Endocrine and Growth Disorders in Children with Haemoglobin-SS

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Summary

BACKGROUND

Suboptimal growth and certain metabolic disorders are commonly presented by children with *Sickle Cell* Disease (SCD). SCD is an autosomal recessive genetic condition common in regions with intense malaria prevalence. The cycles of de-oxygenation and oxygenation of red blood cells producing repeated sickling and unsickling, leading to red cell damage is a concern.

There was need to establish the cause of common triggers for Vaso-Occlusive crises which include dehydration, infections, extreme temperature and emotional stress. Recurrent painful episodes, several physical and biochemical disorders including suboptimal growth, low immunity, anemia and a variety of serious organ system complications that cause life-long disabilities and/or early death in HbSS patients was a challenge. The highest frequencies (3 to 4% of populations) of *Homozygous Sickle Cell* disease occur in Sub-Saharan Africa.

METHODOLOGY

Twenty eight children aged 4-10 years with Hemoglobin-SS (HbSS) and Vaso-Occlusive crisis attending Children Emergency Clinic at Ladoke Akintola University of Technology Teaching Hospital and another 30 healthy sex and age matched children with HbAA (controls) participated in this study. Plasma levels of Growth Hormone(GH), cortisol, prolactin, Total Thyroxin(TT4), Total *Triiodotyronine*(TT3), Thyroid Stimulating Hormone(TSH) and insulin were determined in all respondents using enzyme linked immunosorbent assay methods.

RESULTS

The weight, height and BMI decreased significantly ($p<0.05$) in HbSS children compared with the controls. Plasma levels of GH, cortisol, TT3 and TT4 increased significantly ($p<0.05$) in HbSS-children compared with controls. Plasma levels of prolactin, TSH and insulin did not show significant ($p>0.05$) changes in the HbSS children compared with the controls. There was a significant ($r=0.46$, $p=0.04$) positive correlation between cortisol and GH in the children with HbSS. A negative correlation ($r=-0.45$, $p=0.045$) existed between TT4 and weight of HbSS-children.

CONCLUSION

The lower levels of height, weight and BMI despite increased plasma level of GH could suggest peripheral tissue resistance and/or GH-receptor deficiency in HbSS children. Elevated cortisol levels and the positive correlation between cortisol and GH could suggest a link between metabolic stress and GH secretion in HbSS Children.

Key words: Hormones, growth disorders, Haemoglobin-SS(HbSS)

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Introduction

Sickle cell disease (SCD) is an autosomal recessive genetic condition common in regions of the world with intense malaria prevalence. The highest frequencies (3 to 4% of populations) of *Homozygous Sickle cell* disease occur in mainly in Sub-Saharan Africa [1].

The replacement of glutamic acid of the β-chain by valine at the 6th position accounts for the mutation of the adult haemoglobin to *Sickle Hemoglobin* (HbS). The HbS had the ability to polymerize by hydrophobic interaction at low oxygen concentration [2] leading to *Poikilocytosis* and Vaso-Occlusion. That was the main specific manifestation of SCD, differentiating it from other hemolytic anemias. Common triggers for Vaso-Occlusive crises include dehydration, infections, extreme temperature, and emotional stress.

However, often no identifiable cause was found and pain usually occurred without warning [3, 4]. Cycles of de-oxygenation and oxygenation of red blood cells produced repeated sickling and unsickling, leading to red cell damage [5]. Previous studies showed that at birth, SCD infants are normal in size, but by 2–5 years of age, significant deficits [6, 7] leading to recurrent painful episodes, several physical and biochemical disorders including suboptimal growth, low immunity, anemia and a variety of serious organ system complications that could cause life-long disabilities and/or early death were apparent [7, 8, 9, 10]. Increased energy and micro-nutrient requirements with endocrine abnormalities have been reported in HbSS patients [11].

Growth hormone abnormalities [6, 7, 12], and certain endocrine dysfunctions have been associated with suboptimal growth and development in HbSS children [13, 14]. Even young adults with HbSS are smaller than their age matched peers [6, 15], and have delayed pubertal development [16] reported a delay in menarcheal achievement in HbSS patients which was attributed to *Hypothalamo-pituitary* axis dysfunction. Heightened pro-inflammatory *Cytokine* production was reported in individuals with SCA during the steady state and in Vaso-Occlusive crisis [17, 18]. Several workers therefore reported chronic inflammation in patients with *Sickle Cell* disease [19, 20].

In Nigerian HbSS children, factors responsible for their poor growth and several metabolic disorders had not been well researched. There were contradictory and varied results of endocrine dysfunctions in Hb-SS patients. The study therefore bridged this gap in knowledge by assessing the physical characteristics (weight, height and Body Mass Index), plasma levels of Growth Hormone (GH), prolactin, cortisol, Total Thyroxin (TT4), Total *Tri-iodotyronine* (TT3), Thyroid Stimulating Hormone (TSH) and insulin in HbSS children.

Materials and Methodology

Materials

Twenty eight (28) HbSS children with vaso-occlusive crisis attending Children Emergency Clinic at Ladoke Akintola University of Technology Teaching Hospital were recruited for this study. Another 30 apparently healthy sex and age matched children with HbAA served as controls. Height and weight were measured using standard methods; and body mass index (BMI) of all participants calculated before being included in the study.

The participants aged between (4 - 10 years) were recruited based on the report of [15] that lower height, weight and body mass index are demonstrated by HbSS children at age 4years. Hb-electrophoresis of each participant was carried out and those with HbSS and HbAA were selected for this study. Ethical clearance was obtained from the ethical committee, Ministry of Health, Osun state Government secretariat

Abeere, Osogbo, Osun state, Nigeria. Verbal and written consents were obtained from parents of the children that participated in the study. 5mls of fasting blood sample was collected between 7.00 a.m and 9.00 a.m from every participant through venipuncture into lithium heparin bottles. The sample was centrifuged and the plasma separated into a plain bottle and stored at -20^{o}C until ready for analysis.

Methodology

Determination of :

TT3
TT4
TSH
Prolactin
GH
Insulin and
Cortisol

Plasma concentrations of TT3, TT4, TSH and prolactin were determined using commercially prepared enzyme linked immunosorbent assay (ELISA) reagents by Dialab, Gesellschaft, Vienna ***(cat. numbers Z01208, Z01232, Z01237*** and ***Z05303*** respectively).

GH, insulin and cortisol were determined using commercially prepared ELISA kits by CALBIOTECH, 10461 Austin Drive, Suite G, Spring Valley, CA U.S.A (***cat. numbers HG048H, IS130D*** and ***C0103S)*** respectively.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences (***SPSS***) for windows, ***version 21.*** The data was expressed as Mean ± SD. Student (t) test was used for the comparison of HbSS patients and controls. Pearsonian correlation coefficient (r) was calculated. The changes were considered significant, when p-values were less than 0.05.

Results

Physical characteristics of the participants are demonstrated in ***Table 1.*** The weight, height and BMI were significantly ($p<0.05$) lower in HbSS children compared with controls. As shown in ***Table 2***, plasma levels of GH, cortisol, TT3 and TT4 increased significantly ($p<0.05$) in HbSS children compared with controls. There were no significant ($p>0.05$) differences in the plasma levels of prolactin, insulin and TSH in the HbSS children compared with the controls.

There was a significant (r - 0.46, p - 0.04) correlation between cortisol and Growth Hormone in the HbSS children ***(Table 3)***. A negative correlation (r - -0.45, p - 0.045) existed between TT4 and weight of HbSS patients ***(Table 4)***.

Table 1: *Physical Characteristics of HbSS-Children and Controls.*

	Controls (N=30)	HbSS (N=28)	t-values	p-value
Age (years)	7.45+1.79	6.20+2.28	3.707	0.062
Weight (Kg)	23.9+5.09	11.65+4.20	69.01	<0.001*
Height (M)	1.28+0.15	1.06+0.10	29.50	<0.001*
BMI (Kg/M2)	16.73+1.85	10.51+3.72	44.97	<0.001*

KEY **N** - Sample size
* - Significantly different from controls.

Table 2: *Plasma Levels of TT3, TT4, TSH, Growth Hormone, Prolactin, Insulin and Cortisol in HbSS Children and Controls*

	Controls (N=30)	HbSS (N=28)	p-value
Growth Hormone ***(ng/ml)***	0.92 ± 0.56	1.73± 1.68	0.049*
Cortisol ***(ng/ml)***	99.35 ± 38.81	176.85± 105.87	0.004*
TT3 ***(ng/ml)***	4.25±3.69	7.11+2.73	0.008*
TT4 ***(ng/ml)***	36.97±30.44	59.95±22.41	0.010*
TSH***(mIU/l)***	0.25±0.14	0.23± 0.18	0.690
Insulin ***(mIU/ml***	8.02±2.90	7.81±1.60	0.721
Prolactin ***(ng/ml)***	4.17±2.05	4.04±1.83	2.080

KEY **N** - Sample size
* - Significantly different from controls.

Table 3: *Correlation of Cortisol with GH, Prolactin, Insulin, TT3, TT4 and TSH in HbSS Children (N= 28)*

Group	r-value	p-value
Cortisol / GH	0.46	0.04*
Cortisol / Prolactin	-0.34	0.14
Cortisol / insulin	-0.19	0.43
Cortisol / TT3	-0.03	0.89
Cortisol / TT4	0.05	0.84
Cortisol / TSH	0.11	0.63

Where **N** = Sample size
* = Significant correlation

Table 4: *Correlation of Weight with Growth Hormone, Cortisol, Prolactin, Insulin, TT3, TT4 and TSH in HbSS-Children (N= 28)*

Group	r-value	p-value
Weight/Cortisol	-0.21	0.38
Weight /GH	-0.28	0.23
Weight /Prolactin	-0.34	0.14
Weight /insulin	-0.19	0.43
Weight /TT3	-0.12	0.61
Weight /TT4	-0.45	0.045*
Weight /TSH	-0.03	0.90

Where **N** - Sample size
***** - Significant correlation

Discussion

Previous studies show that HbF is highest (98%) at birth decreasing at 5% per week till 6th month when it wanes off.

Therefore, HbSS children are apparently normal at birth, but by 2–5 years of age when the hemoglobins are predominantly SS, significant physiological deficits are apparent and several characteristics such as suboptimal growth, *Haemolytic* anemia and immune dysfunction manifest [6, 7].

The suboptimal growth was confirmed in this study with significantly reduced height, weight and BMI observed in our HbSS children. Continuous tissue breakdown, increased inflammatory *Cytokines,* malnutrition due to *anorexia, hypoxia* and growth hormone metabolic disorders could contribute to the loss of weight in these HbSS children. Our finding agree with [15] who reported that, lower height, weight and body mass index were demonstrated by HbSS children. The present findings also corroborate that of [21] who reported growth failure and maturational delay remain significant chronic problems in HbSS children.

Under physiological condition, cortisol mobilized *Triglycerides* to Visceral Fat Cells to aid *Adipocytes* development into mature fat cells. It also played a significant role in glucose metabolism, protein metabolism, inhibiting the production of inflammatory *Cytokines* that improved the hemodynamic status of the respondent [22 - 23].

Previous studies show that, excess cortisol suppresses the immune system, decreased amino acid uptake by muscle, inhibited collagen formation and decreased bone formation [24 - 26].

The elevated cortisol level observed in the study could be due to a response to stress, which might have antagonized the physiological effects of Growth Hormone in the HbSS children. This findings did not agree with independent reports of significant lower levels of cortisol in both male and female HbSS patients [27 - 28].

The contradictory reports by previous researchers might be due to inconsistency in the clinical conditions and age status of the HbSS patients used for their studies. For example, induced stress through insulin *Hypoglycaemia* test in HbSS patients during crisis and non-crisis periods, reported lower level of plasma cortisol during painful crises [29]

However, this study seems to ratify the report that, cortisol levels increased significantly during painful crisis in the HbSS patients. It demonstrated increased levels of Growth Hormone in HbSS children despite the suboptimal growth observed in them. Elevated Growth Hormone Level could be due to peripheral tissue resistance or GH-receptor deficiency in those children. Assumption of impairments in the regulatory feedback mechanisms of the *Hypothalamic-Pituitary* axis leading to continuous secretion of Growth Hormone in the HbSS children could not be under-estimated [30].

The correlation between Cortisol and Growth Hormone might confirm the effect of metabolic stress on Growth Hormone secretion in these children. The report does not support several previous researches which reported lower levels of Growth Hormone in HbSS patients while other researches reported that, growth delay was associated with anemia and deficiency of certain nutritional factors rather than endocrine abnormalities [31,32].

The report to partially corroborate with an earlier one which reported a slightly higher level of GH in HbSS patients with age range of 4 - 50 years. The levels of Growth Hormone reported in many of those earlier studies therefore contradict our report, possibly because those facilitators recruited both adults and young HbSS patients and failed to consider only growing children with optimal Growth Hormone secretion in their studies [33].

Several researches have reported significant metabolic changes in human and animal models with *Thyroid* hormone disorders. The T3 is the key metabolic regulator coordinating short-term and long-term energy needs [34]. Elevated levels of plasma TT3 and TT4 in HbSS children recruited for this study might be due to higher basal metabolic requirement, tissue resistance to *Thyroid* hormones and or selective resistance in the pituitary gland.

Since; T3 is 25 % bound to *albumin* and 75 % bound to thyroid binding globulin,
while; T4 is 20 % bound to *Thyroid* binding *pre-albumin*, 75 % bound to thyroid binding globulin and 5% to *albumin*,

the *hyperglobulinaemia* due to immune response to infections might have contributed to the higher levels of total T3 and total T4 in our HbSS children.

A negative correlation reported between TT4 and weight of HbSS patients could confirm the effect of *hyperthyroidism* on weight loss. Our finding seems to agree with [33] who reported that, free T3 and T4 were slightly higher in the HbSS patients compared to the controls. In other studies, the levels of total T3 and T4 were reported to be within the normal range in HbSS patients.

However, the significantly higher levels in many studies have shown that the basal metabolic rates were significantly increased in HbSS patients [31, 32, 35, 36]. This elevated thyroid hormone levels could contribute to the already established increased metabolic rate, and the loss of weight observed in the HbSS children.

Prolactin is a stress induced hormone that plays physiological roles only during pregnancy and in lactating mothers. Apart from Pituitary *Adenoma* and stress that enhance *prolactin* secretion in adults, the plasma level increases in newborn babies due to high level of maternal *estradiol* transferred to the baby in-*utero* and declines after birth. In this study, *prolactin* level did not show significant change in HbSS children. The study confirms the findings of [37] who reported insignificant change in the level of *prolactin* in HbSS patients.

However, the only report of *hyperprolactinemia* in HbSS patients was due to *pituitary adenoma* [38]. Insulin played critical roles in the metabolisms of Growth Hormone, glucose, proteins and lipids [39].

Several studies failed to show the co-existence of insulin metabolic dysfunction and *Sickle Cell* disease. Lack of reported cases of Type 1 diabetes *mellitus* in

patients with HbSS suggested that, HbSS might be protective against the development of Type 1 diabetes *mellitus.* Also in the study, there was no significant change in the level of insulin in our HbSS children. This study contradicts [40] who reported that, *normoglycemic* patients with *Sickle Cell* disease demonstrated impaired β-cell function with reduced insulin secretion.

Meanwhile, our finding seems to support the report of [41] who reported that;

a. There were no cases of *Sickle Cell* disease in the diabetic population.
b. All *Sickle Cell* disease patients demonstrated normal glucose tolerance, with insulin and glucagon responses.

Conclusion

In conclusion, increased levels of cortisol, Thyroid Hormones and Growth Hormones are possible features of children with Hemoglobin-SS *(*HbSS). Peripheral tissue resistance and other unknown factors might account for increased plasma level of growth hormone in the HbSS children.

Authors' Contributions

MOA, MOO, FFA, SSA designed the research, MOA, SSA, HMG, TYO and KAU did the analysis and all authors contributed and approved the final manuscript.

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This study was informed by the reports of Gozashti et al. [42] that certain *endocrinopathies* were present in 65% of their HbSS patients. The authors appreciate Mr. F.J. Abegunde, Department of Biochemistry and Chemistry, Caleb University Lagos, Nigeria for the material and technical supports.

Limitations

They include non-compliance of some HbSS children and their parents, lack of funding to investigate and establish some other facts in HbSS children.

Competing interest

The authors declared that they had no competing interests.

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