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Research Article

Assessment of Plasma Trace Elements Levels and Haematological Indices in Sickle Cell Patients Attending Federal Medical Centre Abakaliki, Ebonyi State, Nigeria

***Ejiofor, Dominic Chinedu[1*], Edward-Ejiofor, Bridget[2], Azunna Uchenna[3], Iheukwumere Barry Chinedu[3], Ideyi, Chikezie[4], Ngwuta, Victor Sochima [1], Etomike, Obianyo[5] Obi, Augustine Uchechukwu[6], Ndiweogu, Chukwuebuka Junior[1], Alagwu, Nnaemeka Da-Silva[6], Obialor, N Ambrose[6], and Obi Dorin Ifunanyachukwu[7]**

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, David Umahi Federal University of Health Sciences, Uburu Ebonyi State, Nigeria
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Imo State University, Owerri, Nigeria
[3]Department of Human Physiology, College of Basic Medicine, Abia State University Uturu, Abia State, Nigeria
[4]Department of Internal Medicine, Federal University Teaching Hospital Owerri, Nigeria
[5]Department of Internal Medicine, Ahmadu Bello University Teaching Hospital, Zaria
[6]Department of Human Physiology, Faculty of Basic Medical Sciences, Imo State University Owerri, Imo State, Nigeria
[7]Department of Internal Medicine, Faculty of Health Sciences and Technology, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
****Corresponding Author's email*: dominicedwardejiofor@gmail.com; Phone: +2348107357039**

ABSTRACT
Various medical conditions can influence how the body interacts with trace elements, resulting in either deficiencies or toxicities that may play a role in the onset of diseases and pathological conditions. Consequently, proper management of trace element imbalances necessitates a comprehensive evaluation of an individual's trace element levels. Therefore, this study assessed the levels of some trace elements and haematological indices in sickle cell patients. Fifty-eight (58) adults, twenty-nine of whom were sickle cell patients attending the haematology clinic at Federal Medical Centre (FMC) Abakaliki, Ebonyi State, and 29 apparently healthy controls, mainly staff at the facility who had consented to participate, were recruited into the study. Trace elements zinc, selenium, manganese, iron, and cobalt, and haematological indices PCV, MCH, and reticulocytes were determined using standard procedures. Plasma Zn and Fe, as well as Hb, PCV, and MCH, reported for sickle cell patients, were significantly ($p<0.05$) lower than those reported for their control counterparts. A contrary observation was made on manganese, while the value reported for cobalt was the same in the two groups. In conclusion, it was deduced from this study that trace elements and haematological indices are greatly reduced in the sickle cell condition.

Keywords: Manganese; Patients; Reticulocyte; Sickle cell; Zinc

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INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder caused by mutations in the β-globin chain of haemoglobin, resulting in the production of haemoglobin S (HbS). This abnormal form of haemoglobin causes red blood cells to become rigid and sickle-shaped, thereby obstructing blood flow and leading to severe pain, anaemia, organ damage, and increased susceptibility to infections (Colombatti *et al*., 2023). SCD is inherited in an autosomal recessive manner, with the homozygous state (HbSS) referred to as sickle cell anaemia (Payane *et al*., 2020). It represents a significant global health burden, affecting millions of individuals worldwide—particularly in sub-Saharan Africa—where it accounts for a substantial proportion of childhood mortality. Approximately 300,000 infants are born with SCD each year, with the highest incidence observed in Africa, where it contributes to 5-10% of under-five mortality (Groose *et al*., 2014).

Haematological parameters are fundamental to the clinical investigation of red blood cell disorders such as SCD, as they enable the measurement of metabolites associated with specific disease states. These assessments are crucial for evaluating the nutritional, pathological, and physiological status of affected individuals (Etim, 2014). Trace elements also play a vital role in maintaining health, influencing numerous bodily functions. They frequently act as catalysts or cofactors for enzymes involved in essential biochemical reactions (Stohs *et al*., 1995). Various disease conditions can alter the body's interaction with trace elements, potentially resulting in deficiencies or toxicities that contribute to pathological developments (Stohs *et al*., 1995). Therefore, the effective management of trace element imbalances necessitates a comprehensive evaluation of an individual's trace element status (Skalny, 2014).

Accordingly, the aim of this study is to assess the levels of selected trace elements and haematological indices in individuals with sickle cell disease

MATERIALS AND METHODS

Study Location and Design

This study employed a case-control design to compare selected variables between individuals with sickle cell disease and healthy controls.

The research was carried out at the Federal Medical Centre (FMC), Abakaliki, located in Ebonyi State in the South-East geopolitical zone of Nigeria. The study period spanned from February 2023 to June 2024**.** According to the 2006 national census, Ebonyi State has an estimated population of approximately 3.24 million people, with Abakaliki serving as the state capital and its most populous urban centre. The population is predominantly of Igbo ethnicity**,** although other ethnic groups are also represented. Geographically, Abakaliki lies between latitudes 6°20'N and 8°06'E.

FMC Abakaliki is a recognised tertiary healthcare institution providing comprehensive medical services to residents of Abakaliki and neighbouring communities, making it a suitable setting for clinical research involving diverse patient populations.

Study Population

This study involved adults with sickle cell disease (HbSS genotype) of both sexes who were attending the haematology clinic at the Federal Medical Center in Abakaliki, Ebonyi State and had consented to participate. The control group comprised healthy individuals who were workers at the facility (HbAA genotype) with no known haematological disorders.

The control group mainly staff at the facility comprised individuals confirmed to have the HbAA genotype (normal haemoglobin) through haemoglobin electrophoresis. They were aged between 5 and 40 years, appeared to be in good health with no known chronic conditions or recent acute illnesses, had no personal or family history of sickle cell disease or its trait, and were willing to provide written informed consent or assent with parental or guardian consent in the case of minors.

Inclusion criteria

Individuals recruited for this study were confirmed to have sickle cell disease through haemoglobin electrophoresis and were between the ages of 5 and 40. They had been regular attendees at the haematology clinic of FMC Abakaliki for the previous 6 months before being recruited for the study and had not experienced any acute sickle cell crises, infections, or blood transfusions in the past month (in a steady state).

Exclusion criteria

Individuals who had received a blood transfusion within the past month and showed signs of infection, illness, or inflammation during sample collection,

and did not have any known chronic conditions such as diabetes, liver problems, or chronic kidney disease that could impact trace element metabolism, were excluded from the study. Additionally, individuals who were taking mineral or vitamin supplements, pregnant or lactating women, and those who were unwilling to follow the procedures or provide informed consent were not included in the study.

Sample Size Determination

A total of fifty-eight (58) adults participated in the study, comprising twenty-nine (29) sickle cell patients receiving care at the Haematology Clinic of the Federal Medical Centre (FMC), Abakaliki, Ebonyi State, and twenty-nine (29) apparently healthy individuals who served as controls. The sample size was determined based on a 95% confidence level, a 5% margin of error, and an assumed population proportion of 50%. The total population size was 67 using the formulae stated below

$$n = \frac{Z^2.\mathrm{p}.(1-p)}{e^2} . \frac{N}{N-1+\frac{Z^2.p.(1-p)}{e^2}}$$

Where:

n= represents the sample size required

N= the size of the population (67)

Z= Z-score is the the desired confidence level (1.96 for 95%)

p = estimated proportion of the population (0.5 is commonly used for maximum variability)

e= Error margin (0.05 for 5%)

Ethical Issues

We obtained approval from the Ebonyi State Hospital Management Board to conduct a study at the Department of Internal Medicine. Participants provided informed consent before enrolling in the study.

Sensitization of participants

Participants were guaranteed confidentiality of their information throughout and after the study. They were informed of their right to withdraw at any time without consequences. Their decision not to participate would not affect their access to clinical services. A questionnaire was provided to collect medical history, ensuring anonymity by excluding personal identifiers.

Sample Collection

A blood sample was obtained through venipuncture of the antecubital vein, collecting 4 ml of blood, with 2 ml each placed into ethylenediaminetetraacetic acid (EDTA) and Lithium Heparin bottles for the determination of haemoglobin genotype, haemoglobin, haematocrit, reticulocyte count, and iron, zinc, manganese, and cobalt levels. The samples in the Lithium Heparin bottle were centrifuged at 2500 rpm, separating the plasma from the packed cells.

Determination of minerals

In the determination of zinc, cobalt, and iron, exactly 1.0 mL of serum sample was added to a clean digestion tube. A mixture of 5.0 mL concentrated HNO_3 and 1.0 mL perchloric acid $HClO_4$ was then added. The mixture was gently swirled and placed on a hot plate under a fume hood at 120°C until digestion was complete. The final volume was adjusted to 10.0 mL with deionized water. A calibration curve was created using an iron standard (Hupfer *et al*., 2009).

$$MCH\ (pg) = \frac{hemoglobin}{RBC} \times 10 \quad \ldots\ldots 1$$

Determination of reticulocytes

This test was conducted using the Sysmex R-1000 series automated reticulocyte analyzer manufactured by Sysmex Corporation Japan (Takani, 1988).

Data analysis

SPSS (Version 23) was used to show data as Mean ± Standard Deviation. An unpaired T-test was used to compare the mean values obtained by male and female subjects. P-values of less than 0.05 were considered statistically significant.

RESULTS

Trace elements and haematological indices of sickle cell individuals are shown in Table 1, indicating that serum zinc (Zn) and iron levels (Fe) in sickle cell individuals were significantly lower (P<0.05) than those in the control group. However, a contrary observation was made for manganese, which was significantly higher (p<0.05) in sickle cell individuals compared to the control group. Plasma cobalt levels in sickle cell patients were not significantly different (p<0.05) from those in the control group. Haemoglobin concentration (Hb), Mean Corpuscular Haemoglobin (MCH), and Packed Cell Volume (PCV) in sickle cell individuals were significantly lower (p<0.05) than those in the control group. This was

not the case for reticulocyte count, which was significantly higher ($p<0.05$) in sickle cell patients compared to the control group.

Table 1. Trace elements and Haematological Indices in Sickle Cell in case and control subjects

Parameters	Patients	Control	P-values
Zinc (µg/dl)	120.70±0.80	136.90±0.97	0.0001
Manganese (µg/dl)	68.76±0.72	63.0±0.79	0.0001
Iron (µg/dl)	47.50±1.06	86.80±3.62	0.0001
Colbalt	68.30±2.68	69.30±2.55	0.3914
Hb (g/dl)	7.41±0.22	13.20±0.25	0.0001
PCV (%)	22.60±0.69	40.40±0.78	0.0001
MCH (pg)	24.60±0.75	30.60±0.37	0.0001
Retics	1.46±0.09	0.81±0.04	0.0001

Values are expressed as mean ± standard deviation. ($P<0.05$) is significantly different

DISCUSSION

Sickle cell disease can affect trace element levels in the body, often leading to their deficiencies. The decreased levels of trace elements observed in sickle cell individuals may be due to the increased oxidative stress experienced by individuals with sickle cell disease (SCD), which depletes antioxidant trace elements needed to combat the damage caused by abnormal red blood cells (Obeagu, 2018). Sungu *et al*. (2018) found that the Hb-SS group had slightly lower mean levels of zinc and magnesium compared to their Hb-AA counterparts. This study supports the findings of Nnodim *et al*. (2014), which concluded that sickle cell anaemia significantly decreases trace element levels. Sickle cells are destroyed more easily, leading to a shortage of red blood cells in individuals with SCD. Additionally, sickle cell disease introduces multi-systemic disturbances and affects the bone marrow, resulting in various changes in the red blood cell line and white blood cells (Obeagu, 2018). The decreased levels of Hb, PCV, and MCH observed in patients with sickle cell disease (SCD) are consistent with the findings of a study by Boasiako *et al*. (2018), which showed that SCD patients had lower Hb and RBC levels compared to their control counterparts. This is also in line with the findings of Swem *et al*. (2018), which reported reduced levels of Hb, PCV, and lymphocytes in sickle cell patients with osteomyelitis.

CONCLUSION

The study revealed that individuals with sickle cell disease had notably lower levels of trace elements and blood indices compared to healthy controls. Patients with sickle cell disease showed significantly lower serum zinc and iron levels, while manganese levels were higher. However, cobalt levels did not show a significant difference between the two groups. In terms of blood health, sickle cell disease patients had significantly reduced levels of haemoglobin concentration, mean corpuscular haemoglobin. The study revealed that individuals with sickle cell disease had notably lower levels of trace elements and blood indices compared to healthy controls. Patients with sickle cell disease showed significantly lower serum zinc and iron levels, while manganese levels were higher. However, cobalt levels did not show a significant difference between the two groups. In terms of blood health, sickle cell disease patients had significantly reduced levels of haemoglobin concentration, mean corpuscular haemoglobin, and packed cell volume, along with a notably higher reticulocyte count, indicating ongoing haemolytic activity. These findings underscore the impact of sickle cell disease on micronutrient levels and blood health, underscoring the importance of regular monitoring and potential dietary interventions to support patient management. Haemoglobin, and packed cell volume, along with a notably higher reticulocyte count, indicating ongoing haemolytic activity. These findings underscore the impact of sickle cell disease on micronutrient levels and blood health, underscoring the importance of regular monitoring and potential dietary interventions to support patient management.

Conflict-of-Interest

Authors hereby declare that no conflict of interest exists

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