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Sex Hormones and Prolactin Ranges in Sickle Cell Disease Subjects in Southern Nigeria

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Abstract
The severity of the sickling phenomenon has been observed to be more at pre puberty, but at puberty, the level of crises becomes fairly stable. This has been attributed to the sex hormones that are responsible for development of the sexual characteristics in both male and female. This study was designed to establish the normal level of these hormones (estradiol, progesterone, testosterone and prolactin) in the serum of persons with sickle cell disease in a steady state and in the other haemoglobin genotypes (HbAA, HbAS) with respect to age and gender. This is with the aim to establish sex hormone ranges and if there is significant difference in the level of these hormones in homozygous SS subjects as a result of the sickling phenomenon in southern Nigeria. Enzyme linked immunosorbent Assay (ELISA) method was used to estimate the level of the hormones. From the result, the level of the hormones showed a significant difference ($P<0.05$) in the gender, the age groups and the HbSS Subjects. The level of the hormones studied also increased with age in the three human haemoglobin electrophoretic patterns. The hormones were decreased significantly in the serum of the HbSS subjects and there was no significant difference ($P>0.05$) in the values obtained for the control (HbAA and HbAS) subjects. The findings indicated gonadal hypofunction in the sickle cell patients but with varied deviations from the mean result.
Keywords: Estradiol; Progesterone; Testosterone; Prolactin; Sex hormones; Sickle cell; Haemoglobin electrophoresis; genotypes.

INTRODUCTION

Sickle cell disease is the most common genetic disorder in persons of African Origin (Huisman, 1981) and the disorder comprises a spectrum of syndromes that range from the almost completely benign trait or carrier state (The AS genotype) to the most severe syndrome, the sickle cell anaemia due to the homozygous presence of the β-haemoglobin (producing the HbSS genotype). Sickle cell anaemia, an inheritance of mutant haemoglobin genes from both parents is globally wide spread. According to World Health Organisation (WHO) report, (2006), about 5% of the world's population carries genes responsible for haemoglobinopathies and each year about 300,000 infants are born with major haemoglobin disorders. More than 200,000 cases of sickle cell anaemia are in Africa (WHO, 2006). In Nigeria, the most populous country in the sub-region with about 150 million inhabitants (Nigeria census, 2006), 24% of the population are carriers of the mutant gene and the prevalence of the sickle cell anaemia is about 20 per 1000 births. The public health implications of sickle cell anaemia are obvious as it causes either death or disability. Its impact on human beings may therefore be assessed against the background of infant and under five mortality. Although an increasing proportion of affected children now survive past five years of age, they have a chronic disease and are at risk of premature death (Platt *et al*., 1994). For reasons that are not clear, sickle cell disease is of very variable severity. The severity of the sickling phenomenon has been observed to be more at pre-puberty, but at puberty, the level of crises becomes fairly stable. This has been attributed to sex hormones that are responsible for development of sexual characteristics in both male and female (Orudugba, 2012). The female attains puberty earlier and better sickling stability than the male and this might be as a result of the female sex hormone, estrogen. Some experts have postulated that the sickle cell disease also delay puberty in comparison with persons of HbAA genotype (Olutunbosun, 1978; Zemel, *et al*., 2007; Kim, 2014).

The hormones are chemical substances which travel from a special tissue, where they are relieved into the blood stream to distant responsive cells where the hormone exerts their characteristic effects (El-Hasmi *et al*., 1991; Schimike *et al*., 1995). The sex hormones are responsible for the development of sexual characteristics in men and women and have been established to play a lot of physiologic and metabolic roles in the body (Goldfien, 1998) (Lee, 2005).

Sex steroids, also known as gonad steroids are steroid hormones, which interact with vertebrate androgen or estrogen receptors. Natural sex steroids are made by the gonads (ovaries or testes), by adrenal glands or by conversion from other sex steroids in other tissues such as liver or fat (Daisely, 2006). Sex steroids play important roles inducing the body changes known as primary sex characteristics and secondary sex characteristics. This development is controlled by sex hormones after the Y-chromosome and/or the SRY gene determines development. In many contexts, the two main classes of sex steroids are androgens and estrogens of which the most important human examples are testosterone and estradiol respectively. Other contexts will

include progestagen as a third class of sex steroid, distinct from androgens and estrogens. The conventional view of prolactin, as argued in some quarters of its sex origin and multifunctional nature, is that its major target-organ is the mammary gland and stimulating mammary gland development and milk production and was included in this study as part of sex hormone workup.

The significance of establishing sex hormones and prolactin ranges in sickle cell disease lies in the fact that sickle cell patients are sexually active and are also interested in living a normal sex life and getting pregnant. As medical advances improve survival, reduce disease –related morbidity and improve quality of life, reproductive issues will take higher priority in the sickle cell disease community (Kim, 2014). The issue of whether steroid contraceptives are appropriate for women with homozygous sickle cell disease remains unresolved. Historically, women with sickle cell disease have experienced difficult pregnancies, characterised by high rates of maternal mortality and morbidity and poor infant outcomes (Freie, 1983), therefore unresolved questions about steroidal contraceptives in women with sickle cell disease (Young, 2001), sickle cell men with erectile dysfunction (Dada and Nduka, 1980), include whether using them may promote blood clots (Briggs and Briggs, 1972).

The aim of this study therefore was to establish the levels of these hormones (estradiol, progesterone, testosterone and prolactin) in the blood of subjects with sickle cell disease (HbSS) in a steady state, the carriers (the HbAS) and normal genotype (HbAA) with respect to age and gender groups. This is with a view to establish the normal ranges and if possible any dysfunction associated with sickle haemoglobin and was primarily aimed at a comparative study of the three human haemoglobin electrophoretic patterns. Reproductive issues in women and men with sickle cell disease include a wide range of complications that are relatively common, however data from well designed, large clinical studies are limited and many studies are quite old, but remain relevant because they describe clinical complications and problems that persist in the sickle cell disease populations today despite advances in medical therapy.

MATERIALS/METHODS

Study Area

The study was carried out in the southern part of Nigeria states of Bayelsa , Rivers and Cross Rivers. Blood samples were collected from subjects and volunteers at the university Teaching Hospital, Okolobiri; Federal Medical center, Yenegoa; University Teaching Hospital, and the General Hospital, all in Porthatcourt, Rivers State, from Univerity of Calabar teaching hospital and from patients that were indentified after counselling and genotyping to determine their haemoglobin electrophoretic pattern at the sickle cell centres and clinics. The control samples were collected from consented members of the public that has the genotype group HbAA and HbAS from the same environment especially the university community.

Study Population

A total of eight hundred and forty (840) subjects were used for this study and were distributed into 3 age groups of 2 -7, 8 -14, and 15 – 40 years for both male and female. This comprised of 40 female HbSS subjects in each age bracket, and 43, 39, 38 male HbSS subjects in 2 -7, 8 -14 and 15 -40 age groups respectively. The study subjects also included 600 males and females of HbAS and HbAA genotypes, with 50 subjects in each age and gender group. The subjects group were gender and age matched and excluded from the study were subjects indentified with different endocrine dysfunction (Stephen *et al*., 2008). Institutional ethical approval was received from the various departments of the hospitals and centres for this study. This study lasted for a period of 5 years and covered the age range of 2 to 40 years.

Sample Collection and Preparation

About 5.0ml of blood were collected from each subject using the standard venepuncture technique. The samples were discharged into a clot-activator tube and then centrifuged for 10 minutes at 3,000 rpm. The serum collected was stored frozen at -20°C and analysis was done within 5days of collection. The subject's age and gender were noted after collection with history of menstrual cycle for females above age 14 years. Samples for age group above 14 years were collected twice at both follicular and luteal phase (Day2 and 21). This was because the study did pick interest in the luteal phase of their cycle because of the elevated levels of estradiol and progesterone at this phase. The essence and details of the study were explained to the subjects and their guardian and informed consent was obtained from them after approval from the management of the hospitals.

Method of Assay

The genotypes of the subjects were confirmed using haemoglobin electrophoresis. The enzyme linked immunosorbent (ELISA) method was used for the hormones (Amballi *et al.,* 2007). The ELISA test is used on the principle of solid phase enzyme linked immunosorbent assay, where the antibody to be measured is incubated with specific antigen coupled to a solid phase (Uotila *et al*., 1981; Peter *et al*., 2001).The product kit was acquired from Micro well laboratories USA. The components of the ELISA kit were specifically designed to analyse the various sex hormones; estradiol, progesterone, testosterone and prolactin. It applies to in-vitro quantitative determination of the hormone concentration in serum.

Sampling method

To achieve this reference range establishment, a random sampling of the different haemoglobin genotype class from the population was used. This took into consideration the age and gender of the subjects, excluding those that have different endocrine dysfunctions as established. The apparently healthy subjects were selected for the study and with a method that has been established to give a precise range of results in hormonal assay (Amballi *et al*., 2007).

Statistical Analysis

The data are expressed as mean ± standard deviation and normal ranges. Correlation between the groups studied was tested using the regression analysis. The results obtained from this were subjected to statistical analysis using standard computerized analysis tool of ANOVA: two factors without replication and standard t-test: pair two samples for mean. 95% confidence level ($P<0.05$) were used and considered significant.

Results

The results of the study on the levels of sex hormones and prolactin in the serum of HbAA, HbAS and HbSS subjects as grouped are presented in the tables below: The differences in levels (age group, male and female) were statistically calculated and its significance shown in the tables: The result showed significant decrease ($P <0.05$) in the values obtained for homozygous SS subjects and without any significant difference ($P >0.05$) between the heterozygote AS and homozygous AA.

TABLE 1: Mean ± S.D results and the normal range for serum female estradiol in the three human haemoglobin electrophoretic patterns with respect to Age**.**

Hb Genotype	Age Groups(yrs)	No. of subjects(n)	Mean ± S.D (pg/ml)	Lower limit - Upper limit	P Value
HbAA	0 – 7	50	14.64 ± 7.50	7.14 – 22.14	
	8 – 14	50	33.16 ± 14.06	19.10 – 47.22	P > 0.05
	15 – 40 (Day2)	50	48.95 ± 20.61	28.34 – 69.56	
HbAS	0 – 7	50	13.95 ± 3.98	9.97 – 17.93	
	8 – 14	50	28.36 ± 13.59	14.77 – 41.95	P > 0.05
	15 – 40 (Day2)	50	51.04 ± 23.09	27.95 – 74.13	
HbSS	0 – 7	40	6.88 ± 2.17	4.71 – 9.05	
	8 – 14	40	19.24 ± 5.80	13.44 – 25.04	P < 0.05
	15 – 40 (Day2)	40	38.65 ± 15.10	23 .55 – 53.75	

TABLE 2: Mean ± S.D results and the normal range for serum female estradiol in the three human haemoglobin electrophoretic patterns with respect to Age **(DAY 21)**

Hb Genotype	Age Groups(yrs)	No. of subjects(n)	Mean ± S.D (pg/ml)	Lower limit - Upper limit	P Value
HbAA	15 – 40	50	73.95 ± 25.61	48.34 – 99.56	P > 0.05
HbAS	15 – 40	50	68.04 ± 23.09	44.95 – 91.13	P > 0.05
HbSS	15 - 40	38	48.63 ± 15.10	33.53 – 63.73	P < 0.05

TABLE 3: Mean ± S.D results and the normal range for serum male estradiol in the three human Haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No. of subjects(n)	Mean ± S.D (pg/ml)	Lower limit - Upper limit	P Value
HbAA	0 - 7	50	9.39 ± 3.43	5.96 – 12.82	
	8 - 14	50	13.13± 8.80	4.33 – 21.93	P > 0.05
	15 – 40	50	25.99 ± 15.97	10.02 – 41.96	
HbAS	0 – 7	50	8.36 ± 4.68	3.68 – 13.04	
	8 – 14	50	13.09 ± 3.44	9.65 – 16.53	P > 0.05
	15 – 40	50	24.43 ± 11.56	12.87 – 35.99	
HbSS	0 – 7	43	4.09 ± 2.29	1.80 – 6.38	
	8 – 14	39	9.02 ± 2.64	6.38 – 11.66	P < 0.05
	15 - 40	38	11.24 ± 4.29	6.95 – 15.53	

TABLE 4: Mean ± S.D results and the normal range for serum female progesterone in the three Human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No. of subjects(n)	Mean ± S.D (ng/ml)	Lower limit - Upper limit	P Value
HbAA	0 – 7	50	0.29 ± 0.13	0.16 – 0.42	
	8 – 14	50	0.61 ± 0.10	0.51 – 0.71	P > 0.05
	15 – 40 (Day2)	50	0.85 ± 0.32	0.53 – 1.17	
HbAS	0 – 7	50	0.23 ± 0.09	0.14 – 0.32.	
	8 – 14	50	0.56 ± 0.27	0.29 – 0.83	P > 0.05
	15 – 40 (Day2)	50	0.79 ± 0.35	0.44 – 1.14	
HbSS	0 – 7	40	0.16 ± 0.09	0.07 – 0.25	
	8 – 14	40	0.38 ± 0.17	0.21 – 0.55	P < 0.05
	15 – 40 (Day2)	40	0.63 ± 0.38	0.25 – 1.01	

TABLE 5: Mean ± S.D results and the normal range for serum female progesterone in the three human haemoglobin electrophoretic patterns with respect to Age **(DAY 21).**

Hb Genotype	Age Groups(yrs)	No. of subjects(n)	Mean± S.D (ng/ml)	Lower limit - Upper limit	P Value
HbAA	15 – 40	50	12.08 ± 8.53	3.55 – 20.61	P > 0.05
HbAS	15 – 40	50	10.95 ± 7.28	3.67 – 18.23	P > 0.05
HbSS	15 – 40	38	7.58 ± 5.06	2.52 – 12.64	P < 0.05

TABLE 6: Mean ± S.D results and the normal range for serum male progesterone in the three human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No of subjects(n)	Mean± S.D (ng/ml)	Lower limit – Upper limit	P Value
HbAA	0 – 7	50	0.23 ± 0.09	0.14 – 0.32	
	8 – 14	50	0.33 ± 0.14	0.19 – 0.47	P > 0.05
	15 – 40	50	0.85 ± 0.32	0.53 – 1.17	
HbAS	0 – 7	50	0.18 ±0.05	0.13 – 0.23	
	8 – 14	50	0.27 ± 0.10	0.17 – 0.37	P > 0.05
	15 – 40	50	0.49 ± 0.25	0.24 – 0.74	
HbSS	0 – 7	43	0.09 ± 0.06	0.03 – 0.15	
	8 – 14	39	0.20 ± 0.10	0.10 – 0.30	P < 0.05
	15 – 40	38	0.32 ± 0.11	0.21 – 0.43	

TABLE 7: Mean ± S.D results and the normal range for serum male testosterone in the three human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No of subjects(n)	Mean ± S.D (ng/ml)	Lower limit – Upper limit	P Value
HbAA	0 – 7	50	0.12 ± 0.09	0.03 – 0.21	
	8 – 14	50	0.62 ± 0.48	0.14 – 1.10	P > 0.05
	15 – 40	50	8.70 ± 5.82	2.88 – 14.52	
HbAS	0 – 7	50	0.11 ± 0.04	0.07 – 0.15	
	8 – 14	50	0.52 ± 0.42	0.10 – 0.94	P > 0.05
	15 – 40	50	8.24 ± 5.13	3.11 – 13.37	
HbSS	0 – 7	43	0.06 ± 0.04	0.02 – 0.1	
	8 – 14	39	0.29 ±0.13	0.16 – 0.42	P < 0.05
	15 – 40	38	4.73 ± 2.56	2.17 – 7.29	

TABLE 8: Mean ± S.D results and the normal range for serum female testosterone in the three human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No of subjects(n)	Mean ± S.D (ng/ml)	Lower limit – Upper limit	P Value
HbAA	0 – 7	50	0.08 ± 0.02	0.06 – 0.10	
	8 – 14	50	0.18 ± 0.03	0.15 – 0.21	P > 0.05
	15 – 40	50	0.65 ± 0.14	0.51 – 0.79	
HbAS	0 – 7	50	0.07 ± 0.03	0.04 – 0.10	
	8 – 14	50	0.12 ± 0.06	0.06 – 0.18	P > 0.05
	15 – 40	50	0.55 ± 0.18	0.37 – 0.73	
HbSS	0 – 7	40	0.05 ± 0.03	0.02 – 0.08	
	8 – 14	40	0.08 ±0.03	0.05 – 0.11	P < 0.05
	15 – 40	40	0.41 ± 0.13	0.28 – 0.54	

TABLE 9: Mean ± S.D results and the normal range for serum male prolactin in the three human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No of subjects(n)	Mean ± S.D (ng/ml)	Lower limit – Upper limit	P Value
HbAA	0 – 7	50	2.69 ± 0.57	2.12 – 3.26	
	8 – 14	50	4.93 ± 1.55	3.38 – 6.48	P > 0.05
	15 – 40	50	10.82 ± 4.52	6.30 – 15.34	
HbAS	0 – 7	50	2.47 ± 0.79	1.68 – 3.28	
	8 – 14	50	3.97 ± 0.62	3.35– 4.59	P > 0.05
	15 – 40	50	8.34 ± 3.05	5.29 – 13.39	
HbSS	0 – 7	43	1.67 ± 0.96	0.71 – 2.63	
	8 – 14	39	2.58 ±1.20	1.38 – 3.78	P < 0.05
	15 – 40	38	4.15 ± 1.63	2.52 – 5.78	

TABLE 10: Mean ± S.D results and the normal range for serum female prolactin in the three human haemoglobin electrophoretic patterns with respect to Age.

Hb Genotype	Age Groups(yrs)	No of subjects(n)	Mean ± S.D (ng/ml)	Lower limit – Upper limit	P Value
HbAA	0 – 7	50	3.40 ± 1.82	1.58 – 5.22	
	8 – 14	50	8.80 ± 3.89	4.91 – 12.69	P > 0.05
	15 – 40	50	12.43 ± 3.66	8.77 – 16.09	
HbAS	0 – 7	50	3.22 ± 1.75	1.47 – 4.97	
	8 – 14	50	6.48 ± 3.59	2.89 – 10.07	P > 0.05
	15 – 40	50	11.60 ± 4.48	7.12 – 16.08	
HbSS	0 – 7	40	2.39 ± 1.55	0.84 – 3.94	
	8 – 14	40	5.16 ±1.29	3.87 – 6.45	P < 0.05
	15 – 40	40	7.65 ± 2.51	5.14 – 10.16	

Discussion

The age of puberty and menarche is known to be generally delayed in African Sickle Cell Patients (Olatunbosun, 1978; Konotey-Ahuhu, 1981). Sex hormones are responsible for some of the most dramatic changes that occur in the body. They control puberty, egg and sperm production, pregnancy, birth and lactation (Kim, 2014). The findings from the measurement of the sex hormones (estradiol, progesterone, testosterone and prolactin) in some sickle cell subjects in southern Nigeria made some revelations. Analyses of the findings as illustrated in the result section (table 1 – 10), showed that the serum level of the hormones increased with age and differ significantly with respect to age and gender. The findings also showed that there was a significant decrease in the production of these hormones in sickle cell disease when the subjects are gender and age matched. The differences in levels obtained were significant for both male and female and different age groups. The result is in agreement with the previous findings (Olatunji and Frasier, 1975; Abudu *et a*l, 2011). Comparative studies have shown that in all human haemoglobin genotype classes studied (HbAA, HBAS, HBSS) variables existed in the order HbAA>HbAS>HbSS subjects. Statistical analysis showed the differences that existed in the HbAA and HbAS subjects for these hormone levels were not significant (P>0.05). But significant differences (P<0.05) existed between the levels in HbAA and HbSS and the levels in HbAS and HbSS subjects. The differences exhibited by the levels in the hormones are more pronounced after puberty (see result section), with the normal ranges as shown in tables 1 -10.

Gonadal malfunction can be genetic, as the function of the testes and the ovaries were determined by the integrity of the hypothalamic pituitary-gonadal axis. Certain genetic disease might affect the gonadal function that might have various clinical manifestations of the hormone effects (Abbasi *et al*., 1976; Andrea, 2005; Taddesse *et al*., 2012). Prepubertal development decreases sex hormones binding globulin (SHBG) (Asterios and Feudon, 2005). Sex hormones and other physiologic differences between male and females therefore will alter the expressivity of a gene (Andrea, 2005). After birth, sexual development does not occur until puberty. Infertility is known complication in males and females with sickle cell disease. This has been attributed to relative primary gonadal failure, impotence and priapism or delayed or impaired sexual development (Olatunji and Frasier, 1975). Low level of the sex hormones and prolactin may be a reflection of hypogonadism secondary to hypopituitarism (Davies and Brosovick, 1989) (Abbasi *et al*., 1976) (Dada and Nduka, 1980).

The result suggested that the sickle cell gene abnormality might have an adverse effect on the endocrine functions.

Conclusion

The findings from the study indicated that the hormones were low in serum of HbSS subjects and there was no significant difference in the values obtained for HbAS with respect to HbAA subjects. There was a significant difference in the values obtained for male, females and the age groups. The low level of the sex hormones and prolactin has been attributed to gonadal hypofunction in the sickle cell subjects. In reproductive endocrinology, reference hormonal values are needed, especially in the clinical evaluations of cases of infertility in both male and females. This study will help in sorting out various approaches to health maintenance and complications of patients with sickle cell disease, particularly in the prescription of steroids in the management of crises and infertility in female patients with sickle cell anaemia that have not been really compared to healthy subjects in southern Nigeria and therefore forms the basis for comparison for health professional practice.

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