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Some Haematological Parameters and Micronutrients among Sickle Cell Disease Children in Sokoto

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List of abbreviations

WBC - white blood cell
RBC - red blood cell
LYM - lymphocyte
GRA - granulocyte
HGB - haemoglobin
HCT - haematocrit
MCV - mean cell volume
MCH - mean cell haemoglobin
MCHC - mean corpuscular heamoglobin concentration
PLT - platelet
ZN - zinc
CU - copper
SCD - sickle cell disease
WHO - World Health Organisation
FBC - full blood count

Abstract

Sickle cell disease (SCD) is an inherited red blood cell disorder that leads to forming the mutated haemoglobin S, resulting in a wide range of sickness. The aim of the study is to check the status of some haematological parameters and trace elements in 60 SCD and 30 non-SCD children in Sokoto metropolis. The result obtained from the study showed that the mean WBC (13.63/7.61),GRA(47.59/45.51),LYM(40.73/44.25),RBC(2.28/3.98),HGB(7.30/11.09),HCT(19.42/31.30),MCV(79.15/74.92)MCH(30.46/28.15),MCHC(39.55/37.34),PLT(584.30/410.50), ZINC(21.93/35.80) and CU(0.68/0.27) for SCD and non-SCD respectively. The mean values for

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WBC, GRA, MCV, MCH, MCHC, PLT and CU are higher in Sickle cell disease children and the mean value for LYM, RBC, HGB, HCT and ZN are lower in sickle cell disease children compared to non-sickle cell disease children. The WBC, MCH and ZN values of Sickle cell disease subject and controls showed a negative but statistically significant comparison ($p < 0.05$). The RBC, HGB and HCT values showed a positive but statistically significant comparison ($p<0.05$). The WBC, MCV, MCH and PLT values of Sickle cell disease subject and controls showed a negative but statistically significant correlation with Zinc ($p<0.05$). The RBC, HGB and HCT values showed a positive but statistically significant correlation with Zinc while WBC showed a positive but statistically significant correlation with copper ($p<0.05$). In conclusion, the serum copper level was significantly higher among sickle cell subjects compared to controls and the serum zinc level was significantly lower among sickle cell subjects compared to controls. Hence, copper, zinc and haematological parameters be monitored routinely among sickle cell disease children to optimize the care offered to these individuals.
Keyword: *micronutrients; sickle cell disease; children*

Introduction

Sickle cell disease (SCD) is an inherited red blood cell disorder that causes mutated haemoglobin S to form. This mutation can cause a variety of signs and symptoms, such as sequestration crisis, repeated infections, chronic hemolytic anaemia, and sporadic pain episodes primarily caused by vaso-occlusive phenomena [1-3]. Long-term consequences of SCD include heart attacks, strokes, sickle nephropathy, lung issues, kidney damage, cardiomyopathy, delayed puberty, and stunted growth [4-5]. An imbalance between the generation and removal of reactive oxygen species is the cause of oxidative stress in sickling and ischemia reperfusion injury linked to sickle cell disease (SCD). Moreover, patients with sickle cell disease experience oxidative stress due to the increased autoxidation rate of haemoglobin S [6-7].

Several micronutrient deficiencies may arise in SCD patients due to the high energy consumption linked to the fast rate of red cell turnover, which may influence the severity of the disease. It has been noted that individuals with sickle cell disease (SCD) typically have low amounts of certain micronutrients [8–9]. Although sickle haemoglobin (HbS) is present from birth, most infants do not exhibit symptoms until six months of age or less, as foetal haemoglobin (HbF) is the predominate haemoglobin at this stage [10]. As a result, measuring haemoglobin SS (HbS) is typically done six months after delivery rather than at birth.

A trace element or micronutrient is a chemical element such as iron, copper, magnesium or zinc that occurs in very small amounts in living things and is necessary for normal growth and development [11].

Zinc and copper are essential cofactors for the optimal performance of superoxide dismutase, a scavengering enzyme responsible for detoxifying anion superoxide to hydrogen peroxide. However, copper could act as a prooxidant and promotes free radicals when it presents in high concentration in the state of impaired zinc bioavailability, a condition that has been previously described in various diseases, including SCD [6, 12-13]. In addition to controlling immune system

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responses, bone metabolism, and heart rhythm, magnesium also affects endothelial inflammation [14]. Data on the status of these micronutrients in SCD were provided by a number of research, however for greater precision, these data need to be summed up and analysed. The objective of this review was to present a quantitative, all-encompassing picture of the condition and degree of deficits in zinc, copper, and magnesium in patients with sickle cell disease.
The aim of the study was to determine some haematological parameters and micro-nutrients (Zinc and Copper) among sickle cell disease children in Sokoto, Sokoto State.

Materials and Methods

Study Area

The study was carried out in Specialist Hospital Sokoto within the Sokoto Metropolis, Sokoto State, Nigeria. The State is located in the extreme Northwest of Nigeria. Near the confluence of the Sokoto River and the Rimas River. It shares borders with Niger Republic to the North, Zamfara State to the East, Kebbi State to the South-East and Benin Republic to the West. The major indigenous tribes in the state are the Hausa and Fulani and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Igbos and others. The Majority of the Hausas' are farmers while Fulanis are nomadic and are engaged in animal rearing. The State is in the dry Sahel, surrounded by sandy Savanna and isolated hills, with an annual average temperature of 28.3°C (82.9°F). Sokoto is on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40°C (104.0°F) and the dryness makes the heat bearable. The warmest months are February to April when day time temperature can exceed 45°C (113°F). The rainy season is from June to October during which shower are a daily occurrence. Sokoto city is a major commerce center in leather crafts and agricultural products. As at 2007, the state has a population of 3.6 million [15]. However, based on population annual growth of 2.5%, the calculated projected population for Sokoto state for the year 2022 should be around 5.2 million.

Study Design

A case-control study involving age and gender-matched 60 children who are confirmed Sickle Cell Disease Children (subjects) and 30 non-Sickle Cell Disease Children as control.

Study Population

A total of 90 participants were selected for the study. These consisted of 60 sickle cell disease children and 30 apparently healthy children as control that met the inclusion criteria and consented to participate in the study.

Inclusion Criteria

- Subjects with confirmed Sickle Cell Disease, age (1 - 12 years).
- Willingness of parents/guardians to offer verbal or written informed consent for their ward to participate in the study.

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Exclusion Criteria

- Sickle Cell Disease children above 12 years and below 11 months old.
- SCD children who had a recent transfusion in the last 4 months.
- SCD children whose parents or guardian refused to provide verbal or written informed consent for their ward to participate in the study.

Informed Consent

Written informed consent was sought from parent or guardian of all participants using a standard protocol.

Ethical Approval

Ethical approval for the study was obtained from Research and Ethical Committee of Specialist Hospital, Sokoto (Reference number: SHS/SUB/l33/VOL.1 14th December, 2022)

Sample Size Determination

The sample size was determined using the COCRAN's formula = (z^2pq/d^2) (Pourhoseingholi *et al*., 2013).

n = minimum sample size.
z = standard normal deviation and probability.
p = prevalence or proportion of value to be estimated from previous studies.
q = Proportion of failure (=1 − P).
d = precision, tolerance limit, the minimum is 0.05.

Therefore, n = (z^2pq/d^2)

Where,
Z = 95% (1.96).
P = % (0.06) (Lema *et al*., 2022)
q = 1 – 0.06 = (=0.4).
d = 5% (0.05).

Therefore, n = $(1.96)^2$ (0.06) (0.94)/$(0.05)^2$.

n = 87
Approximately, n = 90.

Sampling Techniques and Method of Data Collection

A conservative sampling method was employed for the selection of the study subjects. The study was carried out on blood sample that was collected from participants in Specialist Hospital Sokoto Sickle Cell Clinic. All subjects who meet the inclusion criteria were given a written informed consent for this study. A semi structure interviewer questionnaire was administered to all consenting Parent/Guardian of participants to obtain information on subject socio-demography such as history of transfusion.

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Specimen Collection and Processing

Blood sample was collected from the cubital fossa vein using a 5ml syringe and needle, 2mls into ethylene diamine tetraacetic acid (EDTA) container and 3mls into plain container, and Serum harvested into cryovial after cloting. These samples were tested in the Pathology Laboratory of Specialist Hospital and in the Biochemistry Laboratory of Usmanu Danfodiyo University, Sokoto, Nigeria. The following laboratory investigations were carried out on K_3EDTA anticoagulated blood and serum.

Laboratory Analysis

Full Blood Count Parameters

Haematological Analyzer was used for the full blood count. Coulter Counter method developed by Wallace H. Coulter 1940.

The EDTA anticoagulated blood samples was analyzed using sysmex haematology analyser. The machine is a three-part auto analyser able to test 19 parameters per sample, including RBC count, Haemoglobin concentration, Haematocrit (HCT), Total White Blood cells and 3-differentials, Platelets counts and other related parameters.

Procedure of Full Blood Count

EDTA blood sample was placed on a blood mixer for proper mixing of the anticoagulated blood sample. The mixed sample tube was placed on a tube holder. The blood was aspirated into the tube analyzer by pressing the aspiration button. The analyzer was allowed to measure the various parameters. The result was printed out using an inbuilt printer on the analyzer.

Trace Element Estimation

Trace elements (zinc and copper) were determined using atomic absorption spectrophotometry method.

Method: Spectrophotometry

The technique makes use of the atomic absorption spectrum of sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert law.

Procedure for Zinc Estimation

Wet digestion for Zinc for Atomic Absorption Spectrometry

Procedure

A volume of 1ml of serum was put into a clean test tube**.** A volume of 10ml of nitric acid was put into the same test tube**.** Test tube was boiled at 200c for 20 minutes**.** A volume of 39ml of de-ionized water was added into test tube**.** The test tube was taken for atomic Absorption Spectrometry. Zinc was coloured silver (Fishman and Downs, 1966).

Procedure For Copper Estimation

Wet digestion for Copper for Atomic Absorption Spectrometry

Procedure

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A volume of 1ml of serum was put into a clean test tube. A volume of 10ml of nitric acid was put into the same test tube**.** Test tube was boiled at 200c for 20 minutes**.** A volume of 39ml of de-ionized water was added into test tube**.** The test tube was taken for atomic Absorption Spectrometry. Copper was coloured blue [16].

Statistical Analysis

Data collected was recorded on excel spreadsheet and statistical analysis was done using a statistical software SPSS version 23 on a computer. Results were expressed as mean (standard deviation) and student T- test was used for test statistics at $p<0.05$ significance level.

Results

The study investigated sixty (60) sickle cell disease children and thirty (30) non-sickle cell disease children as control. Some haematological parameters and micro-nutrients were analysed in 60 sickle cell disease children and 30 non-sickle cell disease children as control in paediatric unit of Specialist Hospital Sokoto to determine the various parameters. The results obtained are presented in the following tables:

Table 1 shows Mean ±SD of Zinc, Copper and Haematological parameters level among sickle cell disease children. The mean values for parameters show low values for GRA(47.59) RBC(2.28), HB(7.30), HCT(19.42), ZINC(21.93) and CU(0.68) , high values for WBC(13.63), MCHC(39.55) and PLT(584.30) and normal values for LYM(40.73), MCV(79.15) and MCH(30.46).

Table 1: Mean ±SD of Zinc, Copper and Haematological parameters level among sickle cell disease children

PARAMETER	NUMBER(N)	MEAN±SD
WBC COUNT ($\times10^9$/L)	60	13.63±5.35
LYM	60	40.73±12.47
GRA	60	47.59±13.75
RBC COUNT ($\times10^{12}$/L)	60	2.28±0.66
HGB (g/dl)	60	7.30±1.74
HCT (%)	60	19.42±6.37
MCV (fl)	60	79.15±16.00
MCH (pg)	60	30.46±5.30
MCHC (g/dL)	60	39.55±8.53
PLT ($\times10^9$/L)	60	584.30±433.93
ZINC	60	21.93±10.73
COPPER	60	0.68±1.32

Values are mean ± standard deviation, Key: N = number of subjects; SD = Standard deviation, WBC = white blood cell count, RBC = red blood cell; HGB = haemoglobin; HGT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular Haemoglobin concentration; PLT = platelet.

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Table 2 shows Mean ±SD of Zinc, Copper and Haematological parameters level among non-sickle cell disease children. The mean values for parameters show low values for GRA(45.51), MCV(74.92), MCH(28.15), ZN(35.80) and CU(0.27), high values for LYM(44.25) and MCHC(37.34) and normal values for WBC(7.61), RBC(3.98), HGB(11.09), HCT(31.30) and PLT(410.50).

Table 2: Mean ±SD of Zinc, Copper and Haematological parameters level among non-sickle cell disease children

PARAMETER	NUMBER(N)	MEAN±SD
WBC COUNT ($\times 10^9$/L)	30	7.61±2.37
LYM	30	44.25±11.53
GRA	30	45.51±12.62
RBC COUNT ($\times 10^{12}$/L)	30	3.98±1.26
HGB (g/dl)	30	11.09±2.65
HCT (%)	30	31.30±11.13
MCV (fl)	30	74.92±12.50
MCH (pg)	30	28.15±3.83
MCHC (g/dL)	30	37.34±7.55
PLT ($\times 10^9$/L)	30	4.11±133.90
ZINC	30	35.80±8.51
COPPER	30	0.27±0.27

Values are mean ± standard deviation, Key: N = number of subjects; SD = Standard deviation, WBC = white blood cell count, RBC = red blood cell; HGB = haemoglobin; HGT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular Haemoglobin concentration; PLT = platelet.

Table 3 shows Comparison between haematological parameters, zinc and copper between Sickle cell Disease and non-Sickle Cell Disease children. The p-value shows that there is no significant difference LYM, GRA, MCV, MCHC, PLT and CU while there is significant difference for WBC, RBC, HGB, HCT, MCH and ZN.

Table 3: Comparison between haematological parameters, zinc and copper between Sickle cell Disease and non-Sickle Cell Disease children

PARAMETER	SCD N = 60	Non-SCD N = 30	t-value	P-value
WBC ($\times 10^9$/L)	13.63±5.35	7.61±2.37	-7.38	0.00(s)
LYM	40.73±12.47	44.25±11.53	1.33	0.19(ns)
GRA	47.59±13.75	45.51±12.62	-0.72	0.48(ns)
RBC ($\times 10^{12}$/L)	2.28±0.66	3.98±1.26	6.93	0.00(s)

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HGB (g/dl)	7.30±1.74	11.09±2.65	7.11	0.00(s)
HCT (%)	19.42±6.37	31.30±11.13	5.41	0.00(s)
MCV (fl)	79.15±16.00	74.92±12.50	-1.38	0.17(ns)
MCH (pg)	30.46±5.30	28.15±3.83	-2.36	0.02(s)
MCHC (g/dL)	39.55±8.53	37.34±7.55	-1.26	0.21(ns)
PLT ($\times10^9$/L)	584.30±433.93	410.50±133.90	-2.84	0.06(ns)
ZINC	21.93±10.73	35.80±8.51	-6.66	0.00(s)
COPPER	0.68±1.32	0.27±0.27	-2.38	0.20(ns)

Values are mean ± standard deviation, Level of significance is considered when $p < 0.05$. Key: SCD = Sickle cell disease; WBC = white blood cell count, RBC = red blood cell; HGB = haemoglobin; HGT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular Haemoglobin concentration; PLT = platelet.

Table 4 shows Correlation between haematological parameters, zinc and copper among sickle and non-sickle cell disease children shows that there is no significant difference between ZN and LYM, GRA and MCHC but there is significant difference for WBC, RBC, HGB, HCT, MCV, MCH and PLT. It also shows that there is no significant difference between CU and LYM, GRA, RBC, HB, HCT, MCV, MCH, MCHC, and PLT but there is significant difference for WBC.

Table 4: Correlation between haematological parameters, zinc and copper among sickle and non-sickle cell disease children

Parameters		**Zinc N = 90**	**Copper N = 90**
WBC COUNT ($\times10^9$/L)	r-value	-0.429	0.396
	p-value	0.000	0.000
LYM	r-value	0.060	-0.030
	p-value	0.575	0.782
GRA	r-value	-0.004	-0.098
	p-value	0.971	0.360
RBC COUNT ($\times10^{12}$/L)	r-value	0.597	-0.037
	p-value	0.000	0.726
HGB (g/dl)	r-value	0.483	-0.063
	p-value	0.000	0.554
HCT (%)	r-value	0.449	-0.124

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	p-value	0.000	0.245
MCV (fl)	r-value	-0.221	-0.128
	p-value	0.036	0.230
MCH (pg)	r-value	-0.359	-0.016
	p-value	0.001	0.884
MCHC (g/dL)	r-value	-0.101	0.163
	p-value	0.345	0.125
PLT ($\times 10^9$/L)	r-value	-0.232	-0.050
	p-value	0.028	0.641

r = regression, positive values means both parameters increases and decreases at the same time while negative value means one increases and the other decreases or vice-versa): P<0.05 is significant. Key: N = number of subjects; WBC = white blood cell count, RBC = red blood cell; HGB = haemoglobin; HGT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; PLT = platelet.

Discussion

This study aimed at providing the status of zinc, copper and some haematological parameter in SCD children. The analysis showed that zinc level is lower in SCD children as compared with that of control whereas copper is higher in SCD children as compared with Control. Findings from this study coincide with the known nature of the chronic inflammatory process occurring in SCD associated with ischemia reperfusion injury, excessive production of free radicals like superoxide and hydrogen peroxide [17]. The high copper values in these patients may be attributed to the chronic heamolysis state and aggravated by coexisting zinc deficiency [18]. The GRA, MCV, MCHC, PLT and CU values of Sickle cell disease subject and controls showed a negative but non-statistically significant comparison (t-value) ($p > 0.05$). The LYM values showed a positive but non-statistically significant comparison ($p > 0.05$). The WBC, MCH and ZN values of Sickle cell disease subject and controls showed a negative but statistically significant comparison ($p < 0.05$). The RBC, HGB and HCT values showed a positive but statistically significant comparison ($p<0.05$). The GRA and MCHC values of Sickle cell disease subject and controls showed a negative but non-statistically significant correlation with Zinc ($p > 0.05$). The LYM values showed a positive but non-statistically significant correlation with Zinc ($p >0.05$). The MCV, MCH and PLT values of Sickle cell disease subject and controls showed a negative but statistically significant correlation with Zinc ($p<0.05$). The RBC, HGB and HCT values showed a positive but statistically significant correlation with Zinc ($p<0.05$).

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The LYM, GRA, RBC, HGB, HCT, MCV, MCH and PLT values of Sickle cell disease subject and controls showed a negative and non-statistically significant correlation with copper ($p > 0.05$). The MCHC value showed a positive but non-statistically significant correlation with Copper ($p > 0.05$). The WBC value showed a positive but statistically significant correlation with Copper ($p < 0.05$). The result from the research also shows that there is malnutrition in the SCD and control generally because the levels of zinc and copper did not reach the normal ranges of 68-107ug/dl and 8-19umol/L respectively according to the reference list used in Chemical Pathology Laboratory of Usmanu Danfodiyo University Teaching Hospital, Sokoto [19]. This study's findings are consistent with those of Eikhidir et al. [20], who gathered data from 36 studies published between 1974 and 2019 (eight from Asia, eight from Africa, nine from the United States, and four from Europe) and found that SCD patients had significantly lower zinc levels than controls (SMD=-1.27 [95% CI: 1.67-0.87, p 0.001). The copper level in SCD patients was found to be considerably higher (SMD = 0.68, 95% CI: 0.05-1.32, p 0.004). The study by Temiye et al. (2010) at Lagos University Teaching Hospital (LUTH), Nigeria, on the "Relationship between Painful Crisis and Serum Zinc Level in Children with Sickle Cell Anaemia" is also consistent with this. It found that the control group had a mean packed cell volume of 21.6±4.2% and a haemoglobin concentration of 7.4±1.4 g/dL, respectively, which were significantly higher ($t = 16.9$. $P = 0.00$) than those of the SCA group. Additionally, the control group's mean serum zinc content was substantially greater than that of the sickle cell anaemia group (42.7±13.6/ 32.3±14.0 ug/dl, $t = 5.2$, $p = 0.00$). It is also consistent with studies carried out in Benin City by Idonije et al. [21], where the decrease in serum Zn (120.85±10.29) level when compared to control was substantial ($P<0.05$). Comparing the Cu (68.54±10.49) to the control, it was somewhat higher. The study found that the Mean MCV, MCHC, and CU (0.68±1.32) among SCD sufferers were somewhat greater than the control group's (0.27±0.27) ($p<0.05$), although not statistically significant.

When compared to controls, sickle cell disease sufferers had considerably greater Mean WBC and PLT ($p < 0.05$), according to research by Erhabor et al. [22]. When comparing sickle cell disease patients to controls, the mean RBC, HCT, and HGB was considerably lower ($p < 0.05$). This study also contradicts a portion of research by Erhabor et al. [22], which found that patients with sickle cell disease had significantly lower MCV, MCH, and MCHC values than controls ($p < 0.05$). In Sickle cell disease participants, the Mean Copper value was considerably lower (40.4 ± 1.44 µg/dl and 54.6 ± 1.60 ng/ml) than in controls (75.6 ± 1.30 µg/dl and 86.3 ± 2.30 ng/ml) ($p < 0.05$). An infection is a typical cause of elevated WBC counts in sickle cell disease patients. The cause of the variance in red blood cell indices could be attributed to the ongoing chronic hemolysis that sickle cell anaemia patients experience, which stimulates hemopoiesis and hemopoietic activity to produce young red blood cells more quickly. Fresh red blood cells are known to have a greater MCV. Normocytic normochromic anaemia may be the cause of higher MCHC, MCV, and MCH [23-35].

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Conclusion

Patients with sickle cell disease had far lower RBC, HCT, and HCT values than controls. Subjects with sickle cell disease had considerably greater WBC and red cell indices than controls. In a stable condition, higher red cell index values are suggested. In sickle cell individuals, the serum zinc level was much lower than in controls, although the serum copper level was significantly greater than in controls. Additionally, a statistical association between a few micronutrients and a complete blood count was demonstrated in the study between children with sickle cell disease and those without it.

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