±4.9 years were studied. They included 30 (42.8%) HbSS participants in steady state and 40 controls [20 (28.6%) HbAS and 20 (28.6%) HbAA]. There was significant decrease in mean serum levels of estrogen in individuals with HbSS compared to those of HbAS and HbAA (P<0.05). Also, there was significant decrease in progesterone level when HbSS were compared to HbAS participants (p< 0.05), but the difference didn't reach significance level when HbSS were compared with HbAA participants (P>0.05). There was higher mean serum level of FSH in HbSS when compared to HbAS and HbAA, though not significant (P>0.05). FSH levels was also found to be inversely correlated with disease severity in individuals with HbSS, though not significant.

Conclusion: Female individuals with HbSS have significantly lower mean levels of estrogen and progesterone compared to Hb AA and HbAS individuals but higher FSH levels compared to HbAS and HbAA individuals, though not significant. Among HbSS individuals, FSH level correlated inversely with disease severity, though not significant.

Keywords: Estrogen; follicle stimulating hormone; progesterone; sickle cell anaemia.

1. INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder of haemoglobin resulting from the presence of a mutant form of haemoglobin (HbS) [1]. Inheritance of HbS in a homozygous form gives rise to HbSS, also known as sickle cell anaemia (SCA), which is the most common form of SCD [1]. Haemoglobin S can also occur in combination with other abnormal haemoglobin such as in HbSC, HbSβ-thal and others. Sickle cell disease affects mainly people of African descent, Mediterranean region, and Middle East. However, migration of people from high frequency areas to low frequency areas has increased the frequency of sickle cell disease even in regions with low frequency such as Europe and USA [1]. World Health Organization report has shown that three quarter of sickle cell disease cases occur in Africa with most of them found in sub-Saharan Africa.[2] In Nigeria with an estimated population of 150million, about 2% of newborn were affected, with a total of about 150,000 children with sickle cell disease born in Nigeria annually [2,3]. In addition, about 24% of Nigerian population are carriers of the mutant gene, making Nigeria the country with the highest burden of SCD in the world [4].

Even though SCA has the same genetic mechanism, it has variable clinical manifestation and severity for yet unknown reasons.[4] Some factors that have been suggested for this variation are socio-economic, genetic and elevated fetal haemoglobin levels [5]. Sickling phenomenon and crisis have been observed to be more severe pre-puberty but fairly stable at puberty [6]. Sex hormones which are responsible for development of secondary sexual characteristics has been implicated. Early attainment of puberty by females compared to males with better sickling stability may be due to estrogen, a female sex hormone [7]. People living with sickle cell disease have been reported to have delayed puberty compared with normal population [8].

Hormones are chemical substances synthesized by specialized glands in one part of the body to act as chemical messenger and control the activity of certain cells and organs in another part of the body [9]. Sex hormones have been shown to play important physiological roles in the body and are responsible for the development of primary and secondary sexual characteristics. Female sex hormones include estrogen and progesterone, the secretion of which is dependent on the action of follicle stimulating hormone (FSH). Establishing the level of sex hormones in adult female patients with sickle cell anaemia is very important. Due to improvement in management, people living with sickle cell anaemia are now growing into adulthood, living normal sexual lives and getting pregnant. It has been reported that women with sickle cell disease have bad obstetric history, with high rate of poor maternal and fetal outcomes [10]. There is therefore the need to determine sex hormone characteristics in women with sickle cell anaemia.

Delayed menstrual development because of decreased sex hormone levels consequent upon hypogonadism have been reported by previous studies [11,12]. The ultimate effect of sickle cell disease is multi-organ dysfunction with negative

effect on endocrine function. The aim of this study is therefore to determine the level of estrogen, progesterone and follicle stimulating hormone among adult female individuals with sickle cell anaemia and to correlate hormonal levels with disease severity.

2. METHODS

2.1 Study Design

This was a cross-sectional comparative study.

2.2 Study Location, Study Population and Sampling Technique

This study was conducted at Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria. Adult female individuals living with sickle cell anaemia (HbSS) were randomly recruited from haematology clinic while an age-matched control group (HbAS and HbAA) were recruited from among female hospital staff, students and female patients' relatives.

Sample Size: Using the method described by Charan and Biswas [13], a sample size of 30 was calculated. However, 70 participants were recruited made up 30 participants with HbSS in steady state and 40 controls (20 HbAS and 20 HbAA individuals).

Inclusion Criteria: Adult females with HbSS in steady state, sickle cell trait (HbAS) and individuals with normal adult haemoglobin (HbAA), all 18-50 years of age.

Steady state was defined as absence of acute illness or crisis for at least one month and absence of blood transfusion for at least three months prior to the study.

Exclusion Criteria: Age below 18 years or above 50 years, pregnant women, male individuals, those with comorbid condition such as renal disease, metabolic disorders such as diabetes mellitus, hypertension, cardiovascular and inflammatory disease (autoimmune disease), individuals on medication such as contraceptive pills or hormonal therapy, those who refused to give consent, SCA patients in crisis.

2.3 Data / Sample Collection and Sample Analysis

Questionnaires were used to gather socio-demographic and clinical information. About 5mls of venous blood was collected from each participant aseptically through venipuncture. Two milliliters of blood was dispensed into an ethylene diamine tetraacetic acid (EDTA) container while the remaining 3 mls was dispensed into a plain bottle. The samples were subsequently transported to the laboratory where analysis was done. Blood in EDTA bottles was used for determination of full blood count and haemoglobin phenotype. Blood in plain bottle was allowed to clot and centrifuged at 5000 rpm for 5 minutes. Serum was extracted and put into another plain bottle and stored at -30^0C until analysis for hormonal estimation was done.

Haemoglobin phenotype was determined by electrophoretic method with cellulose acetate paper at a pH of 6.8 using an electrophoresis chamber (Helena Biosciences, UK).

Estrogen, progesterone and FSH were determined by Enzyme Linked Immunosorbent Assay (ELISA) method using ELISA washer and reader as well as ELISA test kit (Accubind ELISA microwells manufactured by Monobind Inc USA), following manufacturer's instruction.

Full blood count was estimated using haematology autoanalyser (KX2IN model, Sysmex Corporation Kobe, Japan). The reagent levels and printer paper were checked, and the analyzer turned on via the power button. The analyzer was allowed to go through its internal checks, and then display 'Ready'. The blood sample was well mixed and presented to the sipper of the machine making sure the sipper was well submerged in the sample and the start switch was clicked. Then a small volume of blood was aspirated and automatically analysed by the machine. A printout of the results was generated.

2.4 Severity Scoring in Sickle Cell Disease

The severity score was calculated using a modification of laboratory and clinical parameters as described by previous studies [14,15]. Anaemia score: Hb ≥ 10 g/dl = 0; Hb ≥ 8 g/dl < 10 g/dl = 1; Hb ≥ 6 < 8 g/dl = 2; Hb ≥ 4 < 6 g/dl = 3; Hb< 4g/dl = 4. White blood cell count score: Count < 9 × 10^9 cells/µl = 0; Count ≥ 9 < 11× 10^9 cells/µl = 1; Count ≥ 11 < 15 × 10^9 cells/µl = 2; Count ≥ 15× 10^9 cells/µl = 3. Number(s) of crisis per year: 0-1 = 0, 2-3 = 1, ≥4 = 2.

Complications score: Each complication was scored 1 except nephropathy and Stroke which were scored 2 each. The complications included

leg ulcer, heart failure, stroke, retinopathy, priapism, acute chest syndrome, nephropathy, avascular necrosis of joint, pulmonary hypertension, liver failure and anaemic heart failure. Transfusion score: number of previous blood transfusion per year: None = 0, 1-2 units = 1, ≥3 units = 2.

The total severity score was calculated as mild (≤ 3), moderate (> 3 – ≤ 7) or severe (> 7).

2.5 Data Analysis

Data collected for this study were analyzed with the use of Statistical Package for Social Sciences (SPSS) software, version 20. Descriptive statistics was used to compute proportions and percentages, means and standard deviation. Analysis of variance (ANOVA) was used to compare means and Pearson correlation coefficient was used to determine relationship between variables. Level of significance was set at 0.05.

3. RESULTS

A total of 70 adult females were recruited for the study. They comprised of 30 (42.8%) HbSS individuals in steady state, 20 (28.6%) heterozygous sickle cell (HbAS) individuals and 20 (28.6%) participants with normal adult haemoglobin (HbAA) who served as the control group. The mean ages of the participants were 24±5.8 years, 23±4.1 years and 24±4.4 years for HbSS, HbAA and HbAS participants respectively.

Mean level of oestrogen, progesterone and FSH is shown in the Table 1.

There was significantly lower mean serum levels of estrogen in female participants with HbSS compared to that of HbAA and HbAS controls (p = 0.021 and 0.001 respectively) (Table 1 and Table 2). Furthermore, there was a significantly lower mean serum level of progesterone in HbSS compared with that of HbAS participants (P = 0.001). Mean serum level of progesterone in HbSS was also lower than that of HbAA participants, though the difference did not reach statistical significance (P = 0.908). There was higher level of mean serum FSH in participants with HbSS compared to those of HbAS and HbAA, though the difference did not reach statistical significance (p > 0.05).

As shown in Table 3, there was negative correlation between mean serum levels of estrogen with age in participants with HbSS, HbAS and Hb AA, though not significant.

Among HbSS participants, those with severe disease had lower mean FSH level compared to those with mild or moderate disease, though this did not reach statistical significance (Fig. 1). However, there was no significant relationship between oestrogen and progesterone with disease severity.

4. DISCUSSION

Endocrine disorders in sickle cell disease are associated with delayed growth and puberty as well as low fertility due to derangement in the level of sex hormones [16]. This study found significantly lower mean estrogen levels among participants with HbSS compared to HbAS and HbAA individuals. Our findings was similar to work done by Phuljhele et al., who observed a decrease in the production of estrogen in sickle cell disease compared with the control group [17]. Similarly, Ezeiruaku as well as Mishra postulated that there was a significant decrease in the mean serum levels of estrogen obtained for homozygous sickle cell subjects compared to heterozygote sickle cell disease and the normal control group [18,19]. The low levels of the sex hormones has been attributed to gonadal hypofunction such as hypogonadism secondary to hypopituitarism [12]. Hypopituitarism in sickle cell anaemia may result from intravascular thrombosis and pituitary infarction [20]. Gonadal malfunction can be affected by the integrity of the hypothalamic pituitary-gonadal axis by which the functionality of the ovary is determined [21]. However, some studies are inconsistent as to whether primary ovarian failure is the cause of the reduced level of estrogen or secondary hypothalamic-pituitary dysfunction [12,22]. Taddesse et al., observed that low serum estrogen levels in female individuals with sickle cell disease with low levels of FSH and LH levels suggest a central mechanism [21]. The theory regarding low level of estrogen in premenopausal women is unusual unless they experience an anovulatory cycle or are supplementing with birth control pills, which can suppress endogenous production of estrogens by the ovaries. A low estradiol level is much more common in postmenopausal women or in women of any age who have had their ovaries surgically removed (oophorectomy). Most importantly, ovarian failure is marked by low levels of estrogen and infertility resulting mainly from diseases or conditions that affect and destroy the ovary [23].

Mean serum progesterone level was also found to be lower among participants with sickle cell anaemia compared to those with HbAS and HbAA, though not significant statistically. This corroborates with the findings from previous studies which reported lower progesterone levels in patients with sickle cell anaemia compared to HbAS and HbAA [18,19]. Lower progesterone level found in sickle cell anaemia may be due to ovarian dysfunction as part of multi-organ dysfunction found in sickle cell anaemia because of repeated vaso-occlusion.

This study also found higher level of FSH among females with sickle cell anaemia compared to females without sickle cell anaemia, though not significant. Similarly, Ibrahim *et al,* observed that there was no significant difference between the mean serum level of FSH among homozygous sickle cell group (HbSS) in comparison with the heterozygous sickle cell subjects (HbAS) and normal control subjects (HbAA) [24]. Conversely, Phuljhele et al., reported a significantly higher serum FSH value in the homozygous sickle cell group (HbSS) in comparison to those with sickle cell traits (HbAS) and normal adult haemoglobin (HbAA) [17]. The rise in FSH is consistent with the decline in ovarian production and estrogen secretion considering that low estrogen levels signal the hypothalamic-pituitary-axis to release more FSH.

Findings from this study also showed negative correlation in serum estrogen level with age among all the three groups, though not significant. The result of this research is similar to the findings of previous studies which reported a negative correlation between age and serum estrogen level in homozygous sickle cell disease, heterozygous sickle cell disease and in normal control individuals [12,17]. Ageing in females is accompanied by a progressive decline of gonadal function, in particular, a decline in total and free estrogen plasma levels. The progressive decrease of plasma estrogen levels has been shown to result from altered neuro-endocrine regulation of leydig cell functions [25].

This study also found decrease in FSH level with increase in disease severity among individuals with sickle cell anaemia. Similarly, previous studies have reported that female patients with the severe form of sickle cell disease showed more frequent reduction of FSH in comparison with individuals with mild disease [11,12]. Increased levels of FSH may be as a result of gonadal failure due to ovarian hypofunction as part of multi-organ failure seen in sickle cell disease. The decrease in FSH with increase in disease severity as found in this study may be due to atrophy of the hypothalamus because of compromised blood supply over time [26].

Table 1. Mean oestrogen, progesterone and FSH levels in HbSS and HbAA participants

	HbSS Mean (SD)	HbAA Mean (SD)	P-value
Oestrogen (pg/ml)	57.24 (±31.04)	82.80 (±42.63)	0.021
Progesterone (ng/ml)	3.47 (±1.65)	3.52 (±1.55)	0.908
FSH (mIU/ml)	5.87 (±2.77)	5.15 (±2.83)	0.349

Table 2. Mean oestrogen, progesterone and FSH levels in HbSS and HbAS participants

	HbSS Mean (SD)	HbAS Mean (SD)	P-value
Oestrogen (pg/ml)	57.24 (±31.04)	96.30 (±34.43)	0.001
Progesterone (ng/ml)	3.47 (±1.65)	5.01 (±1.33)	0.001
FSH (mIU/ml)	5.87 (±2.77)	5.34 (±2.53)	0.192

Table 3. Correlation of the serum levels of estrogen, progesterone and FSH with age in participants with HbSS, HbAS and HbAA

	SS genotype		AA genotype		AS genotype	
	r	p-value	r	p-value	r	p-value
Age versus estrogen	-0.221	0.288	-0.158	0.506	-0.021	0.931
Age versus progesterone	0.217	0.298	-0.419	0.066	-0.322	0.166
Age versus FSH	0.112	0.594	0.201	0.395	-0.217	0.358

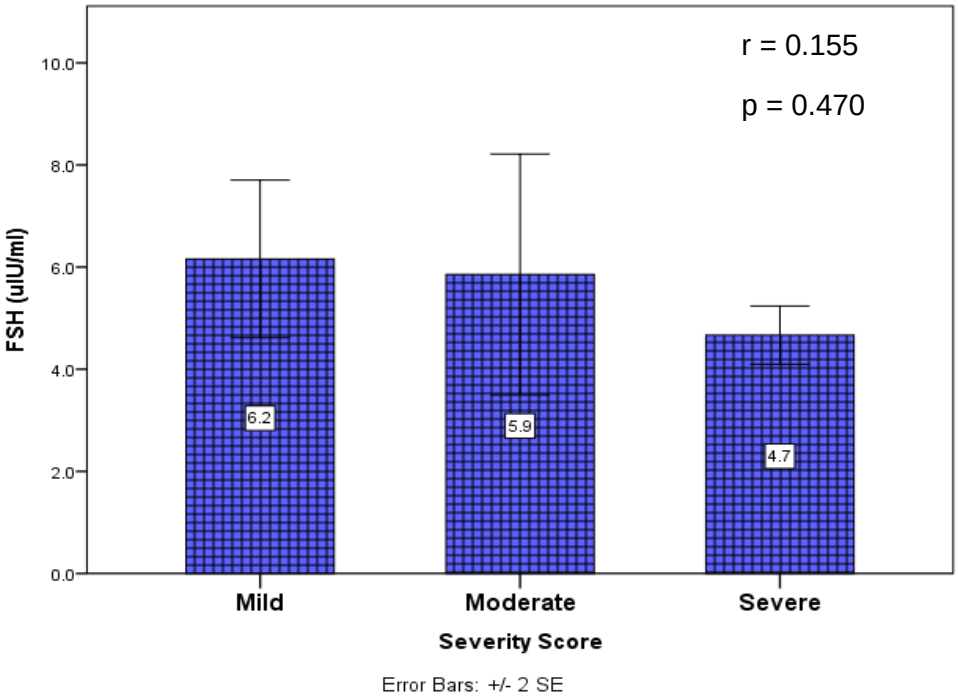

Fig. 1. Serum levels of FSH and disease severity in HbSS individuals

5. CONCLUSION

Adult females with sickle cell anaemia have significantly lower mean serum levels of estrogen compared to individuals with Hb AS and HbAA. In addition, patient with sickle cell anaemia have higher levels of follicle stimulating hormone (FSH) compared to individuals with Hb AS and HbAA, though not significant. Among participants with sickle cell disease (HbSS), FSH level was found to decrease more with severe disease. The authors therefore propose that therapeutic replacement of FSH may be considered in adult female SCD patients that are severely affected to improve their capability to reproduce. Further study with a larger population is recommended to confirm these findings.

SIGNIFICANCE OF STUDY

Due to improvement in healthcare, patients with SCD can now live to adult age and may want to reproduce. This study found that patients with SCD may have derangement of the sex hormones which may be related to severity of disease and may affect their reproductive capability. Early detection and follow up will help to reduce disease severity and affectation of organs including gonads, with improvement in levels of sex hormones. Therapeutic replacement of FSH may be considered in adult female SCD patients that are severely affected to improve their capability to reproduce.

ETHICAL APPROVAL AND CONSENT

Approval for this research was gotten from the Ethics and Research Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State Nigeria, with reference number NAUTH/CS/66/VOL.10/39/2017/009. Before being enrolled into the study, informed written consent was obtained from each participant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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