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The Interactive Roles of Some Toxic Metals, Micronutrients, Antioxidant Vitamin and Sex Hormones in Nigerians with Sickle Cell Disease

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Authors' contributions

All authors designed the study, interpreted the data and approved the final manuscript. Authors MACD and FCO wrote the protocol. Author TSA, a consultant haematologist, recruited the participants after diagnosis. Author FCO managed the literature search, laboratory/statistical analysis and wrote the first draft of the study. Author MACD critically reviewed the manuscript.

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ABSTRACT

Aims: To define the involvement of sex hormones-estradiol, testosterone, toxic metals and micronutrients in individuals with sickle cell disease (SCD).
Study Design: A case control study.
Place and Duration of Study: Departments of Chemical Pathology and Haematology, College of Medicine, University of Ibadan, Ibadan, Haematology Outpatient Clinic, Ring Road State Hospital, Oyo State, Nigeria between Dec 2007 and Jul 2008.
Methodology: One hundred and twenty participants, consisting of 68 with Haemoglobin S (SCD) and 52 with Haemoglobin A aged 17-43 years were recruited for the study. Five ml blood samples

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were obtained from each participant. Anthropometry and reproductive history were obtained by standard methods. Serum estradiol and testosterone concentrations were estimated using enzyme linked-immunoassay methods, Zinc, Copper, Selenium, Lead and Cadmium by atomic absorption spectrophotometry and vitamin E by High Performance Liquid Chromatography.

Results: The mean Zinc, Copper, Vitamin E concentrations in SCD were significantly lower, while those of lead and Cadmium were significantly higher when compared with the corresponding control values (P <.001). The mean testosterone in male and estradiol in female (SCD) were significantly lower than the corresponding control values (P <.03). The mean age at menarche in SCD was significantly higher than the value in control participants. In female SCD, the serum estradiol was positively correlated with height and negatively correlated with lead in the control (P =.05). The incidence of priapism in male SCD was significantly higher than the value in male control subjects (P <.007). In both male and female SCD, the mean body mass index and body weight were significantly lower than the corresponding values in the control group (P <.006).

Conclusion: A significant number of Nigerians with SCD had priapism and the observed oxidative stress in SCD probably due to hypogonadism, may be amenable to micronutrient supplementation.

Keywords: Micronutrients; oxidative stress; testosterone; estradiol; sickle cell anaemia; priapism.

1. INTRODUCTION

Sickle cell disease (SCD), the phenotypic expression of homozygous inheritance of haemoglobin S gene is the most prevalent genetic disease in African region [1,2]. The average life expectancy of individuals with the disease (ISCD) is decreased by 25-30 years and many die before they reach reproductive age [1]. The prevalence of SCD at birth is 2% in Nigeria [3] and 1-10% in Africa [2].

In most of the countries where SCD is a major public health concern, national program for its control does not exist and basic facilities to manage patients are usually absent. Systematic screening for the disease is not common practice and its diagnosis is usually made when a severe complication occurs [1]. The only option for reducing the incidence of SCD is prevention which depends upon education, the detection of carriers, genetic counseling and prenatal diagnosis in couples who are both carriers [2]. Several treatment regimens have been used to alleviate the suffering associated with the disease. However, allogeneic bone marrow transplantation is the effective treatment for the disease. This form of treatment remains inaccessible as two-thirds of people suffering from the disease are in the very low socio-economic class in Nigeria, a developing economy [2].

Acute and chronic vaso-occlusion characterize SCD causing morbidity and mortality [2]. Frequent episodes of crisis, infections and organ damage reduce the quality of life of people with SCD [1]. Men and women with SCD may have impaired fertility, with fertility particularly compromised in younger men [4]. Men with SCD (MSCD) have delayed puberty (sexual maturation and the teenage growth spurt), hypogonadism, sperm abnormalities and priapism [5,6]. Hypopituitarism resulting from intravascular thrombosis and pituitary infarction has also been observed in MSCD [7].

Some MSCD experience priapism, the painful erection of penis in the absence of sexual desire. There is stasis, hypoxia and acidosis of venous blood during normal erection, resulting in sickling of erythrocytes, within the venous sinusoids of corpora cavernosa, causing obstruction of venous outflow. Infarction of the venous outflow tract results in increased blood volume in the paired lateral corpora cavernosa and single corpus spongiosm causing erection. Persistence for longer duration may lead to fibrosis and impotence [7,8].

Delayed puberty, amenorrhoea and many pregnancy-induced complications were reported in females with SCD (FMCD) [4,8]. Risk of maternal mortality are increased, fetal wastage is common and more than one third of pregnancies in FSCD have terminated in spontaneous abortion, still birth or early neonatal death [9].

Sickle hemoglobin exhibits accelerated auto-oxidation under various conditions, enhancing oxidative stress with increased generation of reactive oxygen species in sickle red cells. [10] Individuals with sickle cell disease are sensitive to the effects of cadmium (Cd) and lead (Pb)-common environmental pollutants in Nigeria, which enhance susceptibility to anemia [11,12].

Though Cd and Pd have no known useful biological functions, their competition with zinc for binding sites can interfere with normal red blood cell and reproductive functions [11,13]. Oxidative stress and micronutrient deficiencies prevail in ISCD and may increase susceptibility to infection, poor growth and recurrent episodes of painful vaso-occlusive crises [14].

Delay in the progression and complication of disease in ISCD by antioxidant vitamins supplementation has been suggested [15]. Certain micronutrients- Copper (Cu), iron (Fe), Zinc (Zn), magnesium (Mg), chromium (Cr), Selenium (Se) and some vitamins (A, B, C, E and folate) have been shown to effectively relieve the stress associated with red cell membranes [16-19]. This study is aimed at evaluating sex hormone levels and their association with essential micronutrients and selected heavy metals in ISCD.

2. MATERIALS AND METHODS

2.1 Participants

Convenient sampling method was used to recruit 120 participants aged 17-43 years in this case-control study from Haematology Clinic of the University College Hospital, Ibadan (UCH) and Ring Road State Hospital, Oyo State, Nigeria. Sixty eight of them were ISCD (cases) (32 males and 36 females). These were age matched with 52 apparently healthy Haemoglobin A (HbA) volunteers (controls) (27 males and 25 females). Serum electrophoresis was used to confirm Haemoglobin status. About 88% of the studied population in Nigeria had none and/or very low educational background and are in the very low socio-economic status.

Exclusion criteria were; diabetes, chronic diseases, hormonal medications and pregnant women. The ethical approval of the study was obtained from Epidemiology/Research/Training and Ethical Review Committee, Ring Road State Hospital, Ibadan, Oyo State, Nigeria.

2.2 Demographic Indices

Age and reproductive history were obtained from each participant through a semi pretest questionnaire. Anthropometric parameters-body weight, height and body mass index (BMI) were obtained as described elsewhere [20].

2.2.1 Blood collection

Five ml of venous blood sample was aseptically collected from the antecubital vein using new disposable pyrogen free syringes and needles with minimal stasis. This was put into plain serum tubes and kept for 1-2 hours to clot and centrifuged at 500 g for 5 minutes. The sera obtained were stored at -20℃ for the determination of hormones- testosterone, estradiol and micronutrients- vitamin E, zinc, copper, selenium with heavy metals- cadmium and lead.

2.3 Biochemical Analysis

2.3.1 Serum testosterone and estradiol

Testosterone and estradiol in serum were estimated by enzyme linked immunosorbent assay using a commercial kit (Teco Diagnostics Anaheim, CA, USA).

2.3.2 Serum trace metals

Heavy toxic metals (Pb and Cd), and essential micronutrients (Cu, Se and Zn) were determined by atomic absorption spectrophotometry (Buck Scientific, model 205, East Norwalk, CT, USA) based on the direct method described by Kaneko [21].

2.3.3 Serum vitamin E

Serum Vitamin E was estimated by high performance liquid chromatography (Waters, Milford, MA, USA).

2.4 Statistical Analysis

Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 15 was used for the statistical analysis. Analysis of variance (ANOVA) and Student's t-test were used for comparison of variables. Chi- square test was used for association between variables while Pearson's correlation coefficient was used for relationships between variables. P=.05) was considered significant.

3. RESULTS

Table 1 shows comparison of age, age at menarche, anthropometric and biochemical parameters in cases and controls. Body weight, BMI, Zn, Cu and Vitamin E levels were significantly lower while Se and Pb were

significantly higher in both male and female cases compared with controls (*P*<.006). Age at menarche was significantly higher in female cases while Cd was higher in male cases compared with the corresponding level in the controls (*P*<.002). The mean testosterone concentration was significantly lower in male cases compared with controls (*P*<.001) while the mean estradiol concentration was significantly lower in female cases compared with the corresponding level in the control group (*P*<.025).

Table 1. Biophysical, reproductive history and biochemical parameters of male and female Haemoglobin S (HbS) and Control

Parameter	Males			Females		
	HbS n=32	**Control n=27**	***P***	**HbS n=36**	**Control n=25**	***P***
Age (years)	26.6±0.8	28.9±0.9	.56	25.8±1.1	28.7±1.04	.067
Height (m)	1.7±0.0	1.8±0.01	.112	1.6±0.01	1.7±0.01	.137
Body Weight (kg)	52.4±1.7	65.2±2.01	.000*	48.6±1.6	56.8±2.03	.002*
BM1(kg/m^2)	18.3±0.8	21.9±0.7	.002*	18.8±0.5	21.5±0.8	.005*
AM (years)	_	_	_	16.4±0.4	14.7 ±0.2	.001*
Zinc (µg/dl)	73.3±1.4	109.5±2.6	.000*	69.4±1.2	100.1±1.9	.000*
Copper (µg/dl)	69.9±1.4	108.8±2.7	.000*	66.6±1.5	104.9±2.6	.000*
Selenium (µg/dl)	65.7±1.0	49.3±1.0	.000*	66.9±0.8	50.6±1.2	.000*
Vitamin E (mg/ml)	4.9±0.3	9.8±0.3	.000*	4.5±0.1	10.3±0.3	.000*
Lead (µg/dl)	76.9±1.8	56.8±1.3	.000*	77.8±1.4	57.8±1.1	.000*
Cadmium(µg/dl)	5.7±0.2	2.7±0.1	.000*	6.0±0.1	4.3±1.6	.198
Testosterone (ng/ml)	2.4±0.2	3.4±0.1	.000*			
Estradiol (pg/ml)						
Early follicular phase				**n=11**	**n=8**	
				31.7±7.6	73.7±11.6	.000*
Late follicular phase				**n=5**	**n=2**	
				22.7±10.8	82.5±8.5	.024*
Luteal phase				**n=15**	**n=8**	
				51.9±10.1	103.1±17.0	.011*

*Values are mean ± SE, *: P=.05 is significant, AM: Age at Menarche*

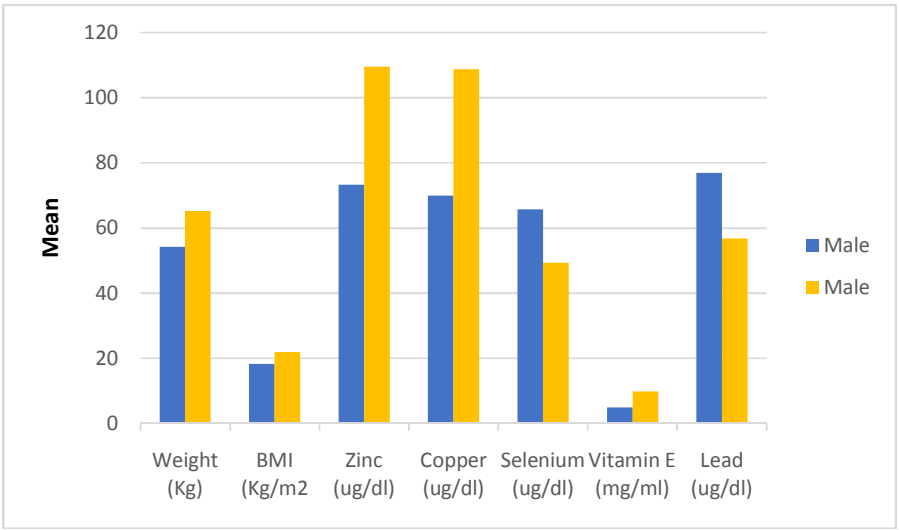

Fig. 1a. Effect of sickling in male HbS and control

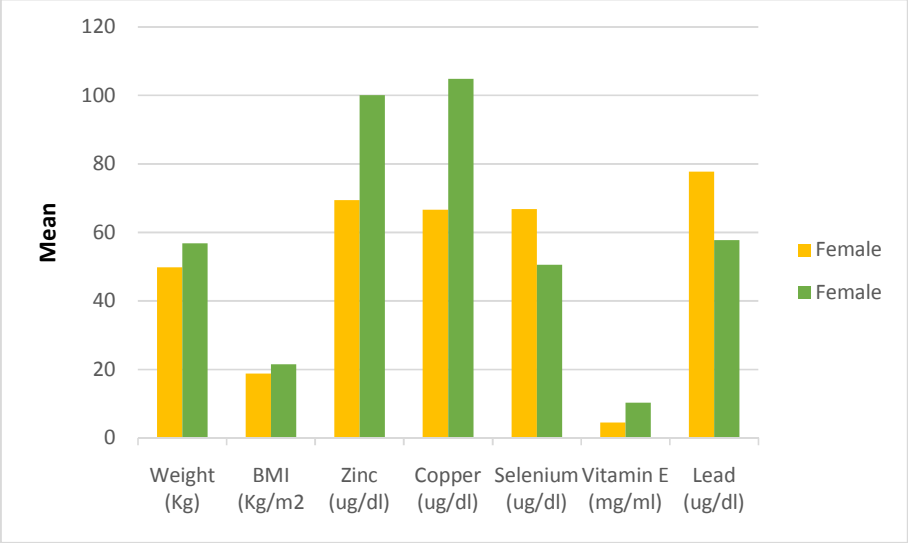

Fig. 1b. Effect of sickling in female HbS and control

In Table 2 biophysical and biochemical parameters in males were compared with females in SCD and control group. Height and Zn level were significantly lower in female HbS than male HbS (*P*<.04) while height, body weight and Zn level were significantly lower in female controls compared with the values in male controls (*P*<.005).

Table 3 shows association of history of priapism in MSCD and controls. 13 (40.6%) of males SCD but none (0%) in the control group had history of priapism. A significant association was observed between SCD and priapism (*P*<.007).

Table 4 shows correlation of anthropometric and biochemical parameters in MSCD and controls. The testosterone was positively correlated with the body weight, while Cu was positively correlated with Vit. E in MSCD (*P*<.04). In male controls, Zn was positively correlated with Vit. E, but negatively correlated with height (*P*< .025).

Table 5 shows correlation of age, age at menarche, anthropometric and biochemical parameters in FSCD and the control group. The height showed a positive correlation with estradiol and copper respectively, while selenium correlated positively with age at menarche and Zn (*P*=.05). In the female control group, Pb negatively correlated with estradiol and Vit. E. respectively. On the other hand there was a significant negative correlation between Cu and selenium and significant positive correlation between Zn, age, body weight and BMI respectively (*P*=.05).

Table 2. Comparison of biophysical and biochemical parameters of male and female HbS and controls

Parameter	HbS			Control		
	Male n=32	**Female n=36**	***P***	**Male n=27**	**Female n=25**	***P***
Age (years)	26.6±0.8	25.8±1.1	.567	28.9±0.9	28.7±1. 04	.067
Height (m)	1.7±0.0	1.6±0.01	.0001*	1.8±0.01	1.7±0.0 1	.0001*
Weight (kg)	52.4±1.7	48.6±1.6	.108	65.2±2.01	56.8±2. 03	.005*
BM1(kg/m^2)	18.3±0.8	18.8±0.5	.589	21.9±0.7	21.5±0.8	.707
Zinc (µg/dl)	73.3±1.4	69.4±1.2	.037*	109.5±2.6	100.1±1.9	. 006*
Copper (µg/dl)	69.9±1.4	66.6±1.5	.115	108.8±2.7	104.9±2.6	.3 05
Selenium (µg/dl)	65.7±1.0	66.9±0.8	.348	49.3±1.0	50.6±1.2	.407
Vitamin E (mg/ml)	4.9±0.3	4.5±0.1	1.325	9.8±0.3	10. 3±0.3	.245
Lead (µg/dl)	76.9±1.8	77.8±1.4	.399	56.8±1.3	57.8±1.1	.563
Cadmium(µg/dl)	5.7±0.2	6.0±0.1	1.387	2.7±0.1	4.3±1.6	.304

*Values are mean ± SE, *: P=.05 is significant*

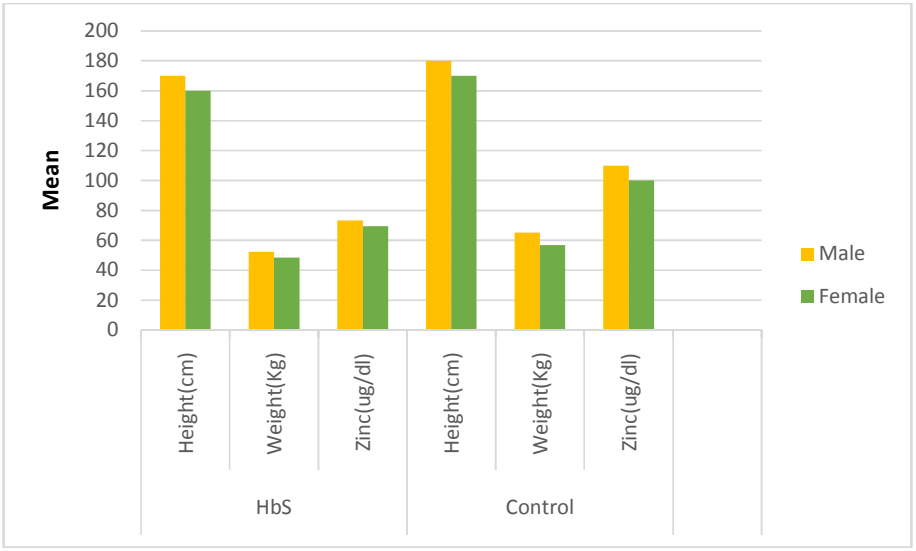

Fig. 2. Effect of gender in HbS and control

Table 3. History of priapism

Index	HbS n= 32		Control n= 27		X^2	P
	With	Without	With	Without	14.1	0.007
Currently	n= 2(6.3%)		0			
In the past 2-6 months	n= 7(21.9%)		0			
In the past 6months- 1year	n= 1(3.1%)		0			
Above 1 year	n=3(9.4%)		0			
Total number of subject	n=13(40.6%)	n=19 (59.4%)	0	27(100%)		

X^2= chi- square test

Table 4. Correlation of anthropometric and biochemical parameters in MSCD

Males			
Index		**HbS n=32**	**Controls n=27**
Testosterone (ng/ml)	Weight (kg)	r=0.368 *P* =.038*	
Copper (µg/dl)	Vitamin E (mg/ml)	r=0.390 *P* =.027*	
Zinc (µg/dl)	Height		r=-0.471 *P* =.13*
	Vitamin E (mg/ml)		r=0.432 *P* =.024*

**: P=.05 is significant*

4. DISCUSSION

Cure for SCD and reduction of its complications still remain a challenge one hundred years after its discovery as a genetically inherited disease [22]. SCD is associated with low lean body mass and fat mass. The effects of chronic illness, anemia, increased cardiac workload, hyperactive erythropoiesis, increased protein turnover, and inflammatory and oxidative stress all contribute to the hypermetabolic state in SCD [23].

The generation of reactive oxygen species which is a steady state cellular event in normal respiring cells, is exacerbated in SCD. Uncontrolled production of reactive oxygen often leads to damage of cellular macromolecules such as lipids, proteins and DNA as well as other

Table 5. Correlation of age, demographic, anthropometric and biochemical parameters in female HbS and their respective controls

Females			
Index		**HbS n=15**	**Control n=8**
Height (m)	Estradiol (pg/ml)	r=0.413 *P*=.021*	
	Copper (µg/dl)	r=0.391 *P* =.018*	
Selenium (µg/dl)	AM (years)	r=0.330 *P* =.049*	
	Zinc (µg/dl)	r=0.432 *P* =.008*	
Lead (µg/dl)	Estradiol (pg/ml)		r=-0.425
Height (m)	Vitamin E (mg/ml		*P* =.043* r= -0.563
			P =.003*
Copper (µg/dl)	Selenium (µg/dl)		r= -0.414 *P* =.039*
Zinc (µg/dl)	Age (years)		r=0.443 *P* =.027*
	Weight (kg)		r=0.722 *P* =.000*
	BMI (kg/m^2)		r=0.635 *P* =.001*

** = P =.05 is significant*

antioxidant molecules [10]. In this study, the mean concentrations of lead and cadmium were elevated in SCD as compared to the corresponding levels in the control subjects. ISCD are sensitive to the effects of toxicants such as cadmium and lead which enhance susceptibility to anaemia [11] and vitamin E supplement has been shown to reduce the effects of lead poisoning [24].

Protection of red cell membrane from free radical – mediated oxidative stress is crucial to the successful management of the sickle cell crises [25]. Micronutrient deficiency has been recognized as a serious complication in ISCD and its recognition in the clinical management of the disease has been suggested. Zn, Cu, Se are essential micronutrients that are cofactors for many enzymes, antioxidants and may play important roles in human growth and development [26]. Deficiencies of zinc and vitamin E malabsorption have been reported in SCD [23]. Nwaoguikpe and Braide [19] reported the antisickling effectiveness of Cu, Zn and antioxidant Vitamin E, which may be important in the management of SCD. Zn, Cu and Vitamin E levels in this study were significantly lower, while Se was higher in both male and female cases compared with controls (P<.006). Cu, Se, Zn and Vit. E, which have been found to effectively relieve the oxidative stress that prevails in SCD, are deficient in these patients [16,25,27].

Growth failure is the most frequent endocrine abnormality observed in patients with SCD. Children with SCD have significantly decreased height, weight, and BMI when compared with healthy, control subjects of comparable age, sex, and ethnicity [23]. In this present study, the mean body weight and BMI were significantly reduced in both male and female cases compared with their respective values in the control group. Body weight correlated positively with testosterone in male cases while estrogen at the luteal phase correlated significantly with height in FSCD. Reduced levels of testosterone in MSCD were observed in this study. Our observations confirm androgen deficiency consistent with the primary testicular failure previously described in adult MSCD by Abbasi et al. [28].

Hypogonadism is one of the most prevalent endocrinopathies in subjects with SCD. Biochemical studies have demonstrated low levels of testosterone and dihydrotestosterone and variable levels of follicle-stimulating hormone

and luteinizing hormone. Much less is known about the prevalence and etiology of infertility in women with SCD [6]. However, menarche is delayed by a mean interval of 2-3 years [23]. Our observations in this study also showed delayed age at menarche for about two years in FSCD, which was significantly higher than the value in the control group. Age at menarche positively correlated with Se in FSCD (P<.05). The mean estrogen level in female cases was significantly lower than the corresponding value in the control group (P<.03) in this study. Estrogen positively correlated with height in cases but negatively correlated with Pb in controls. The ubiquitous contamination of our environment by Pb is on the increase due to unregulated industrial pollution. Endocrine disrupting activity of Pb is well known [12]. The reasons for decreased growth are multifactorial with contributions from abnormal endocrine function, sub-optimal nutrition, an increase in metabolism because of hyperactivity of the bone marrow and chronic inflammation, and hypogonadism. The etiology for hypogonadism in SCD is unclear; however, several causes have been proposed, including primary testicular failure, hypothalamic and/or pituitary dysfunction, zinc deficiency and delay of puberty [23].

Semen analyses in patients with SCD have revealed decreased total sperm counts, reduced sperm density, reduced mobility, and reduced indices of semen quality compared with healthy, fertile control subjects. Infertility seems to be a greater problem among males than females with SCD, because such men have rarely fathered children, whereas many women with SCD have had successful pregnancies [6,23].

Priapism describes a persistent erection lasting longer than 4 hours, which occurs in SCD. It is veno-occlusive with low flow (ischeamic) and could be pathologic. It is a urologic emergency as it results in corporal fibrosis and erectile dysfunction when untreated. It could reoccur as unwanted painful erections in MSCD, who typically awaken with an erection that persists for several hours and becomes painful. 40.6% of MSCD had history of priapism in this study. However, the recommended treatment for ischemic priapism is decompression of the penis by needle aspiration and if needed, injection (or irrigation) with dilute sympathomimetic drugs [23,29]. It is suggested that oxidative stress in SCD may relate to hypogonadism which could be relieved by micronutrient sufficiency.

5. CONCLUSION

Low levels of circulating zinc, copper, vitamin E, testosterone and estradiol are present in SCD patients. The present findings provide additional information on the relationship between zinc, copper, vitamin E with testosterone and estradiol in SCD patients which may be related to oxidative stress and subfertility in these patients. Thus, it is suggested that micronutrient/ antioxidant supplements be included in the therapy for management/treatment of patients with SCD so as to reduce morbidity and mortality and prevent infertility.

CONSENT

All authors declare that 'written informed consent was obtained from each participant of the study.

ETHICAL APPROVAL

All experiments have been examined and approved by the Epidemiology/ Research/ Training and Ethical Review Committee of Ring Road State Hospital, Ibadan, Oyo State, Nigeria and therefore have been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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