University of Zambia
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Assessment of Serum Zinc, Copper and Selenium in Non-Symptomatic Sickle-Cell Anaemia patients at the University Teaching Hospital, Lusaka, Zambia.

By

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A Research Dissertation Submitted to the University of Zambia, in Partial Fulfilment of the Requirements for Master of Science in Pathology (Haematology)

JANUARY 2017

Declaration

I, **ALFRED MACHIKO** this 08th day of January, 2017, declare that this dissertation represents my own work. This work has not been done in Zambia before and neither has it been published for any qualification at the University of Zambia or any other University. Various sources to which I am indebted are clearly indicated in the text and in the references.

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<div align="center">**Certification of Approval**</div>

DISSERTATION TITLE: ASSESSMENT OF SERUM ZINC, COPPER AND SELENIUM IN NON-SYMPTOMATIC SICKLE-CELL ANAEMIA PATIENTS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

This dissertation for Alfred Machiko has been approved as partial fulfilment of the requirements for the award of the Master of Science degree in Pathology (Haematology) at the University of Zambia.

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Abstract

Background: Zinc, Copper and Selenium are important trace elements in human health and disease. They play a vital role as cofactors of enzymes such as superoxide dismutase and glutathione peroxide. These act as first line antioxidants enzymes in red blood cells and in plasma. In sickle-cell anaemia patients, the antioxidant activities of such enzymes is markedly reduced. Despite improvement in sickle-cell anaemia management, morbidity and mortality still remains significant hence the need to assess their levels. The baseline data obtained can be used for further research on the possible supplementation and nutritional options which can improve the patient's antioxidant status and disease management. The study was aimed at determining the serum levels of Zinc, Copper and Selenium in asymptomatic sickle-cell anaemia patients at the University Teaching Hospital in Lusaka, Zambia.

Methods: The study was a Case Control study. Asymptomatic participants were enrolled from the specialized Haematology and Oncology Clinic 4 at the University Teaching Hospital, Lusaka, Zambia. 4 mls of whole blood was collected from 46 sickle-cell anaemia patients and 46 Controls who did not have any major medical condition from Out-Patient Department after consent. Using Atomic Absorption Spectrometry, (ContraAA700® ANELYTIK JENA, Germany) serum levels of Zinc, Copper and Selenium were assayed and determined. STATA version 11.0 was used for data analysis.

Results: The median serum levels of Zinc in patients were lower [85.64±20.46mg/L vs 104.39±43.23mg/L; p<0.028] compared to controls. Copper levels were [150.26±54.82mg/L vs 129.49±54.16mg/L; p<0.191] in patients compared to the controls. Selenium levels were [0.082±0.041mg/L vs 0.083±0.032mg/L; p<0.380] in patients compared to the controls. There was no association between the frequency crises per last one year to the serum levels of Zinc, Copper and Selenium.

Conclusion: Findings show that Zinc, is markedly reduced in sickle-cell anaemia patients compared to apparently health normal individuals. There was no direct association between the sickling crises frequency and levels of Zinc, Copper and Selenium.

Keywords: Sickle-cell anaemia, Zinc, Copper, Selenium, Antioxidants.

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Dedication

This work is dedicated to my late mother Virginia Zulu MHSRIP, my son Farai Machiko and my wife Nsama Mwango Machiko.

Acknowledgements

I would like to acknowledge the following for the role they played in my work:

God Almighty for making all this possible,

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LIST OF ABBREVIATIONS

SCA ………………………………… Sickle-Cell Anaemia

BMT ………………………………….. Bone Marrow Transplant

CBC …………………………………. Complete Blood Count

Hb ………………………………….. Haemoglobin

ROS ………………………………….. Reactive Oxygen Species

RBC ………………………………….. Red Blood Cells

EDTA ………………………………….. Ethylene Diamine Tetra-Acetic Acid

H_2O_2 ………………………………… Hydrogen Peroxide

O_2^- ………………………………… Oxygen Radical

MMP ………………………………… Matrix Metalloproteinase

HCT ………………………………… Haematocrit Concentration

NF-κβ ………………………………… Nuclear Factor Beta

MCHC………………………………… Mean Corpuscular Haemoglobin Concentration

MCV ………………………………… Mean Corpuscular Volume

UTH ………………………………… University Teaching Hospital

SOP ………………………………… Standard Operating Procedures

SLE ………………………………… Systemic Lupus Erythematosus

UNZA ………………………………… University of Zambia

AAS   ……………………………………   Atomic Absorption Spectrometry

FDCL ……………………………………   Food and Drugs Control Laboratory

ICP   ……………………………………   Inductively Coupled Plasma

OES   ……………………………………   Optical Emission Spectrometry

GFAAS…………………………………..   Graphite Furnace Atomic Absorption Spectrometry

FAAS …………………………………..   Flame Atomic Absorption Spectrometry

Chapter 1.0 Introduction

1.1 Background

Zinc, Copper and Selenium are among the trace minerals important in human health and diseases (Engle and Sellins, 1999). Essential elements (micronutrients) are cardinal in various biological processes such as immune system, haemopoiesis, wound healing, gene expression for Matrix-Metalloproteinase, balancing hormones and secretion among others. They are also important in haemostatic regulation involving processes of absorption, storage and excretion (Prashanth et al., 2015). They act as Cofactors to enzymes which in turn enhances the enzymes activity and function.

Sickle-cell anaemia is the most common inherited monogenic disorder in Arabian Gulf countries, India, the Caribbean and Africa (Elamin, 2007). In Africa, prevalence of the sickle-cell anaemia trait is around 5% to 30% (WHO, 2006). In Zambia, the prevalence of sickle-cell anaemia trait is 6% to 17.5% (Mbinga, 2009). Sickle-cell anaemia involves the inheritance of a mutated β-globin chain at position 6 where Valine is substituted for Glutamic acid there by changing the biochemical properties of the beta chain with devastating consequences (Queiroz and Lima, 2013). Sickle-cell disease (SCD) has six major genotypes depending on the combination of the sickle gene in which haemoglobin S (HbS) makes up part of the haemoglobin present (Elamin, 2007). In homozygotic patients (HbSS), sickle-cell anaemia (SCA) only HbS is present. Another type is HbS- beta – thalassemia which is a severe form of disease almost indistinguishable from SCA phenotypically. This type of sickle-cell with a combination of S and C haemoglobin (HbSC) disease is a condition with intermediate clinical severity. HbS/hereditary sickle-cell has a persistence of foetal haemoglobin and a mild form of sickle-cell anaemia. Sickle-cell with HbS/HbE syndrome is a rare condition with generally mild clinical course. Lastly there are rare combinations of HbS with HbD and HbO Arab among other forms of sickle-cell diseases. The severity of the disease depends on the percentage content of the haemoglobin S (El-hazmi et al., 2011);(Grosse et al., 2011).

Sickle-cell anaemia (SCA) is a heterogeneous group of genetic inherited haemoglobinopathies manifesting as a myriad of clinical complications and currently has no cure widely and readily available (Samir, 2005). Despite having a number of pharmacotherapeutic agents, care of sickle-cell anaemia patients has largely remained supportive. Hydroxycarbamide (hydroxyurea) is one of the disease modifying agents that increase foetal haemoglobin thereby lessening the severity of the disease (Neville and Panepinto, 2011). Analgesics such as Paracetamol, Ibuprofen, Codeine and

Morphine together with antibiotics such as Phenoxymethylpenicillin, Cefotaxime with vaccines such as the Polyvalent Pneumococcal Vaccine are used in patient management (Neville and Panepinto, 2011). Recently, further research in the management of sickle-cell anaemia has led to the developments of Bone Marrow Transplantation (BMT) (also known as stem-cell transplantation) though it poses immunological risks due to repeated bone marrow transfusions and Gene Therapy in which certain cases have been cured. Despite the positive developments, these procedures are restrictive in terms of applications and coverage, and has largely remained in developed countries and are at an experimental research stage (Oni et al, 2012).

This biochemical feature of the β-chain from acidic to hydrophobic characteristics results in a number of clinical features. These include Vaso-occlusive painful events, Acute Chest Syndrome, Bacteraemia/Sepsis, Pulmonary Hypertension, Central Nervous System Disease, Priapism, Renal effects, Avascular Necrosis, Leg Ulcers and Stroke (Neville and Panepinto, 2011); (Olujohungbe and Burnett, 2013). Pathogenesis and pathophysiology of these clinical symptoms has been linked to an altered reduction-oxidation (redox) environment in various literatures where patients with sickle-cell anaemia have increased Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Khanna et al., 2010). In sickle-cell anaemia erythrocytes produce twice as much superoxide, hydrogen peroxide and hydroxyl radicals compared to normal healthy individuals (Titus et al., 2004) which are all known to cause increased damage to Lipids, Proteins and Nucleic acids. The Lipids, Proteins and Nucleic acids make up many membranes and structural components of the cell thus damage to any of them results in cell injury.

Zinc

Zinc is a coenzyme in over 200 matrix-metalloproteinase (MMPs) and important in immune function, wound healing, metabolic homeostasis and antioxidant activity (Rashidi et al., 2011). It is a key constituent or Cofactor in over 300 mammalian proteins (Ho, 2004). Zinc functions as an essential component of the Cu/Zn Superoxide Dismutase (Cu/ZnSOD) of which is the first line of defence against reactive oxygen species which are abundant in sickle-cell anaemia patients (Queiroz and Lima, 2013). It achieves this by removing the Superoxide anion (O_2^{-}) converting it to Hydrogen Peroxide (Ho, 2004). It is able to antagonize with the redox-active transition metals such as Copper or Iron preventing oxidation of sulfhydryl groups. This results in the protection of sulfhydryl containing proteins such Dehydroorotase, Tubulin, Zinc fingers, Alanyl tRNA synthase

and Farnesyltransferase. Zinc can directly bind to MTF-1 (Metal transition factor 1) which binds to the metal-response elements to induce gene expression of proteins such as Metallothionein which has antioxidant activity all helping to mop-up excess reactive oxygen species in sickle-cell anaemia (Ho, 2004). Zinc is needed in the normal development and function of cell mediated, innate immunity, Neutrophils and Natural Killer cells (Osredkar and Sustar, 2011). Zinc affects the secretion and functions of cytokines, the basic messenger of the immune system and this is seen in sickle-cell anaemia as chronic infections due to a depressed immunity (Prasad, 2009). Deficiency in Zinc leads to atrophy of the thymus and lymphoid tissue in experimental animals (Prasad, 2009). This affects the growth, maturation and function of the B and T lymphocytes further potentiating the immune system suppression.

Copper

Copper is critical to normal physiology and is second only to Zinc in terms of the number of enzymes that require this metal for appropriate functions (Engle and Sellins, 1999). It is important for connective tissue development through collagen cross linking and important in wound healing (Engle and Sellins, 1999). It is also an essential component of the Cytochrome Oxidase System and is thus critical in the Electron Transport Chain, making it essential to cellular respiration and converting oxygen to water. This process leads to production of an unpaired electrons which can be a source of oxidation and reduction to protein molecules. This may result in as increased oxidative stress observed in sickle-cell disease. Copper is a component of Ceruloplasmin which is important in haemoglobin synthesis were Iron is converted to ferric form and incorporated in the haemoglobin molecule (Rajeswari and Swaminathan, 2014). Therefore anaemia is associated with deficient Copper levels. Copper/Zinc Superoxide Dismutase is a strong antioxidant which helps in scavenging of reactive products of Oxygen and Nitrogen (Mahabir et al., 2006). Copper stimulates the production of Thyroxine (T_4) and prevents over absorption of T_4 in the blood cells by controlling the body calcium levels. This is very important in sickle-cell anaemia where red blood cells sickle at reduced oxygen tension (Rajeswari and Swaminathan, 2014). Calcium is required for the stabilization of the cell membranes and reduces cell permeability. This can reduce Chronic Haemolytic Anaemia seen in sickle-cell patients whose red blood cells are highly fragile. Copper deficiency inhibits differentiation and self-renewal of CD34[(+)] hematopoietic progenitor cells and this connects Copper to anaemia seen in certain patients (Rajeswari and Swaminathan, 2014).

Selenium

Selenium is another micronutrient with various roles in the body. It is important in fat metabolism and helps in Vitamin E and Zinc functions which all are very strong antioxidants. It is a component of various enzymes most notably Glutathione peroxidase (Klotz et al., 2003). This enzymes helps inactivate oxygen radicals such as Hydrogen Peroxide (H_2O_2) and thus prevents it from causing cellular damage especially to fragile red blood cells containing haemoglobin S (Engle and Sellins, 1999). Selenium plays a critical role in the functioning of the thyroid gland and in every cell that uses thyroid hormone. By participating as a cofactor in the three of the four known types of thyroid hormone deiodinases, it activates and deactivates various thyroid hormones and metabolites (Venturi and Venturi, 2007). These thyroid hormone deiodinases are iodothyroinine 5' – deiodinases type I, II, III and are involved in thyroid metabolism (conversion of T_4 to T_3). Other molecules such as Thioredoxin Reductase, Selenoprotein P (Selenium transporter), and Selenoprotein W serve as important antioxidants (Turanov et al., 2015).

With these critical functions, these essential elements are critical to the normal physiology. Deficiency states, in sickle-cell anaemia can be associated with various clinical manifestations earlier alluded to. In this present study the serum levels of Zinc, Copper and Selenium was measured to assess the serum levels in these patients using a sensitive and reliable method, Atomic Absorption Spectrometry. Furthermore the study will provide baseline information to be used for further research such as possibility of supplementation in sickle-cell anaemia patients and as documented evidence of the essential trace elements levels in the Zambian sickle-cell patients.

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1.2 Statement of the problem

With treatment of sickle-cell anaemia mostly supportive, most of the clinical symptoms result in hospitalization of most of the patients. Despite the improvements in management of sickle-cell anaemia, morbidity and mortality has still remained significantly high. Sickle-cell anaemia contributes 5% of under-five deaths on the African continent, more than 9% of such deaths occur in West Africa and up to 16% of under-five deaths occur in individual West African countries (WHO, 2006). In Sub-Saharan Africa, Sickle-cell anaemia has a high child mortality rate of 50% – 90% though there is lack of reliable consolidated and published data (Grosse et al., 2011). At the University teaching Hospital, overall mortality rate is 3.8% with 66.6% of the deaths occurring in paediatric patients (HIS,UTH unpublished data 2015).

With sickle-cell anaemia patients having an increased oxidative stress due to increased and abnormal amounts of reactive oxygen species, it is important to assess the levels of the trace elements in these patients. Few studies have looked at these trace elements in relation to oxidative stress and the potential link to sickling crises in sickle-cell anaemia patients. It is thus very important that this study be carried out to provide this knowledge especially in this country, Zambia.

1.3 Study justification

Effective management of sickle-cell anaemia is important through the understanding of the pathophysiology of the disease. Increased oxidative stress due to excess oxygen reactive species such as Hydrogen Peroxide and oxygen radicals result in a disturbed reduction - oxidation environment in sickle-cell anaemia patients. The internal environment is in a pro-inflammatory state and has increased oxidative stress due to increased reactive oxygen species and reactive nitrogen species, neutrophil activation with endothelial dysfunction and reduced antioxidant activities (Klings and Farber, 2001; Arruda et al., 2013).

Zinc, Copper and Selenium together with other vitamins such as A, C ,E, Carotenoids, Flavonoids and enzymes such Glutathione Peroxidase have been shown to have antioxidant activity and reducing oxidative stress in Blood and Red Blood Cells coupled with many physiological functions (Prashanth et al., 2015);(Hamid et al.. 2010); (Khanna et al., 2010);(Roos et al., 2015). It is crucial that the frequency of the clinical manifestations be reduced thereby decreasing frequent hospitalization and morbidity. Therefore, sickle-cell anaemia patients do not have the normal haemostatic functions making it imperative to probe and investigate if there is a statically significant difference in healthy individuals and Sickle-cell anaemia patients. From the study baseline information was collected and shed more insights and knowledge in trace element levels in sickle-cell anaemia patients at the University Teaching Hospital.

1.4 Literature Review

1.4.1 Sickle-Cell Anaemia

The genetic changes occurring in sickle-cell anaemia include red blood cell sickling presence of stressors e.g. dehydration, infections, fever and hypoxemia, generation of increased amounts of oxygen radicals and a decrease in the rate of physical development (Okochi and Okpuzor, 2011). A mutation on the 6th codon of the β-gene produces a defective haemoglobin containing the HbS. This haemoglobin polymerizes under hypoxic condition and ultimately results in the anaemia due to RBC destruction (Ray and Mondal, 2014). In sickle-cell anaemia (SCA) (HbSS), clinical signs and symptoms are severe as there is almost complete absence of HbA that is the normal beta globin chains. Patients with sickle-cell anaemia have complications like enlarged spleen in children, infections, painful episode and vaso occlusive crises. Inflammatory mediator activation, chronic haemolytic anaemia, increased oxidative stress and endothelial dysfunction are reduced by increased concentration of foetal haemoglobin (HbF)(Bhagat and Kumar, 2012).

Chronic haemolytic anaemia is associated with increased turnover of haemapoetic cells which results in cell death and red marrow expansion. This leads to a hyper metabolic rate and increased nutrient demand (Araba, 2005). A study done at Bagdad University showed wide spread evidence of apoptosis using Annexin V-Fluorescein Isothiocyanate (FITC) method in 18 sickle-cell anaemia patients compared to 10 healthy controls. This proved a theory of increased red blood cell destruction which ultimately leads to chronic haemolytic anaemia (Saud, 2013).

1.4.2 Pathophysiology and Reactive Oxygen Species generation in Sickle Cell Anaemia

Sickle-cell anaemia is characterized by heterogeneous clinical complications. Precipitating factors include upper respiratory tract infection, fever, dehydration, and hypoxemia, exposure to cold temperatures, weather changes and emotional stress. Mechanical or traumatic events result in an interruption of blood flow to tissue and can also result in ischemia and subsequent severe pain (Elamin, 2007).

A pro-inflammatory state and endothelial activation results in an elevated White Blood Cell count and this is associated with early death in sickle-cell anaemia. Because neutrophils are larger and more difficult to deform than red blood cells, attachment to endothelium particularly in the microcirculation would impede passage of RBC and WBC increasing the risk of vaso occlusive

crises (Roos et al., 2015). In a study done in USA by Klings and Farber, facts showed that red blood cells in what is known as "auto oxidation" produce increased levels of reactive oxides especially reactive oxygen radicals (O_2^-). This mechanism involves oxygen radical formation through deoxygenation of haemoglobin through the electron transfer between Iron (Fe) and Oxygen O_2 (Fenton reaction) leading to the production of Oxygen radicals (O_2^-) and Methemoglobin. This occurs physiologically to a small extent but in HbS auto oxidation occurs at 1.7 times the rate of haemoglobin A (Klings and Farber, 2001); (Queiroz and Lima, 2013). Decrease in the antioxidant defences in sickle-cell anaemia patients are accompanied by activation of enzymatic NADPH Oxidase, Xanthine Oxidase and other molecules which are sources of Oxygen radicals resulting in oxidative stress. This leads to dysfunction/activation of arteriolar and venular endothelial cells, resulting in impaired vasomotor function and blood-endothelial cell activation (Wood and Granger, 2007).

In a study done at University Teaching Hospital, 89% of patients had a high White Blood Cell Count (WBC) from a population of 55 sickle-cell anaemia patients. This finding confirms that an elevated WBC is a feature of sickle-cell anaemia as part of the pathophysiology in the disease (Mbinga, 2009). A review article by Nur et al showed the increased levels of Reactive Oxygen Species such as Hydrogen Peroxide (H_2O_2), Superoxide (O_2), and Hydroxyl radicals (OH) in sickle-cell disease patients. Deficient and dysfunctional defence mechanism of enzymes (Superoxide Dismutase, Catalase, Glutathione Peroxidase and many other enzymes) are associated with the disease features. The trace elements under study are part of the enzymatic defence system thus the association with their concentration is critical for the signs and symptoms observed (Nur et al., 2011).

A short communication by Nnodim showed that in 100 HbSS steady state patients aged 5-30 years, the level of antioxidant Vitamin C and E were significantly reduced. This shows that the severe clinical symptoms in the sickle-cell anaemia patients (HbSS) are due to the severe depletion of Vitamin C and E in these patients (Nnodim et al., 2014). In another study done in the USA, in sickle-cell anaemia patients, Total Glutathione (and its precursors) and Glutamine were assayed in plasma and erythrocytes of 40 sickle-cell anaemia patients and 9 healthy volunteers. Results showed that erythrocyte Glutathione and Glutamine were significantly lower in sickle-cell anaemia patients than in volunteers (Morris et al., 2008).

1.4.3 Zinc, Copper and Selenium

The role of essential trace elements in health and in disease remains key and cannot be over emphasized. As part of many antioxidant cofactor, these trace elements play a crucial role in abating severe clinical course of the disease and many other conditions. In a cohort of sex and age matched study of 1676 lung cancers and 1676 healthy controls, study showed that Zinc, Copper and Selenium had a protective effect ($p < 0.05$) (Mahabir et al, 2006).

As part of Glutathione Peroxidase, Cu/Zn Superoxide Dismutase, Ceruloplasmin, Cytochrome c Oxidase and many other enzymes and non-enzymes molecules, these trace elements are at the centre of controlling oxidative stress in blood and cell systems. In a cross section of 130 healthy pregnant women in different trimesters and 30 non-pregnant controls, copper levels showed an increase ($p < 0.018$) while Selenium levels decreased ($p < 0.0001$) (Nwagha et al., 2011). In a study in Sudan, homozygous sickle-cell anaemia patients had an assessment of renal functions, Copper and Selenium levels. With 70 sickle-cell anaemia patients and 30 healthy controls Copper mean levels where significantly increased to the mean level of controls (p value 0.00) but serum Selenium levels were significantly decreased (p value 0.00) (Khalid and Idris, 2015).

In another cohort in Nigeria, the levels of antioxidant enzymes in adult sickle-cell anaemia patients was evaluated. These included Glutathione Peroxidase, Superoxide Dismutase, Catalase and other molecules which are indicative of inflammation such C-reactive protein and Fibrinogen in 144 patients (68 males and 76 females) in steady state and 80 apparently healthy age/sex matched controls. Results showed that serum Glutathione Peroxidase, Superoxide Dismutase and Catalase were significantly lower in sickle-cell anaemia patients compared to controls. C-reactive protein and Fibrinogen were significantly increased in sickle-cell anaemia patients compared to controls in both sexes. A decrease in antioxidant enzymes shows that the antioxidant defence mechanism is disturbed and these trace elements are components of these enzymes. C-reactive protein and fibrinogen levels are an indication of the underlying inflammatory environment in sickle-cell anaemia patients (Emokpae et al., 2010).

In another study on evaluation of antioxidant levels and trace elements status in sickle-cell anaemia patients with plasmodium parasitaemia. Trace elements were significantly reduced in sickle-cell anaemia patients including Zinc, Copper and Selenium compared to controls (Arinola et al., 2008).

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From all these studies, a pattern has emerged which under pins the vital and critical role of Zinc, Copper and Selenium in sickle-cell anaemia and many other diseases which involves a disturbed redox environment and increased oxidative stress thus makes it imperative to measure and think of a possibility of supplementation.

1.4.4 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry is currently the most widely used method for elemental analysis especially for Zinc, Copper and Selenium (Al-assaf, 2010). For Selenium there is a slight modification with the flame production, instead Graphite Furnace (Nitric oxide) Atomic Absorption Spectrometry (GFAAS) is mostly used. Atomic absorption spectrometry is mostly suited for routine measurements although superior techniques such as ICP-MS and ICP/OES are much faster and with a high throughput, they are still expensive and highly complicated thus are mostly used in research institutions. AAS continues to be widely used for routine measurements as it is cost effective offering good result specificity and sensitivity thus a good choice for use in this present study (Arnaud et al., 2008).

1.5 Research Question

What are the serum levels of Zinc, Copper and Selenium in Sickle-Cell Anaemia patients at the University Teaching Hospital?

Chapter 2.0 OBJECTIVES

2.1 General Objective

To determine the serum levels of Zinc, Copper and Selenium in Sickle -Cell Anaemia patients at the University Teaching Hospital.

2.2 Specific Objectives

1. To determine the median levels of Zinc, Copper and Selenium in Sickle-Cell Anaemia patients and in controls.
2. To find an association between the levels of Zinc, Copper and Selenium and the frequency of previous sickling crises for past one year.

Chapter 3: Materials and Methods

3.1 Study Design

This study was a Case Control Study conducted from April to July, 2016.

3.2 Study Site

The study was conducted at a referral hospital, University Teaching Hospital, Lusaka, Zambia. Haematology and Oncology Clinic 4, is a specialist clinic consisting of sickle-cell anaemia patients that are seen routinely and some who are have been referred to University Teaching Hospital for further management from all patients over the country. Controls were recruited from Out-Patient Department within the hospital.

3.3 Target Population

The target population consisted of sickle-cell anaemia patients (HbSS) in steady states whose routine specimens were used and controls were those patients without sickle-cell anaemia with minor medical condition from the same population being seen at University Teaching Hospital. All age groups were selected so that the results should be applicable to almost all sickle cell anaemia patients unlike just to a narrow age group of patients.

3.4 Study Population

Individuals with sickle-cell anaemia were sequentially enrolled into the study. A study control group of consisted of that did not have clinically or laboratory diagnosed sickle-cell anaemia, any inflammatory condition and non-pregnant, were recruited. These individuals came to the University Teaching Hospital for any medical services at any Out-Patient Department in the hospital. The study sample and the control group were matched for age and sex in order to minimise bias.

3.4.1 Inclusion Criteria - Patients

➢ Individuals with sickle-cell anaemia of all ages confirmed by electrophoresis

➢ Male and female

➢ Individuals giving personal consent without undue duress

➢ Assent from children were gotten where applicable and if they are very young assent was obtained from parents or guardians

3.4.2 Exclusion Criteria - Patients

➢ Individuals with sickle-cell anaemia on Zinc, Copper and Selenium supplementation
➢ Patients who had received a blood transfusion in the last 2 months
➢ Individuals who had undergone any major surgery within the past three month.
➢ Patients with serious inflammatory conditions such as SLE
➢ Patients in active sickling crises

3.4.3 Inclusion Criteria - Controls

➢ Individuals giving personal consent without undue duress

➢ Assent from children were gotten where applicable and if they were very young assent was obtained from parents or guardians

➢ Patients who report to UTH and do not have clinically or laboratory diagnosed sickle cell anaemia
➢ Patients without any oedema and non-pregnant

3.4.4 Exclusion Criteria - Controls

➢ Individuals with sickle-cell anaemia
➢ Patients who had received a blood transfusion in the last 2 months.
➢ Individuals who have undergone any major surgery within the past three month
➢ Non-consenting patients/individuals
➢ Patients with serious inflammatory conditions such as SLE

3.5 Sampling and Sample Size

3.5.1 Sampling Method

Convenience sampling of routine blood samples of patients and controls who met the inclusion criteria were used.

3.5.2 Sample Size

A total sample size of **92** participants (**46 patients and 46 controls**) was calculated using the formula for determination of sample size for comparative research studies between two groups as given below;

$$N = \frac{4\sigma^2(z_{\text{crit}} + z_{\text{pwr}})^2}{D^2},$$

$$N = \frac{4 \text{ x } 1^2 \text{ x } (1.960 + 0.842)^2}{(0.58)^2}$$

N = **93.35557669**

N = **92** participants for the two equal groups, patients and controls.

Where; N is the total sample size (the sum of the sizes of both comparison groups), σ is 1; the assumed SD of each group (assumed to be equal for both groups), the z_{crit} value is 1.960 as given in tables for Standard Normal Deviation (z_{crit}) corresponding to the desired significance criterion of 0.05 or 95% confidence interval (CI), the z_{pwr} value is 0.842 as given in Standard Normal Deviation (z_{pwr}) tables corresponding to 80% statistical power, and D is the minimum expected difference between the two means which has been estimated at 0.58mg /L (in reference to copper). Three papers where looked at were the average mean difference of copper was calculated at 0.58 and this was the estimate used as D which is the minimum difference between the two means. Both z_{crit} and z_{pwr} are cut-off points along the x axis of a standard normal probability distribution that demarcate probabilities matching the specified significance criterion and statistical power, respectively. The two groups that make up N are assumed to be equal in number, also that the outcome variable of a comparative study is a continuous value for which means are compared, and a two-tailed statistical analysis was used (Eng, 2003).

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3.6 Data Collection

3.6.1 Clinical and Demographical data collection

Participants (patients) were recruited when they come to the Haematology and Oncology Clinic 4 for their routine visit to the doctors. As the participants were being seen by the clinician they were been informed and enlightened about the study by the clinician who also provided the participants with the study information sheet. If a patient autonomously agreed to be part of the study, they were required to sign the consent form, and assigned a serial number. Thereafter, information on the patient's demographic and clinical data was collected and included information such age, sex, number of crises in the past year and last time the patient received blood transfusion.

3.6.2 Specimen Collection, Storage and Preparation

3.6.2.1 Specimen Collection

Blood samples was collected from 46 sickle-cell anaemia patients and 46 healthy individuals (control group). Blood samples (4 ml) was drawn into a tube from each participant. Blood samples was collected from research participants via venepuncture from an antecubital vein or other visible veins in the forearm using the Evacuated Tube System (ETS). Blood was collected into a serum tube (plain container) for element analysis. Specimens were collected according to the Clinical Laboratory Standards Institute (CLSI) procedures for collection of diagnostic blood specimens by venepuncture (Dennis et al., 2007).

3.6.2.2 Specimen Preparation and Storage

3.6.2.2.1 Preparation and Storage of serum specimens

The blood in a serum container was left for a minimum of 15 minutes to allow blood to clot and then centrifuged at 4000 rpm for 3 minutes. Serum specimen was transferred into vials and kept at -20 degrees Celsius until analysis.

3.7 Quality Control

To ensure reliable results, quality control was performed on all the reagents, procedures and analytical instruments and analysers to be used for specimen analysis according to the established quality control guidelines. Quality control included equipment calibrations and analytical control runs on all analysers before each test analysis.

3.8 Specimen Analysis/Test Protocol

Serum Zinc, Copper and Selenium was analysed using the ContraAA® 700 HR-CS-AAS from the Food and Drugs Control Laboratory, UTH, Zambia.

METHOD   :   ZINC AND COPPER ANALYSIS

Calibration

Calibration standards were prepared from Multi – Element Calibration standards traceable to NIST in intermediate ranges of:

 0 / 25/ 50/ 100/ 150/ 200/ 250 µg/L from 100ppm stock standard.
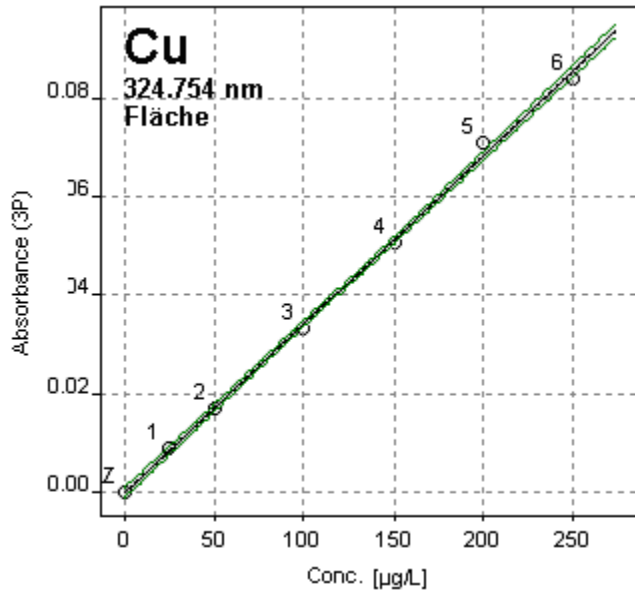
$$R^2 = 0.996$$
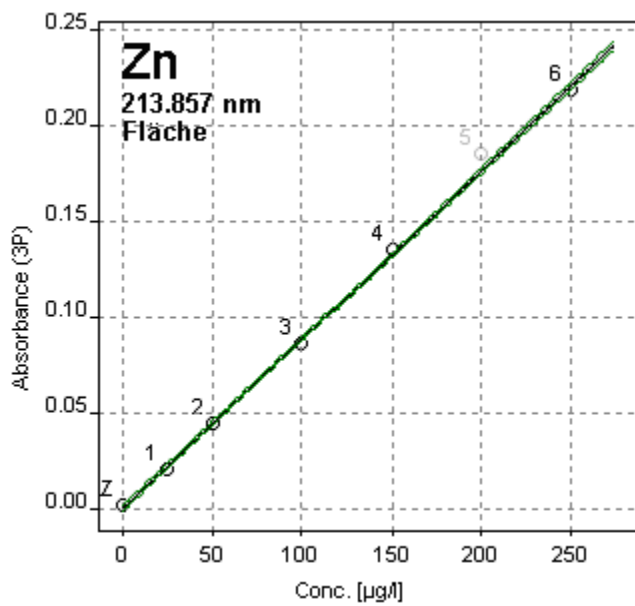
$$R^2 = 0.975$$

A correlation coefficient of 0.930 to 0.999 was acceptable.

Procedure

- For the determination of Cu and Zn the samples were centrifuged at 4000 rpm for 3 minutes to separate serum

- 0.5ml of serum was diluted to 10mls with deionised water in 50mls volumetric flasks

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- Diluted samples were treated with 1ml of 0.1 % KCl (Potassium Chloride)

The dilution factor was 20.

After the Reagents were added, the samples were ready to be analysed using flame AA under the following Parameters;

ELEMENT	WAVELENGHT	FLAME TYPE	GAS FLOW RATE L/h	BURNER HEIGHT (mm)
CU	324.7540	Air/C_2 H_2	45	9
Zn	213.8570	Air/C_2 H_2	45	8

METHOD   :   SELENIUM ANALYSIS

Calibration

Calibration standards were prepared from Multi – Element Calibration standards traceable to NIST in intermediate ranges of:

0 / 25/ 50/ 100/ 150/ 200/ 250 µg/L from 100ppm stock standard.

A linear Calibration curve was obtained with Correlation Coefficient R^2 = 0.997

Procedure

- For the determination of Se, the samples were centrifuged at 4000 rpm for 3 minutes to separate serum.
- 0.5ml of serum was diluted to 10ml with deionised water in 50mls volumetric flasks
- 0.1% Acetic acid was added to the samples to denature the proteins and 0.1% Triton X to reduce surface tension.

The dilution factor was 20.

After the Reagents were added, the samples were ready to be analysed using flame AA under the following parameters;

ELEMENT	WAVELENGHT	FLAME TYPE	GAS FLOW RATE L/h	BURNER HEIGHT (mm)
Se	196.0267	Air/$C_2 H_2$	50	9

3.9 Statistical Analysis

Data was analysed using STATA (version 11.0). The unpaired student t-test was used to compare the median values of Zinc, Copper and Selenium between the two groups (sickle-cell anaemia patients and controls). All statistical tests was performed at 5% significance level, and the difference was considered significant if two-tailed $P<0.05$. Previous sickling crises was associated to the concentrations of Zinc, Copper and Selenium using logistic regression.

3.10 Ethical Considerations and Permissions

3.10.1 Ethical Consideration

The study protocol was approved by the University of Zambia Biomedical and Research Ethics Committee (UNZABREC). And consent was granted by the University Teaching Hospital Administration. Permission to collect samples was granted by each participant/guardian.

3.10.2 Utilization of Results

The study is expected to provide baseline data on level of trace elements in Zambian patients with Sickle-cell anaemia (SCA) through measuring levels of Zinc, Copper and Selenium. If levels are indeed low, intervention measures may be considered including modified treatment approaches, supplementation and dietary modifications. Further research could be conducted on patients in a sickling crisis to determine if supplementation with Zinc, Copper and Selenium can reduce the complications and severity of sickling.

Chapter 4.0 Results

4.1 Mean Age Distribution

The figure below shows the mean age distribution of patients and controls and shows most of them to be below 20 years.
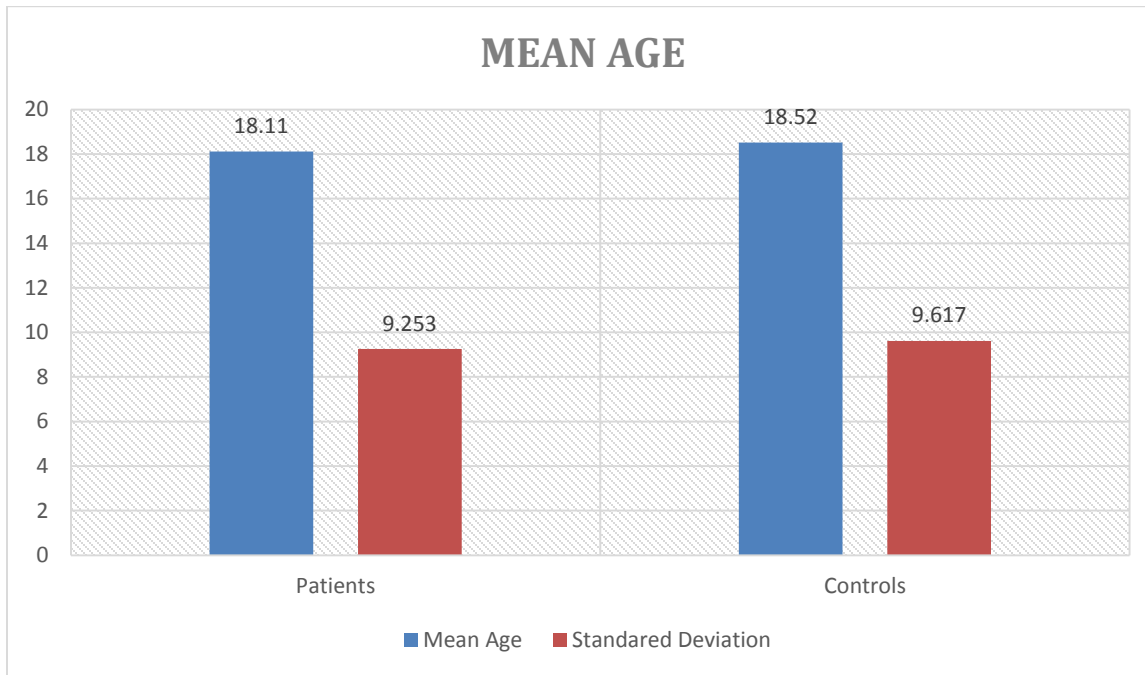
Figure 1 shows a mean age distribution**.**

4.2 Median Serum levels of Zinc and Copper

The figure 2 below shows a low median concentration of Zinc serum in patients compared to controls (P = 0.028). The median serum concentration of Copper shows no difference though it is elevated in patients compared to controls (P = 0.191).
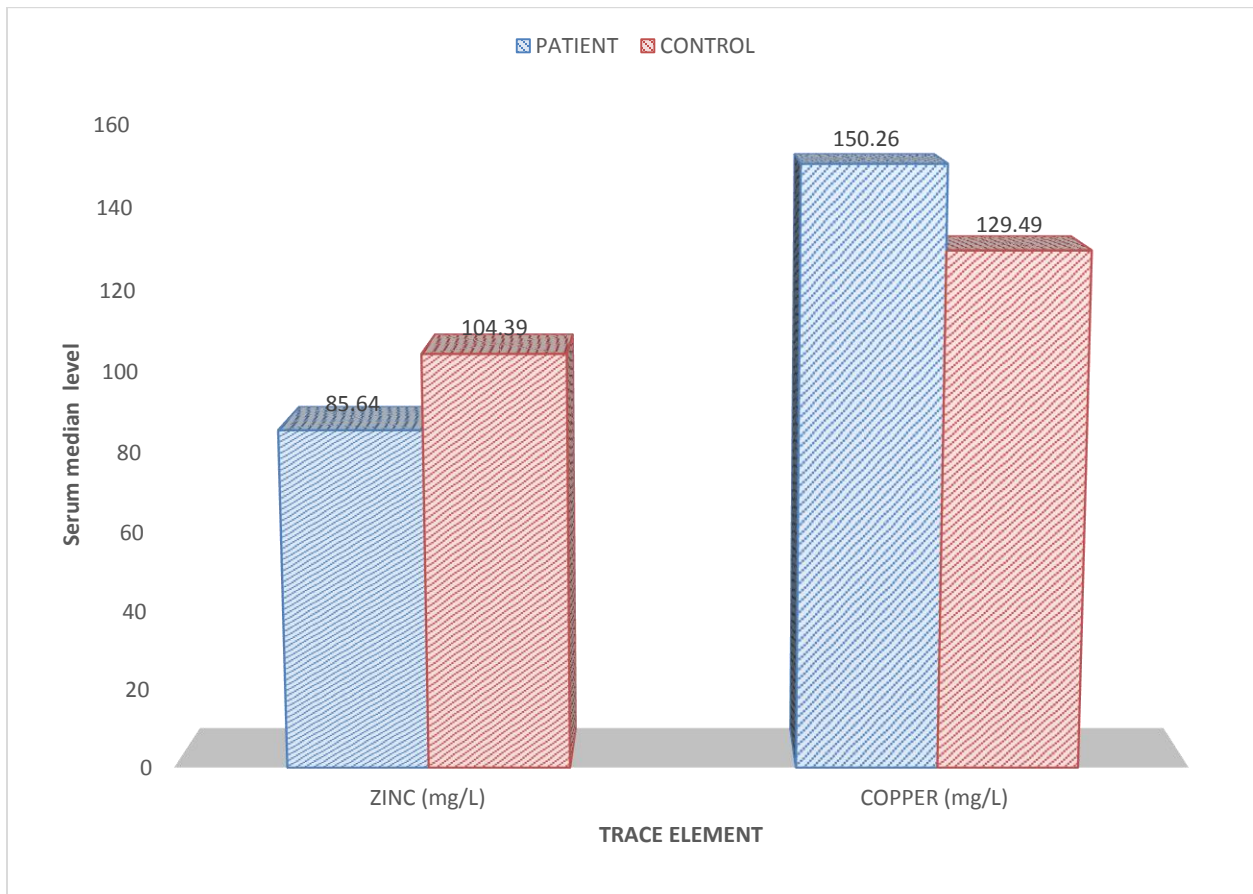
Figure 2 shows the median serum levels of Zinc and Copper in sickle-cell anaemia patients and controls.

4.3 Median Serum levels of Selenium

The figure 3 below shows no difference in median selenium levels in sickle-cell anaemia compared to health individuals although it is lower in patients compared to controls (P = 0.380).
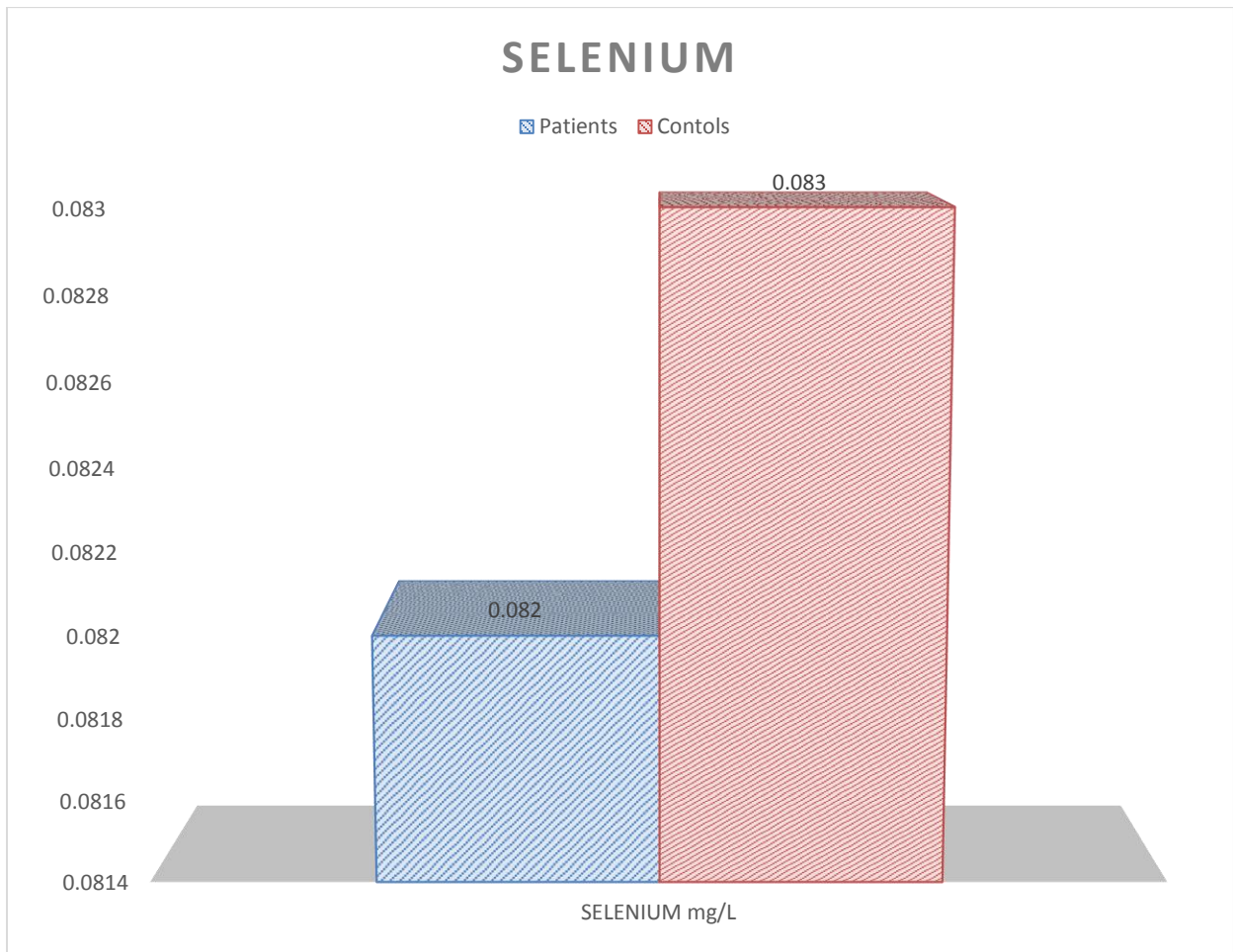
Figure 3 shows the median serum levels of Selenium in sickle-cell anaemia patients and controls.

4.4 Serum Median levels and standard deviation (median ±SD)

The table 1 below shows low serum Zinc in sickle-cell anaemia patients compared to health individuals. Similar Copper and Selenium levels are found in both patients and controls.

	Patient Group	Control Group	P-Value
Zinc (mg/L)	85.64 ±20.46	104.39 ±43.23	**0.028**
Copper (mg/L)	150.26 ±54.82	129.49 ±54.16	0.191
Selenium (mg/L)	0.082 ±0.041	0.083 ±0.032	0.380
N	46	46	

TABLE 1 shows the median serum levels of Zinc, Copper and Selenium, Standard Deviation and the P-Values.

4.5 Sickle cell crises per year

4.5.1 Table 2 shows the STATA analysis of the association between Crises/year to the levels of Zinc, Copper and Selenium**.** The P-Values shows no association.

	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf. Interval]	Crisis/year
Selenium	980.8383	8386.976	0.81	**0.420**	.0000517	1.8610
Zinc	.9875446	.0101439	-1.22	**0.222**	.9678791	1.00761
Copper	1.006763	0.0071939	0.94	**0.346**	.09927611	1.020962

Chapter 5: Discussion

Sickle-cell anaemia is a hereditary autosomal recessive haemoglobinopathy affecting both males and females. It is characterised by varying symptoms including pain syndromes, anaemia, infections and many comorbid conditions (Samir, 2005). This results in a decreased life expectancy and is seen in the mean age of our patients used in the study with an average of 18 years.

Zinc

The findings of the present study indicates a significant reduction in serum Zinc in sickle-cell anaemia patients compared to apparently healthy individuals. The study shows no association between the frequency of the sickling crises from the last one year and the serum levels of Zinc in sickle-cell anaemia patients. Zinc is part of over 300 mammalian proteins and a cofactor of over 200 metalloproteinase (Rashidi et al, 2011; Ho, 2004). This suggest that Zinc may play an important physiological role in determining the severity of the sickling crises and other clinical complications such as vaso-occlusive painful events, acute chest syndrome, bacteraemia/sepsis (Neville and Panepinto, 2011). This is because Zinc is important in the maturation and function of B and T lymphocytes and in the normal function of natural killer cells and neutrophils (Osredkar and Sustar, 2011). Sickle-cell anaemia patients despite having a normal diet have inadequate Zinc due to chronic pain, haemolysis and reduced appetite. Sickle-cell anaemia patients have increased demand and consumption for Zinc which may result in Zinc deficiency. The third reason for Zinc deficiency is increased urinary excretion due to impaired renal function and hypoxanthinuria (Arruda et al, 2012; Wood and Granger, 2007). Oxidative stress in sickle-cell anaemia patients is increased and this may be due to a decrease in Zinc which forms part of the first line of antioxidant defence enzyme such Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX) (Emokpae et al, 2010). This may suggest an imbalanced redox internal environment and although it may not trigger a sickling crises it may complicate the severity of the sickling crises. Zinc reduces inflammatory cytokine production through the Zinc-induced up-regulation of a Zinc-finger protein, A20, which inhibits NF-kB activation via TRAF pathway. This shows that Zinc can also be used an anti-inflammatory agent in sickle-cell anaemia patients whose internal environment is in a constant inflammatory state (Prasad, 2009).

Our findings in this study agrees with a study by Rashidi et al whose findings had a significant reduction in serum Zinc in sickle-cell anaemia patients and beta-thalassemia patients. Many other studies have showed a marked reduction in antioxidant vitamin A, C, E and Zinc and an elevation of Copper in sickle-cell anaemia (Hasanato, 2006; Mahdi, 2014). In a double blind, placebo controlled trial by Bao et al, results showed that Zinc as a therapeutic agent may be very useful in patients with sickle-cell disease (Bao et al, 2008). This study provides solid evidence of the therapeutic effects of Zinc in sickle-cell anaemia.

Copper

Copper serum levels were elevated in sickle-cell anaemia patients compared to apparently healthy individuals though there was no statistical difference between the two groups. A study conducted in Sudan showed an increase in Copper levels in homozygous sickle-cell patients which affirms our results (Khalid and Idris, 2015). Another study by Hasanato had similar significantly raised Copper serum levels. Copper is part of the enzyme Superoxide Dismutase (Cu/ZnSOD) and play an important role in Ceruloplasmin which helps in Iron metabolism. Though it plays many physiological roles it has been found to be significantly raised in many other conditions such as chronic obstructive pulmonary disease (COPD), malignancy and psychosis (Goyal et al, 2006). Although the mechanism is poorly understood this may be due to Copper toxicity associated with excessive accumulation in particular tissues and organs. Sickle-cell anaemia patients have impaired renal function that results in a disturbed excretion process which may lead to increased copper retention. This may further potentiate the pathophysiology process in sickle-cell anaemia patients (Voskou et al, 2015). As a part of the enzymes Cytochrome C oxidase Copper plays a role in energy production, as part of dopamine β-hydroxylase with a role in conversion of dopamine to norepinephrine, and with Factor IV helps in blood clotting. This increased nutritional demand may be another reason for increased levels of Copper in sickle-cell anaemia patients (Osredkar and Sustar, 2011). Zinc competes with Copper for binding sites on proteins thus, increased Copper levels previously observed is a result of Zinc deficiency observed in sickle-cell anaemia patients (Prasad, 2009; Prashanth et al., 2015). Copper is a pro-oxidant metal sometimes known for producing unpaired electrons and increased levels lead to increased oxidative stress and this may further increase the severity of a sickling process (Mahabir et al, 2006).

Selenium

In the present study selenium was slightly lower in sickle-cell anaemia patients compared to apparently healthy control group although there was no statistical difference between the two groups and this is in conflict with many other studies. The study shows that there is no association between the Selenium levels and the frequency of sickling crises/year. Selenium is an important microelement in the antioxidant enzymes like Glutathione Peroxidase (Zhao et al., 2013). This enzyme is cardinal as it helps reduce the increased oxidative stress observed in sickle-cell anaemia patients due to excessive oxygen radicals. A study done showed a statistically significantly reduction in the serum levels of Selenium in sickle-cell anaemia patients compared to controls while another study done in India showed significant reduction of Selenium in oesophageal carcinoma (Goyal et al., 2006; Khalid and Idris, 2015). This disparity can be due to the soil and diet content of Selenium as well as the number of the patients and participants involved in our study.

Precipitating factors of sickling crises include upper respiratory tract infections, fever, dehydration, and hypoxemia, exposure to cold temperatures, weather changes and emotional stress. Mechanical or traumatic events result in an interruption of blood flow to tissue and can also result in ischemia and subsequent severe pain (Elamin, 2007). Although Zinc, Copper and Selenium might not directly be linked to the precipitating factors of sickling crises, it clear that these micro elements play a vital indirect role which may help ameliorate the sickling episodes and improve the morbidity of the sickle-cell anaemia patients.

Our findings from this study shows no association between the levels of Zinc, Copper and Selenium and the sickling crises previously experienced by the patients. This can be due to the fact the patients were in steady state and not in active sickling were the concentration of Zinc, Copper and Selenium would have been extremely much lower. The lack of association may be also due to the fact that Zinc, Copper and Selenium are trace elements which occur in minuet concentrations affecting the functions of macromolecules such as enzymes. It therefore may not precipitate a sickling crises but may affect the severity outcome of a sickling crises. It can also be due to a relatively smaller study population.

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Chapter 6: Conclusion and Recommendations

6.1. Conclusion and Recommendations

Findings show that Zinc, is markedly reduced in sickle-cell anaemia patients compared to health normal individuals. There was no direct association between the sickling crises frequency and levels of Zinc, Copper and Selenium. Further studies on Zinc supplementation are recommended in sickle-cell anaemia patients.

6.2 Strengths and weaknesses

One of the strengths of this study is its novelty; no work has been done and published on the levels of trace elements Zinc, Copper and Selenium on sickle-cell anaemia patients in Zambia. This study is a baseline study and can be used for policy change towards sickle-cell anaemia patient management such as supplementation and dietary modifications.

The weaknesses of the study are the use of sickle-cell anaemia patients who are in steady state and not those in active sickle-cell crises, not measuring the antioxidant enzymes such as vitamin A, C and E due to the cost of HPLC, this would have given a comprehensive antioxidant status in sickle cell anaemia patients in Zambia. The other is that the control group used were apparently healthy. They had minor medical conditions that mighty have affected the overall outcome of the research findings.

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APPENDIX A: **Information sheet: participant – English form**

This form gives you information on the study in which you are being requested to participate in. To make sure that you have all the facts about this study you must read this form or have someone read it for you. If you agree to participate in this study, you must sign the consent form or put your thumbprint in the space provided, if you cannot write. If you feel that you cannot take part in this study, you are free not to participate in it and your refusal will not in any way jeopardize the care you will receive from the health providers.

PURPOSE OF THE RESEARCH

My name is Alfred Machiko studying for a Master of Science degree (MSc.) in Pathology (Haematology) at the University of Zambia- Ridgeway Campus. I am carrying out a research as a requirement for fulfilment of Master of Science (MSc) degree in Pathology (Haematology). You have been invited to participate in this study which seeks to determine the serum levels of Zinc, Copper and Selenium in blood. You have been requested to participate in this study because one of the following reasons;

(a) You have been diagnosed/already are diagnosed with sickle cell anaemia and also based on the clinician's assessment with regard to the study inclusion criteria.

(b) You as a control have been invited to participate in this study not because you have sickle cell anaemia but you match in age and sex with the patients and from your specimen we can draw valuable comparisons in the levels of these trace elements which can help improve clinical management of the sickle cell anaemia patients.

If you decide to take part in the study, you will be requested to give 8mL (1 EDTA container and 1 Plain container) of blood as part of your specimen. The study requires at least 92 people to participate.

RISKS, DISCOMFORTS AND BENEFITS OF THIS STUDY

There are no risks associated with being a participant in this study. However, you will experience some pain when blood is being drawn from the vein. Every effort will be made to reduce the pain that you feel as the blood is being collected. There are no costs to you for being in this study and

the study may not benefit you directly. However, the study results will provide valuable information which can be used to improve the health of sickle cell anaemia patients in the future.

CONFIDENTIALITY

The information you will give in this study will remain confidential and will not be made available to anyone who is not connected with the study. Furthermore, your name will not be written on any data collection tool for confidentiality purposes.

Please if you have any queries do not hesitate to contact the following:

1. **Machiko Alfred**
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4. The Chairperson

University of Zambia Biomedical Research Ethics Committee (UNZABREC):

Contact Number: 260-1-256067

Email Address: unzarec@zamtel.zm

NOTE: THE ABOVE SECTION SHOULD BE GIVEN TO THE PARTICIPANT

APPENDIX B: papala ya mau a ofunikakufunsiwa – Nyanja/chewa

Iyi ni papala yama phunziro yamene nifuna kukufunsani, ndipo nikupe mphani kuti mukale amodzi ofunina kuti muavela za mpuazilo iyi, mufunika kubelenga olo munthu akubelengelani pepala iyi. Kapene mubvomela kukala amodzi ofuasi wa muphunzilo iyi, mufunika ku saina fomu yobvomekeza kuti mwabvomela kufunsiwa olo kufwatika chikumo. Kapena simufuna kukhala amodzi ofuasiwa, muli na mphanvu yosavomera, ndipo osaganiza kuti simazayamba kulandira thandizo ihyonse kuno ku chipatala chifukwa mwakana.

CHIFUKWA CHAMAFUNSO AWA

Zina langa ndine Alfred Machiko mwama wasukulu mumaphunziro apamwamba ochedwa Masters of Science in Pathology zokhuzana nankhani yaku laboratory. Nichitila sukulu iyi pa university of Zambia – Ridgeway campus. Mwaitaaidwa kumafunso akufufuza ndikuona ma mpimowa zinc, copper na selenium mu ma gazi.

Ndipo mupenphedwa kukhala mumaphunziro awa kupitila mumafunso chifukwa;

  (a) munapimiwa kale kuti muli na matenda a sickle cell anaemia, ndiponso kulondola malamu amafunso aphuaziro iyi.
  (b) Imwe ndimwe osanikila chabe osati kuti mudwala, kumo muli mu zaka zimodzi nakuti muli anthu amozi amuna or akazi olingwana ndi odwala. Chifukwa tifuna kukutenga magazi anu osadwala nolingani nabuja odwala kuti tilinganize kuona mipimoya zinc, copper na selenium, kuti tipeze njila notithandiza ku njila yothandizilamo banthu pali namatenda awo.

Mukabvomera ku kukhala amodzi othandizila muzapemphedwa ku chosedwa magazi okwana ngati 5mls mu ma contena yabili kuti tipime zinc, copper na selenium mu magazi anu. Aya maphunziro afuna anthu okwan angati 92 ku apimiwe.

CHOYOFYA, KUSAMVELA BWINO NA PHINDU YA PHUNZIRO IYI

Kuli zoyofya zhkhuzana ndi kufufuza kwa phunziro iyi. Muzatungiwa na nyereti pochosa magazi. Zaose zofuaikila kuchepesa ukali pochosa magadzi zizachitika. Kulibe kulipilisiwa mukukufuza uku, ndipo phiaelu simungaiwone apa, kuma kasuma lidwe kamatenda abu kaza sinthu kusogolo.

KUSUNGA CHISINSI

Zonse zomwe munzatiuza sitiza uza muntu aliyense amene Sali mugulu yaanu.

Ndiposu zina yanu sitizalemba pali ponse potenga nkhani chifukwa chakusungu chisinsi.

Kapena muna funso iliyonse, osalephela kutumila banthu aba;

1. **Machiko Alfred**
   The Researcher
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4. **The   Chairperson**

   University of Zambia Biomedical Research Ethics Committee (UNZABREC):

   Contact Number: 260-1-256067

   Email Address; unzarec@zamtel.zm

PAPALA IYI YAMAU IFUNIKA KUPASIWA KULI UYO AFUNIKA KUFUNSIWA

APPENDIX C: Informed consent form/assent form - English

Study Title**: Assessment of Serum Zinc, Copper and Selenium in non-symptomatic sickle cell anaemia patients at the University Teaching Hospital, Lusaka, Zambia.**

By signing my name below, I …………………………………….…….. Confirm the following:

- I have read (or had read to me) this entire consent document and all of my questions have been answered adequately.

- The study's purpose, procedures, risks and possible benefits have been explained to me.

- I freely and voluntarily choose to participate OR allow my child/dependant to participate.

- I understand that participating or not will not affect my health care or that of my family members.

- I understand that my rights and privacy OR the privacy of my child/dependant will be maintained.

Participant signature ………………………… Date……………………..

Thumb print if participant can't sign/parent or Guardian………………………….

…………………………… ……………………………….
Witness (Name and Signature) Date

NOTE: **The participant will be provided with a signed copy and dated copy of this consent form will remain with the P.I as evidence of consent. It will help him/her remember what we discussed today.**

APPENDIX D: chivomelezo chowuzidwa – nyanja/chewa

Phunziro**: Assessment of Serum Zinc, Copper and Selenium in non-symptomatic sickle cell anaemia patients at the University Teaching Hospital, Lusaka, Zambia.**

Polemba zina langa apa, ine ……………………………………….. nivomela ku izi

- Nabelenga, kapena bani belengela ndipo ndilovomekezela ndikuti mafunso nayankha bwino.

- Zonse soyofya, kachitidwe ndiponso phindu yapezeka muphunziro iyi namasulidwa.

- Nazisankila nekha kupezaka mu phunziro iyi o nasikila mwana wanga kupezeka muma phunziro

- Niziba kuti kukhala umozi wa ufufuzaza matenda awa kuza sokoneza umoyo wanga kapena banja langa

- Namvela kuti chisinsi na umunthu wanga o wamwana wanga ozasungiwa

Usaina kwa osankhidwa…………………………………. Siku………………………………

Chala chikukulu cha inki kapena simungalembe or osungamwana………………………….

……………………………..   ……………………………………

uboni (Zinanakusaina)   (Siku)

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THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

CLINICAL AND DEMOGRAPHICAL DATA FORM-SCA PATIENTS

Study Title: Assessment of Serum Zinc, Copper and Selenium in non-symptomatic sickle cell anaemia patients at the University Teaching Hospital, Lusaka, Zambia.

Date……………….................

PATIENT ID…………………………………………… SERIAL #......................

Sex……………......... Age……....................... Phone #...

Haematological indices

MCV……………………… MCH…………………… MCHC………………….

Platelets………………… WBC…………………… Hb………………………

DEMOGRAPHIC DATA

a) Marital Status……........… b) Occupation……...........……… c) Residence…...........……

MEDICAL HISTORY AND GENERAL HEALTH

a) When last time did you have a blood transfusion? ………………......... (Indicated Months Since)

b) When last time did you have an active sickle crisis? .... (Indicate #/ year)

c) Do you have hypertension or any cardiovascular condition? .... (YES/NO)

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

CLINICAL AND DEMOGRAPHICAL DATA FORM-HEALTHY CONTROLS

Study Title: Assessment of Zinc, Copper and Selenium in non-symptomatic sickle cell anaemia patients at the University Teaching Hospital, Lusaka, Zambia.

Date………………..............

PATIENT ID…………………………………………………   SERIAL #.....................

Sex……………......... Age……....................... Phone #...

Haematological indices

MCV……………………….   MCH……………………   MCHC………………….

Platelets…………………   WBC……………………   Hb………………………

DEMOGRAPHIC DATA

a) Marital Status……............ b) Occupation……............……… c) Residence….............……

MEDICAL HISTORY AND GENERAL HEALTH

a) When last time did you have a blood transfusion? ………………......... (Indicated Months Since)

b) Do you have hypertension or any cardiovascular condition? .... (YES/NO)

APPENDIX: **G**

RESEARCH WORK PLAN

ACTIVITY	PERIOD					
	Jan-Mar 2015	Apr-Oct 2015	Nov-Dec 2015	Jan-Mar 2016	Apr-Sep 2016	Oct- Dec 2016
Proposal Writing	███	███				
Proposal presentation & submission			███	███		
Data Collection				███	███	
Data Analysis					███	
Dissertation Writing					███	
Dissertation Submission & Presentation					███	
Findings publication					███	███

APPENDIX: H

RESEARCH BUDGET

N0.	ITEM	PACK SIZE	QUANTITY	UNIT COST	TOTAL COST
01	Eppendorf tubes/vial		200	K700.00	K700.00
02	Transport		9 months		K1,800.00
03	Ream of paper	500sheets	5	K 30.00	K 150.00
04	Other Stationary			K200.00	K 200.00
05	All-In-One Printer		1	K 500.00	K 500.00
06	Specimen Processing Assistant		1	K 3000.00	K 3000.00
07	Vacutainer needles 21G	1x50needles	04	K50.00	K200.00
08	Vacutainer needle holder	1x50holders	04	K50.00	K200.00
09	AAS Run/Analysis		92	K270.00	K24840.0
10	Ethics approval				K500.00
	Total				**K32,090**