DRUG MANAGEMENT AND MICRONUTRIENT LEVELS IN ADULT PATIENTS WITH SICKLE CELL DISEASE IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL SHIKA, ZARIA

BY

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AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS OF SCIENCE DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

NOVEMBER, 2012

DECLARATION

I declare that the work in the thesis entitled 'Drug Management and Micronutrient Levels in Adult Sickle Cell Disease Patients in Ahmadu Bello University Teaching Hospital, Shika, Zaria' has been performed by me under the supervision of Dr. A.U. Zezi, Dr. B.B. Maiha and Prof. A.I. Mamman.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for the award of another degree or diploma at any university.

Olorukooba, Bashirat Abimbola _____ _____

Signature Date

CERTIFICATION

This thesis entitled '**DRUG MANAGEMENT AND MICRONUTRIENT LEVELS IN ADULT SICKLE CELL DISEASE PATIENTS IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, SHIKA, ZARIA'** by Olorukooba, Bashirat Abimbola meets the regulation governing the award of the degree of Master of Science in Pharmacology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to Allah (SWT) for His Kindness, Mercy, and Blessings.

To my parents, my husband, my siblings, and entire family; for your constant love, prayers and support; may Allah reward you all abundantly and grant you all the best in both worlds. Amin.

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To all the patients and volunteers who consented to participating in this study, indeed this work would not have been possible without you. May the Almighty bless you.

ABSTRACT

Sickle cell diseases (SCD) have numerous complications which vary widely among patients making drug treatment difficult. In addition, SCDs are associated with deficiencies in some micronutrients; supplementation of which may ameliorate some of the complications and contribute to existing drug management strategies. The drug utilisation of 45 SCD patients in crises and 45 SCD patients in steady state in a Nigerian Teaching Hospital (Ahmadu Bello University Teaching Hospital, Shika, Zaria-ABUTH) was evaluated. 45 HbAA volunteers acted as controls. The medication and clinical history was obtained via questionnaire and interview of patients and their care givers. Plasma magnesium, zinc, copper and iron levels of the SCD patients and 45 age and sex matched HbAA controls was determined using spectrophotometric methods (Beckman Coulter DU 520 Colorimeter). Genotype was determined using haemoglobin electrophoresis following sickle test. While, full blood count was determined using standard methods. The drug management was in line with standard treatment guidelines (2008). The SCD crises group had a higher percentage of analgesic use than the steady state group; however their routine drug use was similar with the exception of liberal fluid intake which was more in the steady state group. Further analysis of the drug utilisation patterns with respect to haemoglobin variants (HbSS, HbSS+F and HbSC) showed a variation, with the HbSS having the highest analgesic utilisation. Non opioid analgesics were more commonly used than opioid analgesics. Amoxicillin was the first drug of choice for treatment of infection. The SCD patients on amoxicillin had a significantly higher ($p<0.05$) white blood cell count than SCD patients who were not on amoxicillin. Full blood counts of the HbAA controls fell within normal limits. There was a statistically significant difference between these values and those found in both SCD groups. The mean plasma magnesium of the SCD patients was significantly lower ($p<0.05$) than that of the HbAA; with the mean plasma magnesium levels of the SCD in crises being significantly lower ($p<0.05$) than that of the SCD in steady state. Although, the mean plasma zinc levels of the SCD patients was lower than that of the HbAA, it was not significant ($p<0.05$). The mean plasma iron levels of the SCD patients was significantly higher ($p<0.05$) than the mean plasma iron levels of the HbAA controls. In conclusion, the drug management strategies in the Haematology department of ABUTH conformed to the standard treatment guidelines. Individualised care will be needed in patients on chronic analgesia in other to prevent co morbid gastrointestinal, hepatic and renal associated toxicities, as well as tolerance and addiction.

Plasma magnesium levels were significantly lower in crises group than other groups suggesting low magnesium levels may be a contributor to the severity of crises. Plasma zinc levels of both SCD groups were also low, suggesting there may be greater zinc utilization in the SCD patients. Plasma micronutrient levels significantly differed from HbAA controls and may present a possible mode for therapeutic intervention.

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GLOSSARY AND SYMBOLS

<	Less than
>	Greater than
=	Equal to
5-Br-PAPS	2-(5-Bromo-2-pyridiylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino] phenol, disodium salt, dihydrate
DiBr-PAESA	[4-(3, 5-dibromo-2-pyridylazo)-N-ethyl-N.sulfo-propylaniline] α- Alpha
A	Absorbance
AAG	Lysine
ABUTH	Ahmadu Bello University Teaching Hospital
ACS	Acute Chest Syndrome
ADHD	Attention Deficit Hyperactivity Disorder
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
ATH	Adeno Tonsillar Hypertrophy
ATP	Adenosine Tri-Phosphate
AVN	Avascular Necrosis
B	Beta
BC	Before Christ
BCG	Bacille Calmette-Guerin

BMT	Bone Marrow Transplant
Ca^{2+}	Calcium ion
CAG	Glutamine
CAL.	Calibrator
CAR	Central African Republic
CAT	Computed Axial Tomography
CBC	Complete blood count
CEMAT	Canadian Early and Mid-Trimester Aminocentesis Trial
Cl^-	Chloride ion
CSSCD	Cooperative Study of Sickle Cell Disease
Cu^{2+}	Copper ion
CVA	Cerebrovascular Accident
CVS	Chorionic villous sampling
δ	Delta
DALY	Disability Adjusted Life Years
DNA	deoxyribonucleic acid
DMSA	Dimercaptosuccinic acid
DTwp	Diphtheria and tetanus toxoid with whole cell pertussis
EDTA	Ethylene Diamine Tetra Acetic Acid
EGTA	Ethylene Glycol Tetra Acetic Acid
Fe^{2+}	Iron ion

γ	Gamma
GDP	Gross domestic product
GAG	Glutamic acid
GTG	Valine
Hb	Haemoglobin
HbA	Haemoglobin A
HbC	Haemoglobin C
HbD	Haemoglobin D
HbF	Haemoglobin F
HbH	Haemoglobin H
HbO	Haemoglobin O
HbS	Haemoglobin S
HDAC	Histone de Acetylase
HDL	High density lipoprotein
HepB	Hepatitis B
HIV	Human Immuno Deficiency Virus
IM	Intramuscular
ISCs	Irreversibly Sickled Cells
IV	Intravenous
K^+	Potassium ion
KCC	Potassium chloride co-transporter

K-Cl	Potassium chloride
$KHCO_3$	Potassium bicarbonate
LDL	low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean Corpuscular volume or mean cell volume
MenAC	Meningococcal AC
MenACWY	Meningococcal ACWY
Mg^{2+}	Magnesium ion
ml	Millilitres
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
NO	Nitric oxide
NPO	"*Nil per os*"- Latin for Nothing by Mouth
NSAID	Non Steroidal Anti Inflammatory Drug
OPV	Oral polio vaccine
OSA	Obstructive Sleep Apnoea
PAH	Pulmonary Arterial Hypertension
PaO_2	Partial pressure of arterial Oxygen
PCV	Packed cell volume
pH	Potential Hydrogen

Pneumo_ps	Pneumococcal polysaccharide
QOL	Quality of Life
RBCs	Red Blood Cells
RDW	Red cell distribution width
RES	Reticuloendothelial System
ROS	Reactive Oxygen Species
SC	Sickle cell
SC	Subcutaneous
SCA	Sickle Cell Anaemia
SCAC	Sickle Cell Advisory Committee
SCD	Sickle Cell Disease
SEM	Standard Error of Mean
S-HPFH	Sickle Cell Hereditary Persistence of Foetal Haemoglobin
SS	Homozygous sickle cell disease
TENS	Transcutaneous Electrical Nerve Stimulation
Thal.	Thalassaemia
TPN	Total Parenteral Nutrition
UTI	Urinary Tract Infection
WBC	White Blood Cells
WHO	World Health Organisation
WR	Working Reagent
Zn^{2+}	Zinc
ZICG	Zinc Investigators' Collaborative Group and others

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sickle cell anaemia (SCA) is a haemolytic anaemia characterized by abnormally shaped (sickled) red blood cells (RBCs) (Ballas, 2007). It is a homozygous state resulting from a lifelong condition in which periods of ill health (crises) intercept varying durations of apparent wellness (steady state). The basis of the clinical presentations of sickle cell disease (SCD) is linked to erythrocyte sickling and haemolysis (Serjeant and Serjeant 2001). The haemolysis is as a result of prolonged retention of the RBCs within the reticuloendothelial system (RES) which reduces the level of oxygen within the RBCs resulting in the adoption of the sickle shape. The sickle cell thus formed is a rigid cell which is susceptible to phagocytosis by cells of the RES. In conditions associated with reduced blood oxygen tension, RBCs containing haemoglobin S (HbS) undergo polymerisation with an increase in stickiness of the RBC surface; this causes the RBCs to adhere to the endothelial surface causing a stockpiling of RBCs with resultant blockade of blood flow (vaso-occlusion) (Gladwin and Vinchinsky 2008). Repeated cycles of sickling and unsickling due to elevation and reduction in arterial oxygen tension ultimately produce the irreversibly sickled cells (ISCs) which are central to vaso-occlusion (Lew and Bookchin 2005).

The presentations of SCD are protean depending on age, social circumstances, background, and medical condition of the patient. In childhood, the hand and foot syndrome (dactylitis) is the commonest presentation due to a highly active marrow (Lonergan *et al*., 2001). With increase in age, bone pain and other manifestations of SCD are restricted to the axial skeleton. The outcomes of SCDs have been reported to be influenced by incidence, severity, and duration of crises (Yale *et al*., 2000). Vaso-occlusive and haemolytic crises appear to occur with greater frequency and severity in SCA patients with micronutrient deficiencies than in patients without deficiencies (Ambrus and Ambrus 2004). While deficiencies in any of the essential micronutrients can result in health problems, there are a few (e.g., zinc, selenium, copper, magnesium, and iron) that are particularly important in SCD (Muller and Brugnara 2001).

The search for an effective treatment for SCDs with less adverse effects have had researchers trying out many nutrients derived from plants and animals as possible and reliable options for the

effective management of SCD. According to Hsu and Muller (2001), patients with SCA have greater than average requirements for both calories and micronutrients. Experts believe that augmenting abnormally low levels of certain micronutrients in sickle cell blood or certain dietary constituents such as thiocyanate, might help ensure that individuals with SCD are healthy. For instance, it has been discovered that magnesium has the ability to reduce sickling of red blood cells (De Franceschi, 2009). In a study by De Franceschi *et al*., (1997), they concluded that oral magnesium supplementation reduces the number of dense erythrocytes and improves the erythrocyte membrane transport abnormalities of patients with SCD. Certain minerals (Copper, Iron, Magnesium and Selenium), some antioxidants and vitamins (C, E, folate, B12, and B6) have been found to effectively relieve the oxidative stress that prevails in SCD (Okochi and Okpuzor 2005). In addition, it is a well known fact that Copper (Cu) is directly involved in the iron (Fe) metabolism; and Fe is important in the synthesis of blood. In SCD, red blood cells are constantly being lysed and constantly need to be replaced. A deficiency of copper will interfere with this process by worsening anaemia (Okochi and Akpuzor 2005).

1.2 Statement of Research Problem

SCD is one of the most common autosomal recessive disorders in the world (Lonergan *et al*., 2001). The burden of SCD is highest in sub-Saharan Africa, especially in Nigeria where approximately six million suffer from SCD, while 25 million others are carriers (Aliyu *et al*., 2008). SCA affects about three percent of children in Nigeria and is the commonest genetic disorder in the country (Ogunride *et al*., 2005). Eight percent of infant mortality in Nigeria every year is due to SCD (Awolusi, 2010).

Of the 200, 000 children born with SCA yearly in Africa, 150, 000 of them are born in Nigeria-making it the country with the highest population of this endemic disease (Awolusi, 2010). In certain areas of sub-Saharan Africa, an estimated 40 to 60% of the population is heterozygous, suggesting that 1 to 4% of babies born in this region have the disease (Aliyu *et al*., 2008). In the United States, it is the most common single gene disorder in black Americans, and about one in 12 Americans of African descent has the heterozygous sickle cell trait (Lonergan *et al*., 2001).

SCD is one of the most prevalent haematological diseases in the world. Despite the immense progress in molecular knowledge about SCD in the last years, few therapeutic sources are

currently available. Nowadays the treatment is performed mainly with drugs such as hydroxyurea or other foetal haemoglobin inducers and chelating agents; most of which have undesirable side effects and are often expensive (Dos Santos *et al*., 2011). The many complications of SCD which vary widely among patients are tiresome to both the patients and physicians (Steinberg, 1999).

Malnutrition and resulting micronutrient deficiencies constitute a growing concern world-wide. Micronutrient deficiencies are increasingly being discovered to play a major role in pathogenesis, progression, and/or severity of various diseases as well as contribute to the morbidity and mortality in certain population groups such as the pregnant women and children; hence it has an impact on the survival and quality of life of patients. Blood levels of several micronutrients are often low in individuals with SCD (Gray *et al*., 1992), and lack of one micronutrient is typically linked with deficiencies of other micronutrients. Deficiencies of micronutrients are associated with increased risks of morbidity and mortality in patients with SCD (Muller and Brugnara 2001); they also cause a significant depreciation in blood-antioxidant status (Blann *et al*., 2003), which results in oxidative stress that may precipitate vaso-occlusion related crises (Klings and Farber 2001).

Although, researches have been carried out which strongly suggest a relationship between micronutrient deficiencies and the severity of SCD; there have been conflicting reports. For example, Olukoga *et al*., (1990) reported low plasma magnesium levels in SCD; in another study, he reported a low erythrocyte magnesium levels. Prasad *et al*., 1976, on the other hand reported a low plasma magnesium levels, but, an increased erythrocyte magnesium level in SCD; while De Franceschi *et al*., (1997) reported low serum magnesium levels in SCD patients; and Oladipo *et al*., (2005) found no significant difference between the plasma magnesium levels in patients with SCD and normal controls.

Similarly, various studies have shown that zinc is deficient in patients with SCD. Prasad *et al*., (1976) showed that oral zinc supplements improved severe crises in patients with SCD. However, a study in Ibadan (Akenami *et al*., 1999), found no relationship between serum zinc levels and the different degrees of clinical severity of the disease; while in Lagos, Temiye *et al*., (2011), found that children with SCA have low serum zinc levels. It is probable that zinc

deficiency may be associated with painful crises, so there is a need to study what is obtainable in our environment.

In low and middle income settings, deficiencies of several micronutrients commonly occur, it is therefore important to find out whether the relationships between these and the burden of disease are independent or overlapping (Black, 2003). Interventions to control these deficiencies can have broad benefits across a range of important infectious diseases, the prevention or treatment of which by other means has proven to be difficult in many settings. Furthermore, the reduction of illness and of disabilities such as cognitive impairment and decreased work capacity can have a strong positive effect on social and economic development in low and middle income countries. Public health interventions that can prevent or correct these micronutrient deficiencies merit the highest priority for national programmes and donor investment.

This research proposes to check the current drug management of sickle cell diseases in Ahmadu Bello University Teaching Hospital, Shika, Zaria; as well as determine the role micronutrients may play in SCD and to check for the relationship between the severity of crises and the level of four micronutrients- magnesium (Mg^{2+}), copper (Cu^{2+}), iron (Fe^{2+}) and Zinc (Zn^{2+}).

1.3 Justification

Micronutrient status in people with SCD may need correction (Gray *et al*., 1992), due to increased requirements and altered metabolism. Previous research strongly indicates a relationship between micronutrient deficiencies and the severity of SCD, but, there is a paucity of information on their role in pathogenesis and management of SCDs (Oladipo *et al*., 2005). There is need to determine micronutrient levels in patients with sickle cell because if indeed deficiencies are discovered to be a contributing factor to ill health, it will provide a treatment intervention which will have a lot of benefits to the patient; who will experience an improved health, better quality of life, less severe episodes of crises, faster recovery from crises; all at a more affordable cost than some currently available treatment/prevention strategies. Some currently available methods for the management and/or treatment of sickle cell crises are either expensive (e.g. bone marrow transplant), have moral or religious ethical issues and conflicts (e.g. antenatal screening), have intolerable side effects (e.g. hydroxyurea), still undergoing research

(e.g. Senicapoc [bis{4-fluorophenyl} phenyl acetamide], or are not readily available. There is need for readily available, cost effective, easily accessible means for management/ treatment.

Micronutrient status of individuals varies from place to place and the phenotypic expression of SCD varies with the environment and genetics. In Saudi Arabia, patients with SCA were found to have near normal levels of copper and zinc, while those in Northern Africa were found to suffer from zinc deficiency (Alayash *et al*., 1987). Also SCD patients in Saudi Arabia are genetically predisposed to having high levels of HbF which accounts for the mild expression of the disease (Sergeant *et al*., 2001). SCD experiences large variability in both clinical and haematological features. Although, patients are believed to have an identical molecular abnormality, the clinical course, which may vary from death in early childhood to a virtually unrecognised condition at the age of 75 years, implies that factors other than SCD (genetics and environment) influence its expression and severity. According to Sergeant *et al*., (2001), it is unrealistic to examine disease manifestations independent of the environment since multiple interactions are inevitable; and it is unreasonable to base the management of diseases in one environment on the studies done in another different environment. It is therefore hoped that the results of this research will improve our understanding of SCD and its management in our environment.

To the best of our knowledge, a study on drug management and micronutrient levels in SCD patients in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria has not been documented. Thus, the outcome of this research may have local, national, and international benefits.

1.4 Theoretical Framework

Drug management of the SCD patients was evaluated by comparing current practices in ABUTH, Zaria; with standard treatment guidelines for Nigeria (2008). Plasma micronutrient levels of SCD patients were compared with apparently healthy volunteers of genotype HbAA. The plasma micronutrient (magnesium, zinc, copper, and iron) levels were determined using spectrophotometric methods at wave length of 520nm (Beckman Coulter DU 520 colorimeter). This was based on the theory that the micronutrients form stable coloured complexes with specific reagents at specific pHs. The intensity of colour formed is proportional to the

concentration of micronutrient in the sample. Serum or plasma levels of Mg^{2+} (Ismail *et al*., 2010), Zn^{2+} (Guerrieri *et al*., 1986), Cu^{2+} and Fe^{2+} (Narasinga, 2003) can be used clinically to determine deficiencies of these micronutrients.

1.5 Importance of the Study

Evaluating drug management of disease conditions is important to ensure that they are up to date with current guidelines. A better understanding of pathogenesis of SCD may enhance its drug management. Pathogenesis is defined as pathologic, physiologic, and biochemical mechanism resulting in the development of a disease or morbid state. By determining the micronutrient levels in our environment, a relationship may be established between these levels and SCD; and this may enable us establish a new management strategy for the treatment of SCD.

1.6 Objectives of the Study

The aim of this research is to determine the current drug management used in ABUTH and assay the micronutrient levels of sickle cell disease patients in Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria in 2011.

The specific objectives are:

1. To check current drug management protocols in SCD patients in ABUTH
2. To determine plasma levels of magnesium, zinc, copper, and iron in:
    a. sickle cell disease patients in steady state,
    b. sickle cell disease patients in crises, and
    c. apparently healthy people with genotype of AA.
3. To determine the relationship(s) between micronutrient levels of sickle cell disease patients and sickle cell crises.

1.7 Research Hypothesis

Micronutrients may play a role in the management of sickle cell diseases (SCDs).

CHAPTER 2

LITERATURE REVIEW

2.1 Sickle Cell Diseases

2.1.1 Introduction

The term sickle cell disease (SCD) is a sickle haemoglobinopathy used in a generic sense to refer to all the clinically severe sickling syndromes (Lane, 1996). Sickle haemoglobinopathies are genetically determined conditions characterized by structurally abnormal haemoglobin variants which give rise to haemolytic diseases by virtue of their property to polymerize and assume the sickle or crescent shape. It applies to all individuals with at least a single HbS chain and one other abnormal β globin chain (Lonergan *et al*., 2001).

The different forms of SCD are determined by the genes inherited from the person's parents. Someone who inherits a sickle cell gene (HbS) from each parent has haemoglobin SS (HbSS) disease, also called sickle cell anaemia (SCA). A person can also inherit a sickle cell gene from one parent and a different kind of abnormal gene from the other and end up with a different form of SCD, such as haemoglobin SC disease or haemoglobin S beta thalassemia. Someone who inherits only one sickle cell gene and a normal gene from the other parent is said to have the sickle cell trait, but not the disease (Miller *et al*., 2009). SCA is inherited as an autosomal recessive condition whereas sickle cell trait is inherited as an autosomal dominant trait (Serjeant and Serjeant 2001).

Sickling of red blood cells is promoted by conditions which are associated with low oxygen levels, increased acidity, and dehydration of the blood (Yale *et al*., 2000). These conditions can occur as a result of injury to the body's tissues, dehydrating states, or anaesthesia. In addition, certain organs are predisposed to lower oxygen levels or acidity, such as when blood moves slowly through the spleen, liver, or kidney. The major features of SCDs include: fatigue, anaemia, pain crises, dactylitis, bacterial infections, progressive damage of organs and tissues, and impaired growth and development usually an end result of the physical and emotional trauma associated with the disease (Yale *et al*., 2000). The life expectancy of persons with SCA

is considered less than the general population; nevertheless, with optimal management, patients now survive beyond the fourth decade (SCAC, 2002).

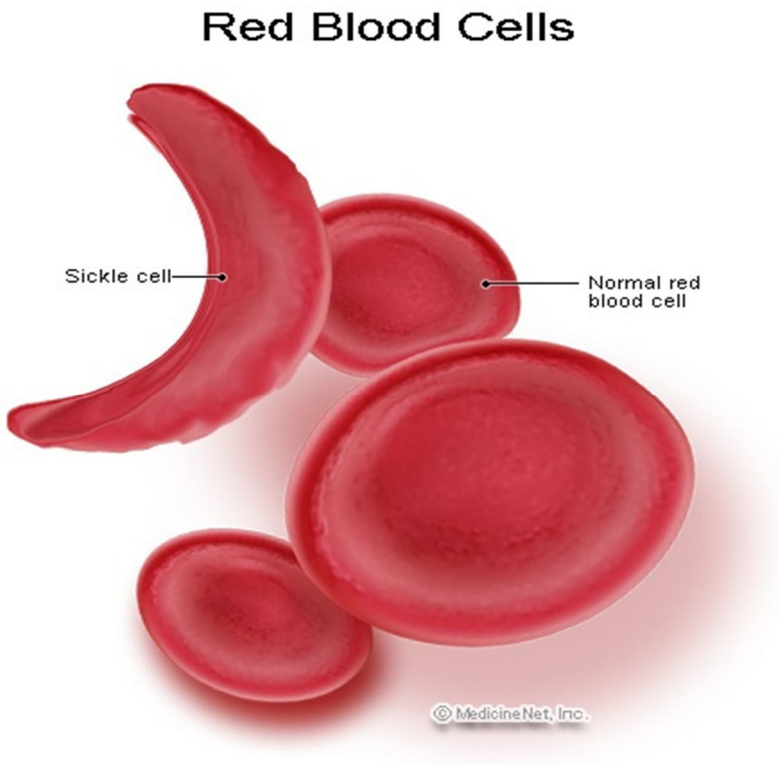


Figure 1: Showing normal and sickle red blood cells (http://www.medicinenet.com/)

2.1.2 History

Sickle cell disease (SCD) was first described by Herrick in 1910, in a dental student who presented with pulmonary symptoms (Herrick, 1910). Herrick coined the term "sickle-shaped" to describe the peculiar appearance of the red blood cells (RBCs) of this patient. However, given the patient's symptoms, he was not sure at the time whether the blood condition was a disease *sui generis* or a manifestation of another disease (Frenette and George 2007). Centuries before this, the people of West Africa knew the disease syndrome, had specific tribal names for it; and also knew that the disease was hereditary (Konotey-Ahulu, 1991). In the next 15 years following Herrick's discovery, several similar cases were described, supporting the idea that this was a new

disease entity and providing enough evidence for a preliminary clinical and pathological description (Frenette and George, 2007). Shortly thereafter, in 1927 suggested that anoxia caused RBC sickling by demonstrating that shape changes could be induced by saturating a RBC suspension with carbon dioxide (Hahn and Gillespie, 1927 in Frenette and George, 2007). Scriver and Waugh (1930) proved this concept *in vivo* by inducing venous stasis in a finger using a rubber band; by showing that stasis-induced hypoxia dramatically increased the proportion of sickle-shaped cells from approximately 15% to more than 95% (Frenette and George, 2007). These studies were noted by Linus Pauling, who was the first to hypothesize in 1945 that the disease might originate from an abnormality in the haemoglobin molecule (Pauling *et al*., 1949). Around the same time, Watson showed that the abnormal response of RBCs in SCA resulted from a primary haemoglobin (Hb) abnormality. He observed that formation of sickle-like spicules on deoxygenation of RBCs from sickle cell infants was absent or minimal until foetal haemoglobin (HbF) was replaced by adult haemoglobin (HbA), at about 3–6 months of age (Watson *et al*. 1948); thus, predicting the importance of foetal haemoglobin (HbF). However, Pauling's hypothesis was not validated until 1949, when by applying moving boundary electrophoresis to haemolysates from individuals with genotypes HbAA, HbAS, and HbSS; he showed that nearly all the haemoglobin in the RBCs of HbSS had a mobility different from that of HbAA, and that HbAS carriers had both types of haemoglobin in their RBCs (about 40% of abnormal HbS and 60% of normal HbA). This crucial experiment identified a molecular disease and explained its patterns of inheritance (Lew and Bookchin 2005). Ingram and colleagues (1959) demonstrated shortly thereafter, in one of the first applications of two-dimensional peptide mapping that the precise molecular difference between mutant sickle haemoglobin (HbS) and normal haemoglobin (HbA) was a single amino acid substitution of valine for glutamic acid in position 6 on the β-chain of HbA (Lew and Bookchin 2005). This was followed by studies that analyzed the structure and physical properties of HbS, which formed intracellular polymers upon deoxygenation (Frenette and George 2007).

2.1.3 Prevalence

Nigeria has an estimated population of 150 million with annual growth rate of 3.2%. The current figure of individuals in Nigeria with this disorder is not known since the majority born in rural community do not survive childhood and for lack of proper statistics. However, about 2.3% of

the Nigerians suffer from SCD and about 25% of Nigerians are healthy carriers of the abnormal hemoglobin gene (Afolayan and Jolayemi 2011).

In broad terms, the prevalence of the sickle-cell trait ranges between 10% and 40% across equatorial Africa and decreases to between 1% and 2% on the north African coast and <1% in South Africa (Fifty-ninth World Health Assembly Report, 2006). In some West African countries like Ghana, the frequency of the trait is 15% to 30%, whereas in Uganda it shows marked tribal variations, reaching 45% among the Baamba tribe in the west of the country (Fifty-ninth World Health Assembly Report, 2006). This distribution reflects the fact that sickle-cell trait confers a survival advantage against malaria (balanced polymorphism). There is much epidemiological evidence that sickle cell trait decreases the risk for all manifestations of severe malaria in malaria endemic regions (Kreuels *et al*., 2009). Although a single abnormal gene may protect against malaria, inheritance of two abnormal genes leads to SCA and confers no such protection and malaria is a major cause of ill-health and death in children with SCA (Oniyangi and Omari 2006). There is increasing evidence that malaria not only influences outcome but also changes the manifestations of SCA in Africa.

2.1.4 Epidemiology

The highest frequency of SCD is found in tropical regions, particularly sub-Saharan Africa, India and the Middle-East (Weatherall and Clegg 2001). However, migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased (Roberts and De Montalembert 2007).

The symptoms related to sickle cell crises were known by various names in Africa, long before they were recognized in the western hemisphere. Symptoms of SCA could be tracked back to year 1670 (Serjeant and Serjeant 2001). The disease had probably been recognised for generations in West Africa, where the chronic recurrent condition was given onomatopoeic names implying ‘cold season rheumatism’. Thus in Ghana, the Ga tribe referred to it as “Chwecheechwe”, the Twi as “Ahututuo”, the Ewe as “Nuidudui”, and the Fante as “Nwiiwii” (Serjeant and Serjeant 2001). In Nigeria, various expressions have been used to describe SCD for many years (Ejiofor, 1998). The descriptions or names are linked to vital phenomena; for example; shortened lifespan [Yoruba terminologies which imply this include-“durojaye”,

"durosinmi" and "abiku" , while the Hausa terminologies include "barmani", "ajuji" (dustbin), and "ayashe" (meant to be dug out), the Ibo's refer to it as "ogbanje" (recurrent death), and the tiv as "vende wanye"], anaemia [Hausa refer to it as "rashin jinni"or "fara"], and for phenomena related to pain/bone pain [the Yoruba refer to it as "lagunlagun", "lagunregbee", or "aromolegun", the Hausa refer to it as "amosanin", the Ibo's refer to it as "aju oyi", and the Fulani's refer to it as "budi"] (Konotey-Ahulu, 1991).

However, the first recorded description in Africa has been attributed to Africanus Horton (1874) who described the fever of crises, the shifting joint pains, the exacerbations during the rainy season, and the constant abnormality of blood (Serjeant and Serjeant 2001). But, it was not until 1910, the scientific community came to know about SCD through Herrick's discovery (Desai and Dhanani 2004). Thus, the disease was occasionally called "Herrick's syndrome". It was later named "Sickle Cell Anaemia" by Vernon Mason in 1922 (Shamsuddeen *et al*., 2007).

2.1.5 Nomenclature and Classification of Sickle Cell Diseases

The principal genotypes include: Homozygous sickle cell disease (HbSS), Sickle cell haemoglobin C disease (HbSC), Sickle cell β^o thalassaemia ($S\beta^o$ thal.), Sickle cell β^+ thalassaemia type I, II, and III ($S\beta^+$ thal. Type I, $S\beta^+$ thal. Type II and $S\beta^+$ thal. Type III). Three other much less common genotypes include: Sickle cell haemoglobin D Punjab (HbD Punjab), Sickle cell haemoglobin O Arab (HbO Arab), and Sickle cell haemoglobin Lepore Boston (HbLepore Boston).

They are all single point mutations in which the codon determining the amino-acid has changed.

- HbSS - Valine replaces glutamic acid at position β^6 i.e. nucleotide is changed from GAG to GTG
- HbSC- Same nucleotide is changed from GAG to AAG, resulting in insertion of lysine in place of glutamic acid at position β^6
- HbD Punjab- Codon CAG is changed to GAG resulting in replacement of glutamine for glutamic acid at position β^{121}

- HbO Arab- Same codon is changed to AAG determining the insertion of lysine in place of glutamic acid at position β^{121}

- The thalassaemias result from a wide variety of DNA (deoxyribonucleic acid) mutations that have in common a reduced synthesis of globin chains. They are classified by the chain they affect into α, β, δβ, or γδβ thalassaemia resulting from a deletion in α, β, and β-complex globin chains respectively with the α and β deletions being the most common.

Beta (β) Globin Haplotypes

The structure of the deoxyribonucleic acid (DNA) surrounding the β globin locus has been found to differ between populations having sickle haemoglobin (HbS) suggesting specific ancestral DNA structures upon which the HbS mutation has arisen as a relatively recent event (Serjeant and Serjeant 2001). These differences in DNA structure or polymorphisms (β-globin haplotypes), believed to represent independent occurrences of HbS mutation have occurred on at least three and possibly four occasions in Africa and are named after the areas where they were first described: Benin, Senegal, Central African Republic (CAR or Bantu), and Cameroon haplotypes. A further DNA structure termed Asian haplotype is associated with most of the β^S genes in the Eastern Province of Saudi Arabia and Central India (Desai and Dhanani 2004). From this primary distribution, the Benin haplotype spread to North Africa, Northern Greece, Southern Turkey, Sicily, Albania, South-West Saudi Arabia; the Bantu haplotype accounts for most of the β^S in Kenya (Serjeant and Serjeant 2001). Analysis of the beta S chromosome of 183 Nigerians showed that 98.4% of them had the Benin haplotype (Akinyanju, 1989).

2.1.6 Geographical Distribution of Sickle Cell Diseases

It is a common misconception that HbS is confined to people of African origin. The disease is more wide spread and has now been globalised due to voluntary and non-voluntary migrations. Notably, HbS appears to reduce susceptibility to malaria (*Plasmodium falciparum* infection); a patient with heterozygous HbAS is more resistant to malaria infection than is a patient with homozygous HbSS, and one with homozygous HbAA is the most susceptible (Lonergan *et al*., 2001). Thus, the distribution of HbSS markedly coincides with the distribution of malaria. According to Serjeant and Serjeant (2001), high frequencies occur in equatorial Africa from which it spread to North and South America, the Caribbean's and lately Europe.

2.1.7 Pathophysiology

A homozygous mutation in the gene for β globin, a subunit of adult haemoglobin A (HbA), in which valine substitutes glutamic acid at position 6, is the proximate cause of SCD (Bunn, 1999). Sickle haemoglobin (HbS) shows peculiar biochemical properties, which lead to polymerizing when deoxygenated (Taher *et al*., 2010). HbS polymerization is associated with a reduction in cell ion and water content (cell dehydration) as well as an increased red cell density which further accelerates HbS polymerization (Bunn, 1999). This leads to formation of a dense RBC (De Franceschi, 2009). Intravascular sickling in capillaries and small vessels leads to vaso-occlusion, impaired blood flow in a variety of organs and tissues, and cell destruction in the peripheral circulation (De Franceschi, 2009). In addition, the polymerization can have a direct impact on the RBC plasma membrane, leading to the extracellular exposure of protein epitopes and glycolipids that are normally found inside the cell increasing the adherence of sickle RBC to vascular endothelium (Frenette and George 2007). There is also a loss of phospholipid asymmetry with externalization of phosphatidylserine in the RBC, which is believed to play a significant role in promoting macrophage recognition with removal of erythrocytes (erythrophagocytosis), cell apoptosis and activation of coagulation explaining the shortened life span of sickled cells (De Franceschi, 2009).

According to Frenette and George (2007), chronic haemolysis in SCD leads to release of plasma free haemoglobin and heme iron from the lysed RBC. When the capacity of protective haemoglobin-scavenging mechanisms (haptoglobin and hemopexin) has been saturated, levels of cell-free haemoglobin increase in the plasma resulting in the consumption of nitric oxide (NO) by haemoglobin-mediated NO scavenging. In addition, arginase released by haemolysed red cells can deplete blood plasma of arginine, the substrate for NO production by NO-synthase. NO plays a major role in vascular homeostasis and is a critical regulator of smooth muscle relaxation and vasomotor tone, expression of endothelial adhesion molecules and platelet activation and aggregation (Walford and Loscalzo 2003). A deficiency in NO may therefore underlie complications associated with SCD (Lin *et al*., 2005). No part of the human body is exempt of the sequel to *in vivo* sickling in SCD. The heme iron is a major cause of oxidative stress that can induce redox-sensitive transcription factors such as nuclear factor kappa-B and activator protein-1; which in turn induce the recruitment of adherent leucocytes in the venules. The presence of

adherent leukocytes in small post capillary venules is emerging as a key factor that contributes to vaso-occlusion during sickle cell crises (Frenette and George 2007). Leukocytes are large cells that are more rigid than the sickled RBC, thus, their role in vaso-occlusion offers an attractive therapeutic target for SCD.

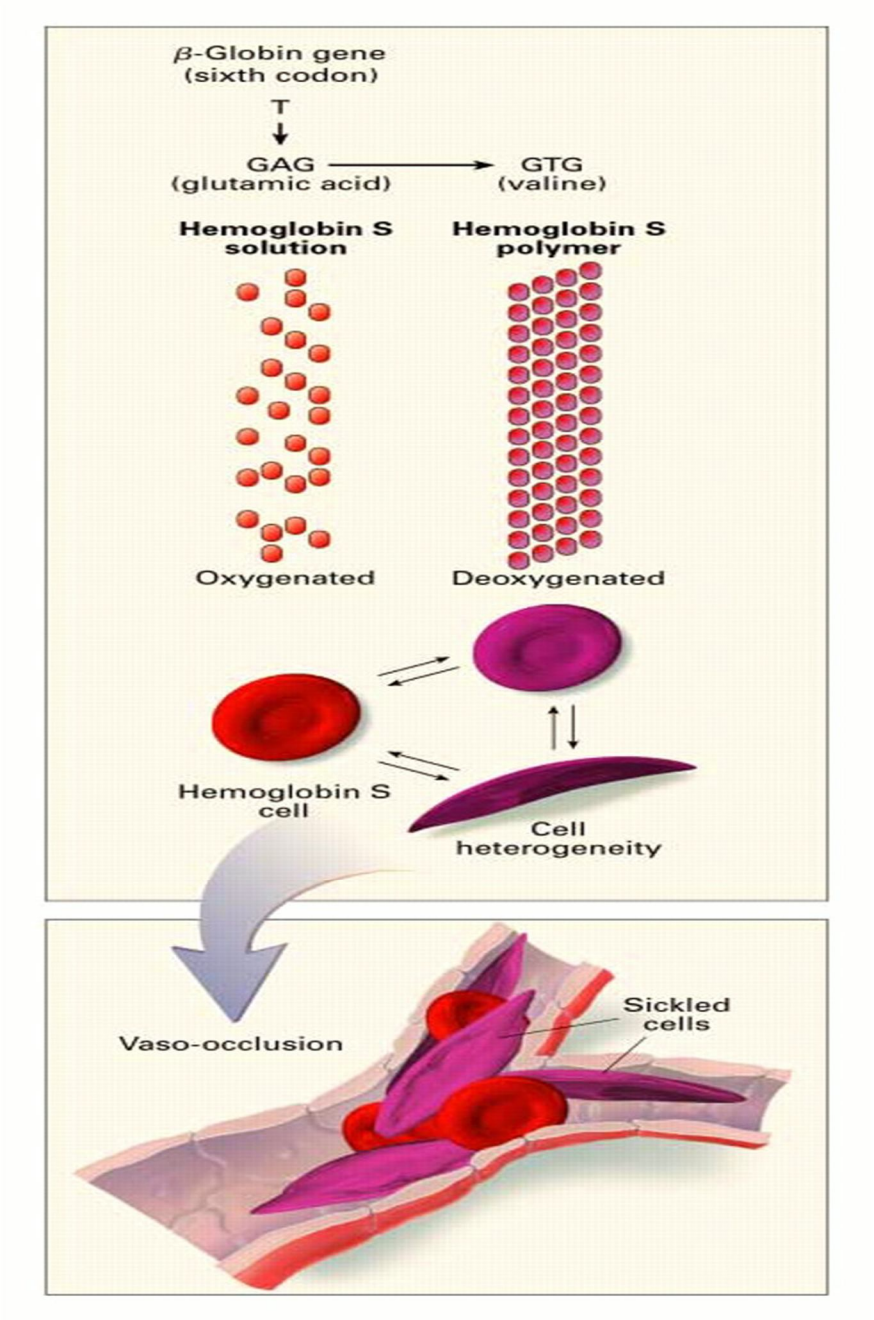


Figure 2: Pathophysiology of sickle cell disease (Steinberg, 1999-*New England Journal of Medicine*. 340:1021-30)

2.1.8 Diagnosis

Diagnosis of the major genotypes of SCD is relatively simple, however, differentiation of the genetic sub-divisions may be complex requiring genetic studies and a variety of sophisticated laboratory procedures (Serjeant and Serjeant 2001).

2.1.8.1 The Sickle test

The standard technique for performing this test is the method of Daland and Castle (1948) in Konotey-Ahulu, (1991). It is based on the morphological changes of the HbS containing red cell when de-oxygenated. One drop of two percent solution of sodium metabisulphite is mixed with one drop of blood on a microscope slide, covered with a coverslip, and sealed with wax to exclude air and to prevent drying. Sickling of the cells is usually apparent under a microscope within an hour, but the result is read after twenty-four hours.

The sodium metabisulphite should be freshly prepared because unless it is stored at $4^{o}C$ (in which case, it can last 6 months), it deteriorates rapidly leading to a false negative. Also, a higher concentration of four percent should not be used because it is a hypertonic solution which will give a false positive (Serjeant and Serjeant 2001).

Limitations include: too insensitive to consistently detect low levels of HbS present at birth.

2.1.8.2 Solubility test

De-oxygenated HbS is relatively insoluble in solutions of high molarity such as concentrated phosphate buffers, lysing and reducing agents. The solubility test is performed by adding 4 drops of blood to 2mls of freshly prepared sodium diothionate and potassium phosphate in a test tube. Appearance of a clear solution indicates HbA, C or D; a precipitate above and a clear solution below indicates HbSS; while a precipitate above and a pink solution beneath indicates HbAS (Serjeant and Serjeant 2001) .

Limitations include: too insensitive to consistently detect low levels of HbS present at birth, inability to differentiate a harmless AS genotype from the clinically significant homozygous and compound heterozygous forms. It also fails to detect HbC, β-thalassaemia trait, and some other

pathological variants. There is no situation in which a positive sickling or solubility test should be reported without further investigation by haemoglobin electrophoresis.

2.1.8.3 Haemoglobin electrophoresis:

Haemoglobin electrophoresis is the major technique used for diagnosis in neonatal and postnatal life. It is performed at an alkaline pH of 8.4-8.9; and is based on the principle that charge changes occur in haemoglobin molecules after certain amino acid substitutions. Thus, the replacement of negatively charged glutamic acid at position β^6 by neutrally charged valine in HbS results in one net positive charge per chain and two net positive charges per molecule relative to HbA; thus, the haemoglobin in HbS will move more slowly towards the anode than the haemoglobin in HbA. HbC in which the negatively charged glutamic acid has been substituted by positively charged lysine has four positive charges relative to HbA and thus moves even more slowly than HbS (Serjeant and Serjeant 2001).

Limitations of electrophoresis include its inability to differentiate abnormal haemoglobins with the same charge.

2.1.8.4 Other methods: Restriction endonuclease analysis of DNA (using foetal blood, amniotic fluid, and chorionic villi) and amniocentesis (Serjeant and Serjeant 2001). However, these methods of antenatal diagnosis are expensive and associated with a number of emotional disorders, ethical, social, and cultural difficulties.

***2.1.8.5 Diagnosis of subdivisions of major genotypes*:** This includes diagnosis of such major genotypes as the heterozygous and homozygous α^+-thalassaemias. The diagnosis is done using the restriction enzyme Bam HI (Serjeant and Serjeant 2001).

2.1.9 Clinical Features and Complications

The versatile clinical features of SCD result from chronic variable intravascular haemolysis, microvascular ischemia and organ damage (Aliyu *et al*., 2006). The severity and nature of tissue involvement depends on several internal environmental factors. These include, the amount of HbS present, the quantity of HbF, haematocrit level, immunological competence of the patient, and the haemodynamics of the organ or tissue affected (Konotey-Ahulu, 1991). All of these

contribute to the end organ damage and other complications which are seen in the SCD patient. Some clinical features and complications seen in the SCD patient are discussed below.

1. Infection, Sepsis and Meningitis

Serious bacterial infections are a major cause of morbidity and mortality in patients with SCD, and they may also enhance susceptibility towards vaso-occlusive complications (Steinberg, 1999). Severe, overwhelming septicaemia/meningitis due to *Streptococcus pneumoniae* is the most common cause of death during early childhood in the United States and other developed countries (SCAC, 2002). In early childhood, 85-95% infections are caused by *Streptococcus pneumoniae* (Serjeant, 2005). However, more recent studies of septicaemia in SCD in Nigeria and Uganda have found that less than 10% of septicaemias in these countries are due to *Streptococcus pneumonia* (Akuse, 1996). Prevention and early aggressive treatment of infection is critical in the management of individuals with SCD. Prophylactic penicillin has proven effective in reducing the number of life-threatening episodes of pneumococcal sepsis in children with SCD under the age of 5years in most states in America. However, pneumococcal vaccines may not be effective in Nigeria where *Streptococcus pneumonia*e is not predominant. In a study carried out in Lagos, Kaduna, Benin, and Ibadan, the predominant organisms found were *Staphylococcus* species, *Salmonella* Species, *Klebsiella*, and *Escherichia coli* (Akuse, 1996; Kizito *et al*., 2007).

2. Splenic Dysfunction and Acute Splenic Sequestration

Splenic sequestration is a significant complication of SCD and a leading cause of death in children (Steinberg, 1999). The spleen possesses a slow, tortuous microcirculation. This increases the susceptibility of circulating RBCs to congestion, sludging, and polymerization, leading to formation of rigid sickled cells (Lonergan *et al*., 2001). The sickled RBCs formed cause occlusion of the reticular networks and interendothelial slits in the spleen, obstructing splenic blood flow which may result in chronic sustained splenic enlargement, acute intermittent enlargement (acute splenic seqeuestration), or a progressive splenic fibrosis (Serjeant and Serjeant 2001). Chronic enlarged spleen which is common in the tropics and subtropics may also be caused by infections such as malaria, leishmaniasis, schistosomiasis, rickettsia, as well as bacterial and viral infections (Konotey Ahulu, 1991). Progressive splenic fibrosis in which the

spleen in the SCD patient shrinks with age into a small fibrotic lump of no functional importance also occurs (Konotey Ahulu, 1991), this seriously compromises its immune functions which include the removal of particulate blood borne antigens and the production of specific antibodies which renders patients with SCD prone to overwhelming blood borne infections (Konotey Ahulu, 1991). At 6 months of age, 14% of patients with SCD are asplenic; at 2 years, 58% are asplenic; and by 5 years of age, 94% are asplenic (Lonergan *et al*., 2001).

3. Anaemia

Chronic anaemia occurs in patients with SCD especially HbSS and $S\beta^0$ thalassemia. Patients with SC disease or $S\beta^+$ thalassemia usually have haemoglobin in the normal or low normal range (SCAC, 2002).

Acute anaemia can be triggered by:

- infection with parvovirus B-19 (aplastic crises),
- suppression of the bone marrow (viral or other infections),
- pooling of blood in the spleen or liver (sequestration),
- increased intravascular haemolysis,
- hyper-haemolytic episodes (e.g., in malaria) (Juwah *et al*., 2004),
- transient suppression of erythropoeisis (transient aplastic crises),
- other causes of anaemia such as blood loss (SCAC, 2002).

4. Chest Pain and Acute Chest Syndrome (ACS)

ACS is a term that describes an acute pulmonary illness characterised by a new pulmonary consolidation with signs of fever, chest pain, cough, dyspnea, and tachypnea (Lane, 1996). ACS affects 40% of all patients with SCD and it is more common in children, but more severe in adults (Steinberg, 1999). ACS is the second leading cause of hospitalization in patients with SCD and has a mortality of approximately 1.8% in children and 4.3% in adults (Vinchinsky *et al*.,

1997). Three major causes of ACS have been proposed: pulmonary infection, embolization of bone marrow fat, and intravascular pulmonary sequestration of sickled erythrocytes (Gladwin and Vinchinsky 2008). Other factors linked to ACS include opioid use, pulmonary oedema, and parvovirus B19 (the latter may cause bone marrow infarction and lead to fat emboli) (Quinn and Buchanan 1999).

5. Pulmonary arterial hypertension (PAH)

The depletion of nitric oxide by free haemoglobin released into circulation during haemolysis leads to an increase in blood pressure which predisposes the sickle cell patient to high blood pressure (Gladwin *et al*., 2003). PAH is a rare blood vessel disorder of the lungs, in which the pressure in the pulmonary artery rises above normal levels and may become life threatening. It has been defined as mean pulmonary artery pressure > 25 mm Hg at rest or > 30 mm Hg during exercise, with normal pressure being 15 mm Hg (Clarke, 2006). Secondary pulmonary hypertension (PAH) has been shown to have a prevalence of 30% in patients with SCD (Aliyu *et al*., 2008). PAH is a risk factor for mortality in SCD (Hecht and Hecht 2010), with death rates significantly higher in sickle cell patients with the disorder as compared to patients without PAH (Castro *et al*., 2002). In the United States, mortality rates of 40% at 40 months after diagnosis have been seen in patients with SCD (Sutton *et al*., 1994).

6. Heart Diseases

Vascular occlusion of small and large vessels can lead to impaired myocardial function including right and left ventricular systolic and diastolic dysfunction, elevated cardiac output, cardiomegaly and myocardial ischaemia (Voskaridou *et al*., 2012). Progressive heart damage from iron overload occurs in patients requiring routine transfusion therapy (Voskaridou *et al*., 2012). Principally, chronic anaemia increases cardiac output and may cause left ventricular enlargement and cardiac insufficiency (De Montalembert *et al*., 2004).

7. Cerebrovascular Accident (CVA)

Approximately 25% of all patients with SCD will have a neurologic complication over their lifetime, 11% of these complications will occur by age 20 years (Lonergan *et al*., 2001). Individuals with SCD are at risk for a CVA at a rate of approximately 0.5% per year (SCAC,

2002). About 10% of all children with SCD have a CVA, and recurrence is likely (Steinberg, 1999). These CVA's comprise: development of aneurysms, intracranial haemorrhage, continued vascular damage and transient ischemic strokes in children.

8. Ocular Abnormalities

Red blood cell sickling can occur in the microvasculature of the eye (SCAC, 2002). Ocular findings, potentially blinding, can occur in the eyes of patients with sickle cell haemoglobinopathy including SS disease, SC disease and Sβ thalassemia (Penman and Serjeant 1992). However, it is most prevalent in the HbSC patient probably due to higher levels of haemoglobin (Konotey Ahulu, 1991). In the United States, of the 0.017% African-Americans with HbSC, as many as 33% present with sickle cell retinopathy (McLeod *et al*., 1997).

9. Priapism

The term priapism is restricted to a condition of persistent and prolonged penile erection, usually painful, unaccompanied by sexual desire, and not relieved by sexual intercourse (Konotey Ahulu, 1991). It can occur at any age and may persist for hours, days, or even weeks. 89% of males with SCD have at least one episode by the age of 20, with an average age of 12 years for the first episode (Bruno *et al*., 2001). The diagnosis of priapism is usually clinical (Sharpsteen *et al*., 1993). The causes of priapism range from trauma/neoplasm of the spinal cord, to infection, tumour, or calculi of the urinary tract, haemorrhage into the penile substance, obstruction of venous drainage from the penis, leukaemia, psychogenic factors, and sickle cell haemoglobinipathies (Konotey Ahulu, 1991).

10. Avascular Necrosis (AVN)

Avascular necrosis (AVN) of the humeral and femoral heads, and less commonly the knees and spine affect patients with SCDs (SCAC, 2002), however, it is more common in the HbSC patient because they have more red blood cells per unit volume of blood and therefore greater blood viscosity when the cells undergo sickling in a localised area (Konotey Ahulu, 1991). The progressive destructive process is accompanied by varying degrees of pain and disability. The diagnosis of AVN is contemplated when the patient seeks medical treatment for acute pain in the

hips, buttocks or shoulders with motion limitation because of the pain. The pain can be intermittent or become persistent.

11. Gnathopathy and bossing of skull bones

Gnathopathy from the Greek word "gnathos" (jaw) is a peculiar growth of the maxilla commonly found in the African presentation of SCD (Konotey-Ahulu, 1991). It is a complication of chronic severe haemolytic states and more commonly occurs in thalassaemias and SCD where it often requires surgical intervention due to its progressive nature of uncontrolled maxillary overgrowth (Konotey-Ahulu, 1991) caused by marrow hyperplasia (Brown and Sebes 1986).

Bossing of the skull bones in SCD is another evidence of the chronicity and severity of anaemia. In most cases, it can be seen clinically and is most marked over the frontal, parietal, and occipital bones (Konotey-Ahulu, 1991). In the skull, thickening of the frontal and parietal bones occur and result clinically in bossing from about the age of three. It is present in about 5% of patients with sickle cell.

12. Liver/Hyperbilirubinemia

Patients with SCD are at high risk of developing pigmented gall stones due to chronic haemolysis. The incidence varies from one population to another and increases with age. The reported prevalence of gall stones in homozygous SCD (Hb-SS) in the United States varied from 34% to 70% and 29% in Jamaica and Africa (Meshikhes and Al-Faraj 1998). Gall stones may be asymptomatic or may present with repeated attacks of biliary colic or with acute cholecystitis. Gallstones may occur as early as two years of age; and are typically most marked in HbSS disease and Sβ° thalassemia and much less in HbSC disease and $S\beta^{+}$ thalassemia (SCAC, 2002). Hepatomegaly is present in 40% to 80% of patients with SS disease (Serjeant and Serjeant 2001). It does not correlate with liver function tests that can be normal or slightly abnormal. Other hepatobiliary complications that occur in SCD include: hepatic necrosis secondary to anoxic damage or blockade of hepatic arteries and rarely hepatic coma.

13. Kidney/Urinary Tract

Renal intravascular sickling is a very common event, which begins early in life and continues for the life of the patient. The combination of hypoxia, hypertonicity and acidosis in the renal

medulla leads to stasis in the vasa recta with consequent ischemia of the renal medulla and papillary tip leading to renal papillary necrosis which can be asymptomatic or accompanied by haematuria or proteinuria. Proteinuria occurs in about 28% of adult SCD patients in Nigeria; while haematuria occurs in about 27% of the SCD patients (Abdu *et al*., 2011). Proteinuria is a more sensitive marker than elevated serum creatinine levels in detecting glomerular injury, and it has been reported to be an early manifestation of sickle cell nephropathy (Aleem, 2008). The kidney of a SCD patient is unable to concentrate urine (hyposthenuria), this is characterised by enuresis and polyuria. This concentration defect makes urine dilute, favouring bacterial growth and eventually a urinary tract infection (UTI) may result. In Nigeria, there is a prevalence of asymptomatic bacteriuria of 6% in children with SCA, which makes them predisposed to UTI (Chukwu *et al*., 2010).

14. Leg Ulcers

Chronic leg ulceration is a common complication of SCA. In the United States of America, leg ulcers occur in 10-20% of patients with SCD between the ages of 10 and 50 years of age and are more frequent in males (SCAC, 2002); while in Nigeria, a prevalence rate of 7.5% has been reported among patients with SCA (Durosinmi *et al*., 1991). Recurrence rates are high and range from 40% in Jamaica (Clare *et al*., 2002), to 71.4% in Lagos, Nigeria (Olayem and Bazuaye 2009), and to 96% in Accra, Ghana (Ankra-Badu, 1992). Their cause is unknown, their prevention is impractical, their management once present is often difficult (Nolan *et al*., 2006), and recurrence is common.

15. Osteomyelitis

The manifestations of SCD in the musculoskeletal system are major causes of morbidity and disability (Nwadiaro *et al*., 2000). These manifestations include avascular necrosis of major bone parts, hypertrophy of bone marrow, osteoporosis, hand-foot syndrome and osteomyelitis (Nwadiaro *et al*., 2000). The prevalence of osteomyelitis among children with SCD is 0.08%; Osteonecrosis occurs in about 10-50% of SCA patients (Steinberg, 1999). The morbidity of chronic osteomyelitis combined with other effects of the haemoglobinopathy decreases the quality of life (Nwadiaro *et al*., 2000).

16. Hand and Foot Syndrome (dactylitis)

Hand and foot syndrome is often one of the first signs of SCD. It is thought to be due to focal capillary stasis involving sickled sludge in the terminal bones causing ischemia and infarction of the red marrow of the fingers and feet (Konotey Ahulu, 1991). There is also an expansion of the bone at the metaphases. The lesions are bilateral and may deteriorate to frank osteomyelitis with chronic discharge or may heal completely without any after effects. It is characterised by painful symmetric swelling of the hands and feet with a shiny reflective surface involving the fingers and dorsa of the hands and feet. It occurs in 60% of patients, presenting between the ages of 9months-4years with painful tender swelling of the hands and feet. The bone changes are reversible within a year following adequate treatment (Onuba, 1993).

17. Vaso-Occlusive / Sickle Cell Crises

The word 'crisis' (Greek *Krisis*, turning point) is defined as a 'sharp turn or definite change in the course of a disease, with the development of new signs and symptoms' (Konotey Ahulu, 1991).

Epidemiologic data indicate that 5.2 % of patients with SCD have three to ten episodes of severe pain every year. In most patients, a pain crisis resolves within five to seven days; while severe crisis may cause pain that persists for weeks to months (Steven *et al*., 2000).

2.1.10 Pain Crises prevention in Patients with Sickle Cell Disease

The following measures can be used to prevent pain crises in SCD patients.

a. Consuming adequate amounts of fluids to prevent dehydration
b. avoiding mountain climbing or air flights in an unpressurized cabin
c. avoiding exposure to extreme cold, exercising to exhaustion or using drugs that can lead to acidosis (e.g., acetazolamide)
d. genetic and newborn screening
e. vocational counselling
f. avoiding hypoxemia in the preoperative period when general anaesthesia is used or when a procedure involves hypertonic radiographic dyes (Steven *et al*., 2000).

2.1.11 Management of Sickle Cell Disease

Optimal management requires a multidisciplinary team (physicians, haematologists, pharmacists, nurses, psychiatrists, physical therapists, pain specialists and social workers) working together to provide empathetic, consistent, longitudinal care in a trustworthy environment (Steven *et al*., 2000). The diversity of SCD complicates its management (Steinberg 1999), thus, the choice of management is both patient and environment specific. Treatment strategies according to the standard treatment guidelines (2008) are:

a. Counselling and health education
b. Encouraging membership of support groups
c. Providing infection prophylaxis
    i. Antimalaria
    ii. Vaccines
d. Providing regular health checks
e. Treating infections (first drug of choice – amoxicillin and its derivatives)
f. Providing folate supplementation for those in steady state
g. Treating pain for those in crises:
    i. Mild pain – paracetamol, aspirin, or ibuprofen
    ii. Moderate to severe pain – diclofenac sodium, diclofenac potassium, or morphine.

1. Specific pain management

Acute episodes of severe pain (crises) are the primary reasons that SCD patients seek medical care in hospital emergency department (Steven *et al*., 2000). Most patients who have episodes of acute pain are neither drug addicts nor drug seekers (Steinberg, 1999). Pain from vaso-occlusive crises is often undertreated because of concerns about narcotic addiction and tolerance, perceived drug-seeking behaviour, excessive sedation, respiratory depression and lack of specific findings on the physical examination (Steven *et al*., 2000). Yet the incidence of opioid analgesic addiction in patients with SCD has been reported to be no higher than 3% (Steven *et al*., 2000). The standard treatment approach includes pharmacologic and non-pharmacologic techniques. Pharmacological management is based on the three-step "analgesic ladder" recommended by the

World Health Organization (WHO) for the treatment of cancer-related pain however, non pharmacological management is also important and includes: psychological supportive care, adequate hydration, rest, physical therapy, heat application, transcutaneous electrical nerve stimulation (TENS), self-hypnosis and diversional techniques (Steven *et al*., 2000).

a. Paracetamol

Paracetamol is a very weak anti-inflammatory drug but is effective as an antipyretic and analgesic agent. It lacks certain side effects of NSAIDs, such as gastrointestinal tract damage and blockade of platelet aggregation (Goodman and Gilman 1996).

Mechanism of action

It is a weak inhibitor of cyclooxygenase (Goodman and Gilman 1996)**.**

Indications

It is the first drug of choice for treatment of mild pain in SCD as well as for lowering body temperature in febrile states. It is a suitable substitute for aspirin for analgesic or antipyretic purposes, particularly where aspirin is contraindicated (EMDEX 2010/2011).

Side effects

It is usually well tolerated in recommended therapeutic dosages. Some side effects include:

- Erythmatous or urticarial rash
- Fever, mucosal lesions, and hypersensitivity reactions
- neutropenia, thrombocytopenia, and pancytopenia
- fatal hepatic necrosis (dose dependent)
- nephrotoxicity (analgesic abuse) (Goodman and Gilman 1996)

Adult dose

Oral: 0.5–1.0 grams every 4 – 6 hours, maximum daily dose is 4 grams (EMDEX 2010/11).

b. Non steroidal anti inflammatory drugs (NSAIDs)

NSAIDs are a heterogenous group of compounds that are often chemically unrelated, but which nevertheless share certain therapeutic actions and side effects. The prototype is Aspirin. Other

examples include ibuprofen, piroxicam, naproxen, diclofenac sodium, diclofenac potassium, and ketoprofen (Goodman and Gilman 1996).

Mechanism of action

Most currently available NSAIDs inhibit both cyclooxygenase 1 (COX-1; constitutive) and cyclooxygenase 2 (COX-2; induced in settings of inflammation) activities, and thereby synthesis of prostaglandins and thromboxane. The inhibition of COX-2 is thought to mediate, at least in part, the antipyretic, analgesic, and anti-inflammatory action of NSAIDs, but the simultaneous inhibition of COX-1 results in unwanted side effects, particularly those leading to gastric ulcers, that result from decreased prostaglandins and thromboxane formation (Goodman and Gilman 1996).

Indications

- Treatment of mild to moderate pain
- As antipyretics to reduce body temperature in febrile states
- As anti-inflammatory agents in treatment of musculoskeletal disorders such as osteoarthritis (Goodman and Gilman 1996).

Side Effects

- Gastrointestinal ulceration and intolerance
- Blockade of platelet aggregation
- Inhibition of uterine motility (prolongation of gestation)
- Inhibition of prostaglandin-mediated renal function
- Hypersensitivity reactions (Goodman and Gilman 1996).

Adult dose

- Diclofenac: Oral: 75-150mg daily in 2-3 divided doses; up to 200mg daily.
- Ibuprofen: Oral: 1.2-1.8g daily in 3-4 divided doses, maximum 3.2g daily.
- Ketoprofen: Oral: 100-200mg daily in 2-3 divided doses; or 200mg slow release once a day.
- Naproxen: Oral: 550-1100mg daily in 2 divided doses; may be increased to 1650mg daily.

- Piroxicam: Oral: 20mg daily as a single dose; maintenance dose, 10-30mg daily as single or divided doses (EMDEX 2010/2011).

c. Opioid analgesics:

Opiates are drugs derived from opium and include morphine, codeine and a wide variety of semi--synthetic congeners derived from them. The term opioid is more inclusive and applies to all agonists and antagonists with morphine like activity (Goodman and Gilman 1996). There are now many compounds with pharmacological property similar to those produced by morphine, but none has proven to be clinically superior in relieving pain (Goodman and Gilman 1996).

Mechanism of action

Morphine and other μ-opioid agonists (methadone, pentazocin) selectively inhibit various nociceptive reflexes and induce profound analgesia. They do this by mediating the inhibition of release of neurotransmitters; including substance P. Morphine also antagonises the effects of exogenously administered substance P (Goodman and Gilman 1996).

Indications

Morphine remains the most valuable analgesic for severe pain. Codeine is much less potent and is used for treatment of moderate pain (sometimes in combination with paracetamol). Tramadol, pentazocin, a partial μ-agonist, and pethidine are used in the management of moderate to severe pain (EMDEX 2010/2011).

Side Effects

Nausea, vomiting, drowsiness, constipation, tachycardia, palpitations, sweating, headache, decreased libido, urticaria, rashes, hallucinations, confusion, respiratory depression, tolerance and dependence (EMDEX 2010/11).

Adult dose

- Tramadol: Intramuscular (IM) or intravenous (IV): 50-100mg every 4-6 hours, not exceeding 400mg per day (EMDEX 2010/11).

- Pentazocin: IM, IV, or SC (Subcutaneous): 30-60mg, 3-4 hourly. Doses in excess of 30mg IV or 60mg IM, SC are not recommended (EMDEX 2010/11).

- Pethidine: IM or SC: 25-100mg, repeated 3-4 hourly if necessary, up to 150mg in severe pain (EMDEX 2010/11).
- Morphine: IM or SC: 10-15mg every 4 hours (EMDEX 2010/11).

2. **Micronutrients and Supplements (Vitamins B_{12}, B_6, folic acid, iron, magnesium, zinc)**

Deficiencies in vitamins B_{12} (Al-Momen, 1995), B_6 (Natta *et al*., 1980), and folic acid (Gothoni, 1995) have been discovered in patients with SCDs. Therefore, daily supplements will be necessary and have proved useful in reversing symptoms of deficiency and in improving the general well being of the patient (Okochi and Akpuzor 2005).

Iron: there are conflicting results as to whether iron deficiency exists in patients with SCA or not and as to whether replacing the iron via iron supplements will be of benefit or harm to the SCA patient (Livrea *et al*., 1996). Some reports suggest that some patients with SCA are indeed iron deficient, however, use of supplements in such patients aggravate the symptoms of anaemia (Carmel *et al*., 1984). In addition, same study stated that, the iron deficiency actually reduces the sickling of red blood cells, reduces symptoms of anaemia, and reduces RBC destruction. Therefore, patients with sickle cell must be carefully evaluated and be given iron supplements only where there is frank clinically detectable iron deficiency.

Magnesium: has been discovered to be deficient in patients with SCA. Lowered blood magnesium leads to dehydration of RBCs which increases the sickling and predisposes to crises. DeFranceschi *et al*., (1997) showed in an experiment that oral magnesium supplements (540mg magnesium per day in form of magnesium pidolate) improved symptoms of patients in crises, reduced the number of pain episodes, and reduced blood abnormalities.

Zinc: Plasma levels of zinc may be low in SCDs due to chronic haemolysis and loss in the urine (Ishir *et al*., 1995). These low levels can be corrected by supplementation (Sindel *et al*., 1990).

Zinc supplementation has a lot of beneficial effects; in a study by Gupta and Chaubey (1995) it was discovered to cause a significant reduction of the mean number of infective episodes and associated morbidity in patients with SCA. It has also been shown in various other studies to have a stabilizing effect on the RBC membrane in people with SCD (Zemel *et al*., 2002), to

increase foetal haemoglobin levels (Wilber *et al*., 2010), and to improve healing of leg ulcers (Konotey-Ahulu, 1991). Care should be taken when correcting zinc deficiencies, because, zinc supplements when taken over a long period of time often lead to copper deficiency which can in turn lead to iron deficiency anaemia due to impaired iron absorption (Ishir *et al*., 1995).

Folic acid and Vitamin B12: folic acid and vitamin B12 are dietary essentials. A deficiency of either vitamin results in defective synthesis of DNA in any cell in which chromosomal replication and division are taking place (e.g. red blood cells). An early sign of deficiency is megaloblastic anaemia.

Indications

- As supplement in sickle cell diseases (folic acid and vitamin B12)
- Treatment of megaloblastic anaemia (folic acid and vitamin B12)
- Prevention of neural tube defects (folic acid)

Side effects

Folic acid: no substantiated reports of side effects (Goodman and Gilman 1996).

Vitamin B12: nausea, headache, dizziness, fever, hypersensitivity (EMDEX 2010/11).

Adult dose

Folic acid: Oral, 400mcg-5mg daily. Vitamin B12: Oral, 5mcg-1mg daily.

3. Antioxidants:

SCD is characterized by a pro-oxidant environment due to high production of reactive oxygen species (ROS) related to increased levels of free pathological iron and heme groups associated with a reduction in antioxidant systems (DeFranceschi, 2009). There are significantly lower plasma levels of the antioxidant vitamins (A, C and E), in patients with severe SCA (Hasanato, 2006). Patients with SCA are under continuous oxidative stress due to sickle cell redox imbalance (Aslan *et al*., 2000). Essien, (1995) in a study in Calabar, Nigeria, found that the plasma concentrations of the antioxidant vitamins A (retinol), C (ascorbic acid) and E (Alpha tocopherol) were significantly low in patients with SCA and he suggested that the deficiency of these antioxidant vitamins could account for some of the observed manifestations of SCD such

as increased susceptibility to infection and haemolysis. The authors then advocated that by making these supplements available to the SCD patient, frequency of infection and crises episodes could be reduced.

4. **Proguanil:**

Proguanil is predominantly a tissue schizonticide and has little blood schizonticidal activity. It is active against pre-erythrocytic intrahepatic forms, particularly of *Plasmodium falciparum*. Proguanil is the drug of choice for malaria prophylaxis in patients with SCD. There is no evidence that proguanil is harmful in prophylactic doses against pregnancy (EMDEX 2010/11).

Mechanism of action

Proguanil (Chloroguanide) exemplifies an antimalarial used primarily for causal prophylaxis of falciparum malaria. It is a tissue schizonticide that acts on primary tissue forms of plasmodia within the liver that are destined within a month or less to initiate the erythrocytic stage of the infection. Invasion of erythrocytes and further transmission of infection are thereby prevented (Goodman and Gilman 1996).

Indications

- It is used in prophylaxis against malaria in areas of low resistance (EMDEX 2010/11).
- Suppresses acute attacks of *Plasmodium vivax* but does not eradicate it (Goodman and Gilman 1996).

Side effects

Mild gastric intolerance, diarrhoea, constipation, occasional mouth ulcers and stomatitis, rarely skin reactions and hair loss, hypersensitivity reactions (EMDEX 2010/11).

Dosage

Oral: 200mg daily (EMDEX 2010/11).

5. **Amoxicillin:**

Amoxicillin and its congeners is the first drug of choice in treatment of infection in SCD patients (Standard treatment guidelines, 2008). It is a broad spectrum penicillin with antimicrobial

activity against gram-negative microorganisms such as *Haemophilus influenza*, *Escherichia coli*, and *Proteus mirabilis*. Unfortunately, amoxicillin can be readily hydrolysed by broad-spectrum β-lactamases that are found with increasing frequency in clinical isolates of the gram-negative bacteria (Goodman and Gilman 1996).

Mechanism of action

Amoxicillin is a β-lactam antibiotic that can kill susceptible bacteria. Although, knowledge of its mechanism of action is incomplete, numerous researches suggest it disrupts the cell wall of the bacteria. Peptidoglycan is responsible for the rigid protective nature of the bacterial cell wall. Peptidoglycan biosynthesis is in three stages, the last stage which is catalysed by transpeptidase is inhibited by β-lactam antibiotics. Inhibition of the transpeptidases causes spheroplast formation and rapid lysis of the bacteria (Goodman and Gilman 1996).

Indications

Urinary tract infections, upper respiratory tract infections, bronchitis, pneumonia, dental abscess and other oral infections, gynaecological infections, gonorrhoea (EMDEX 2010/11).

Side effects

Nausea, vomiting, diarrhoea, thrombocytopenia, neutropenia, hypersensitivity reactions (EMDEX 2010/11).

Adult dose

Oral: 250mg-3g every 8hours, maximum-6g daily.

6. Oxygen Therapy

Oxygen therapy is often used routinely in the management of vaso-occlusive crises, especially in patients with ACS and those with evidence of hypoxemia (headache, fatigue, shortness of breath, euphoria, nausea, cyanosis) (Steinberg, 1999). However, there is lack of evidence supporting the effectiveness of these measures in all patients e.g. in those SCD patients with ACS whose partial pressure of arterial oxygen (PaO_2) is in the normal range (Steven *et al*., 2000). In addition, oxygen may suppress erythrocyte production, depress reticulocytosis and cause rebound sickle cell crises on discontinuation of therapy (Steven *et al*., 2000).

7. Transfusion

Transfusion is done using sickle negative blood. It "dilutes" out sickled RBCs, reduces ISCs count, precludes recurrent stroke if HbS is less than 30%, and reduces abnormal flow in cerebral vessels in children with SCD (Adams *et al*., 2000). Indications for transfusion include pregnant patients with anaemia, acute cerebral syndrome, priapism, acute chest syndrome, surgery, and anaemia. Exchange transfusions should be considered in patients who have a prolonged refractory vaso-occlusive crisis with a stable baseline haemoglobin concentration. The goal is to reduce sickling by reducing the haemoglobin S level to below 20% (Steinberg, 1999).

Risks of transfusion include iron overload, increased blood viscosity, worsening of sickling, alloimmunization, and transmission of infections such as hepatitis or HIV.

8. Fluid replacement

Increased plasma osmolarity from a reduced plasma volume can worsen a vaso-occlusive crisis via intracellular dehydration, haemoglobin polymerization and worsening of haemoglobin sickling. During hyponatremia, the affinity of haemoglobin S for oxygen is increased. Patients with SCD have isosthenuria, which leads to difficulty in excreting sodium load (Okpala, 1998), thus, hypernatremia ensues and the affinity of haemoglobin S for oxygen is reduced. This further reduces the ability of the sickled cells to carry oxygen. Fluids should therefore be administered in a quantity sufficient to correct existing deficits and replace ongoing losses in order to maintain a euvolemic state (Steven *et al*., 2000).

9. Diet and nutrition

The voluntary energy intake of sickle cell patients appears to be similar to HbAA individuals, but since the sickle cell patients have a higher resting metabolic rate, it suggests a suboptimal nutritional state for SCD patients (Singhal *et al*., 1997). Growth of the child and adolescent should be carefully monitored. If there is significant lag, the patient must be assessed to rule out other causes of growth delay (Hyacinth *et al*., 2010). Peripubertal delay of both growth and sexual maturation of as much as two years is frequent in children with SS disease. It is important to reassure the parents and the teenager that there will be catch-up in both, so that adult size and development will be within normal limits. In addition, adequate fluid intake is important.

10. Vaccination and immunization

All SCD patients should be given appropriate doses of standard internationally recommended vaccines. Other vaccines to be added are those dictated by the epidemiologic circumstances which include meningococcal and yellow fever vaccines. In view of the fact that SCD patients suffer from functional asplenia and are susceptible to encapsulated bacteria such as *S pneumoniae, H influenzae* type B, *Salmonella,* and *Klebsiella*, it is recommended that Children with SCA are given prophylactic oral penicillin beginning at 3 months of age and continuing to at least age 5 years (Lonergan *et al*., 2001). This treatment has been shown to reduce the prevalence of bacterial infection by 84% (Lonergan *et al*., 2001). They are also given pneumococcal and *H influenzae* vaccines, beginning between 2 and 5 years of age (Lane, 1996).

Nigeria's Immunization Schedule is as follows (EMDEX, 2010/11):

a. BCG (Bacille Calmette-Guerin) – at birth
b. DTwp (Diphtheria and tetanus toxoid with whole cell pertussis vaccine) – at 6 weeks, 10 weeks, and 14 weeks
c. HepB (Hepatitis B vaccine) – at birth, 10 weeks and 16 weeks.
d. MenAC or MenACWY (Meningococcal AC or Meningococcal ACWY) – for special groups e.g. Pilgrims.
e. OPV (Oral polio vaccine) – at birth, 6 weeks, 10 weeks, and 16 weeks.
f. Pneumo_ps (Pneumococcal polysaccharide vaccine) – special groups
g. Vitamin A – 9 months and 15 months.
h. Yellow fever vaccine – 9 months.

11. Induction of foetal haemoglobin (HbF)

A large number of epidemiological, clinical, and laboratory observations have converged to support the notion that HbF administration can ameliorate the clinical severity of SCD (Frenette and George 2007). In a study in the United States by Platt *et al*., (1994), the Cooperative Study of Sickle Cell Disease (CSSCD) identified HbF as a prognostic factor for several sickle cell complications, including painful events, acute chest syndrome, and death. Also, Serjeant and Serjeant (2001) noted that patients with SCD from the eastern provinces of Saudi Arabia and from India typically have a very mild sickling disorder associated with high levels of HbF.

Furthermore, elegant laboratory studies conducted many years earlier demonstrated that HbF interferes with the polymerization of deoxygenated HbS *in vitro* (Frenette and George 2007). This was further confirmed by Gladwin and Vinchinsky (2008) who stated that, presence of foetal haemoglobin in the erythrocyte reduces the concentration of haemoglobin S and thereby inhibits its polymerization.

a) 5-Azacytidine

5-Azacytidine was the first agent to be used to induce HbF expression via epigenetic silencing of the γ-globin genes in adult life. 5-Azacytidine was shown to induce very high levels of HbF in anaemic baboons. Its ability to stimulate HbF production was also demonstrated in a small number of patients with SCD and β-thalassemia. In spite of these promising results, this drug was never tested in large-scale clinical trials because of concerns about potential carcinogenicity (Frenette and George 2007).

b) Hydroxyurea

Hydroxyurea was first synthesized in 1869 in Germany by Dressler and Stein (Segal *et al*., 2008). Hydroxyurea is an S phase–specific chemotherapeutic agent (Letvin *et al.*, 1984). It was first approved by the Food and Drug Administration (FDA) in 1967 for the treatment of neoplastic diseases (Segal *et al*., 2008). It was shown to result in a marked increase in HbF levels in baboons (Letvin *et al.*, 1984), thereby reducing the severity of SCD by preventing the formation of haemoglobin S polymers (Steinberg, 1999). In February 1998, hydroxyurea was approved by the (FDA) for use in adults with SCD in America (Segal *et al*., 2008). In 2002, the National Heart Lung and Blood Institute issued a recommendation that practitioners should consider using hydroxyurea daily in selected patients with SCD (Segal *et al*., 2008).

Mechanism of Action

The precise mechanism by which hydroxyurea produces its varied effects is unknown (Segal *et al*., 2008); Hydroxyurea inhibits ribonucleotide reductase (Steinberg *et al*., 2003). It was originally proposed that hydroxyurea may elevate HbF levels by accelerating erythroid differentiation in the bone marrow, leading to the appearance of "foetal-like" cells in the peripheral blood. HbF has a higher affinity for oxygen than HbS, thus, in this way hydroxyurea

was said to decrease sickling (Charache *et al*., 1995). More recent studies have shown that it also has the ability to generate nitric oxide (NO) *in vivo*, which results in the vasodilation of the vascular endothelia, nullifying effects of haemolysed cells. Hydroxyurea, has therefore been considered for management and prevention of pulmonary arterial hypertension (PAH) (Aliyu *et al*., 2008). It has also been shown to decrease the adhesion of sickle cells to endothelium (Frenette and George 2007). Owing to its myelosuppressive activity, hydroxyurea reduces circulating white blood cell (WBC) counts and likely the number of adherent leukocytes recruited to the wall of small venules. The reduction of WBC counts was correlated with the clinical benefit from hydroxyurea. It is still not entirely clear how much of the clinical benefit from hydroxyurea could be attributed to its effect on HbF levels compared with its other activities.

Studies have shown that hydroxyurea showed a marked decrease in the frequency of painful crises and acute chest syndrome and a reduction in transfusion requirements and hospitalizations in adults with moderate to severe SCD. It also improved survival, clinical efficacy and short-term safety of hydroxyurea in children with SCD (Frenette and George 2007).

Indications

Hydroxyurea is indicated in adolescents or adults with frequent episodes of pain, a history of acute chest syndrome, severe symptomatic anaemia, or other severe vaso-occlusive complications. At present, hydroxyurea can be considered in patients who have severe complications and who can reliably follow the regimen (Segal *et al*., 2008).

Side Effects

Concerns on use of hydroxyurea include the variable and poor response in some patients (Serjeant and Serjeant 2001), as well as fears of toxicity such as:

a. Neutropenia, skin rashes, hair loss, nausea, and nail changes (Segal *et al*., 2008).

b. Theoretical dangers of mutagenesis, teratogenesis, and leukaemogenesis (Serjeant and Serjeant 2001).

Dosage and administration

It is an orally available drug that is relatively well tolerated and simple to use. Hydroxyurea is initiated in a dosage of 500 mg per day (10-15 mg per kilogram of body weight every morning) for six to eight weeks. If blood counts are acceptable, the dosage is increased to 1,000 mg per day after six to eight weeks, with the patient monitored for a decline in granulocyte or platelet counts. The maintenance dosage is between 1,000 and 2,000 mg per day, depending on the balance between hematologic toxicity and increases in haemoglobin F values. Blood counts should be followed every four to six weeks to detect longer term hematologic toxicities. The long-term effects of hydroxyurea maintenance therapy are not well known. The paucity of long-term studies limits conclusions about toxicity (Lanzkron *et al.*, 2008).

c) Butyrate

Butyrate, a short-chain fatty acid that inhibits histone de acetylase (HDAC) was shown to stimulate embryonic or foetal globin gene expression in chicken, mice, and baboons (Frenette and George 2007). When arginine butyrate was administered to patients with SCD intermittently (four days every four weeks), it resulted in sustained induction of HbF production in a majority of patients (Atweh *et al.*, 1999). In spite of the considerable promise of this agent in the treatment of SCD, the difficulty of administrating large volumes of this drug through central venous catheters poses a major therapeutic challenge. It is unlikely that the full potential of butyrate and other HDAC inhibitors will be realized until an oral preparation is identified (Frenette and George 2007).

d) Decitabine

Small scale clinical trials have shown that treatment with decitabine (5-aza-2-deoxycytidine- a new analog of 5-azacytidine) resulted in significant increases in mean γ-globin synthesis, HbF levels, and the number of F cells (RBC that contain HbF) (Frenette and George 2007). Increased HbF levels were observed in 100% of patients with SCD who received decitabine, including patients who had previously failed to respond to hydroxyurea. The increase in the levels of HbF was associated with significant improvement in RBC adhesion, endothelial damage, and activation of the coagulation pathway (Sauntharajah *et al.*, 2003). Larger and longer-term studies are needed to confirm the efficacy and safety of decitabine in the treatment of SCD.

12. Prevention of dehydration

One of the distinguishing characteristics of SCD is the presence of dense erythrocytes, formed as a result of cell dehydration and loss of potassium (K^+). These dense red cells generally have a low HbF content and a high percentage of irreversible sickle cells (ISCs). An inverse correlation has been demonstrated between percentage of ICSs and erythrocyte survival. In addition, the dense, dehydrated red cells might be easily trapped in post capillary venules, promoting microvascular obstruction. Thus, preventing of red cell dehydration presents an exciting possible new therapeutic strategy (DeFranceschi, 2009). In SCD, the anti-adherence therapeutic strategies can be divided into:

a) **The imidazole antimycotic clotrimazole** - has been shown to be a specific inhibitor of the Gardos channel and to prevent sickle cell dehydration *in vitro* (DeFranceschi, 2009).

b) **Senicapoc: a novel Gardos channel inhibitor -** limits solute and water loss, thereby preserving sickle red blood cell (RBC) hydration (Ataga *et al*., 2008).

c) **L-Arginine** - supplementation of transgenic sickle cell mice resulted in inhibition of erythrocyte Gardos channel activity and amelioration of red cell dehydration. However, phase II studies to test the effect of arginine supplementation have shown no major effects on Gardos channel function and erythrocyte hydration in patients with SCD (DeFranceschi, 2009). It has been used in dose of 0.1 g/kg three times daily to decrease pulmonary artery systolic pressure by 15% in a small study of sickle cell patients (Machado, 2009).

13. Bone marrow transplantation (BMT)

BMT involves replacing defective SC producing bone marrow with bone marrow that produces normal RBCs (Lucarelli *et al*., 1990). For many years, such an approach was considered too risky for a non-malignant disorder such as SCD, since the mortality of the procedure itself was around 20%. The reduction in mortality resulting from recent advances in immunosuppressive therapy and supportive care and the fact that long-term survival of patients with β-thalassaemia after BMT was shown to be greater than 90% (Lucarelli *et al*., 1990), resulted in renewed interest in this therapy for SCD. It can be curative, but it still presents problems. Long-term follow-up is lacking, and the procedure is expensive and not widely available. Another serious

barrier to the use of bone marrow transplantation is the frequent lack of a human leukocyte antigen matched sibling as donor in the individual case (Walters *et al*., 1996) as well as the high cost which will not be affordable by majority in the developing countries like Nigeria.

14. Gene therapy

Autosomal recessive disorders e.g. SCD are good candidates for gene therapy because a normal phenotype can be restored in diseased cells with only a single normal copy of the mutant gene (Olowoyeye and Okwundu 2010). The challenge of replacement gene therapy for SCD is to ensure viral transduction into hematopoietic stem cells (HSCs) and to generate safe, stable, erythroid-specific replacement gene expression at a level that is sufficient to have a clinical effect. The necessity for fulfilling all these criteria may make this genetic disorder among the most complex to treat successfully by gene therapy (Walters, 2005). Gene therapy continues to be studied as a way to inactivate the sickle gene and to increase expression of the gene for HbF (Olowoyeye and Okwundu 2010).

15. Stem cells

Since the first report of successful BMT for SCA 20 years ago, stem cells curative therapy has emerged as an important option in treating individuals who inherit this disorder. While its curative potential distinguishes it from therapeutic alternatives, such as RBC transfusions and hydroxyurea; the acute and late toxicities of transplantation raise concerns about its wider use (Walters *et al*., 2002). The use of cord-blood stem cells might avoid some of the problems of bone marrow transplantation (Brichard *et al*., 1996). Non-ablative marrow infusion, rather than total marrow replacement, also shows promise.

16. Anti adherence therapy

Vaso-occlusive episodes are central events in the pathophysiology of SCD associated with abnormal adhesive interactions between erythrocyte, reticulocytes, endothelial cells, platelets or soluble mediators which may represent a possible new therapeutic target. The end-point of anti-adherence therapy is to interfere with the initialization and/or amplification of adhesive events. These strategies can be divided into:

a) **Molecules interfering with chemical-physical processes during erythrocyte-endothelial adhesion events**

Non-ionic surfactant block copolymer such as RheothRx (Poloxamer 188) lowers viscosity and frictional forces in RBCs which improves microvascular blood flow. Phase II studies have shown a limited favourable effect in treatment of acute pain crises when compared with hydroxyurea in sickle cell children. However, no further clinical development studies are planned for this compound (De Franceschi, 2009).

b) Molecules interfering with sickle cell-endothelial adhesive mechanisms

Sickle cell-endothelial adherence might be blocked by anionic polysaccharides such as high molecular weight dextran sulfate or chondroitin sulphate, or heparin (De Franceschi, 2009) .

c) Molecules modulating inflammatory pathways involved in sickle cell-endothelial adhesion

Chronic inflammatory states have been described in SCD patients. These factors participate to affect leukocyte chemotasis, modulate vascular tone and contribute in sickle cell related tissue damage. Thus, anti-inflammatory therapy has been proposed to interfere with inflammatory storm and abnormal vascular activation. e.g. Sulfasalazine (De Franceschi, 2009).

d) The heme-oxygenase-1 connection

Heme-oxygenase-1 is a cytoprotective gene which has been shown to increase in response to chronic inflammatory stress characterizing SCD. Belcher *et al.,* (2005) have shown up-regulation of cytoprotective gene as heme oxygenase-1 (HO-1) and a reduction of the sickle cell related organ damage when pathological mice were treated with heme oxygenase-1 products. However, this is still being studied (De Franceschi, 2009).

2.2 Micronutrients

Micronutrients are nutrients needed throughout life in small quantities. They are dietary minerals which are essential for normal growth and body functions. They are needed in very small quantities as opposed to macro-minerals which are required in larger quantities. The micronutrients include vitamins, iron, cobalt, chromium, copper, iodine, manganese, magnesium, selenium, zinc and molybdenum.

2.2.1 Introduction

"Micronutrients" is the collective term applied to essential vitamins and trace minerals. Inadequate intake of them is now recognized as an important contributor to the global burden of diseases through increased rates of illnesses and deaths from infectious diseases, and in disabilities such as mental impairment (Black, 2003). Micronutrient malnutrition affects approximately 2 billion people worldwide (Howson, *et al*., 1998). Under-nutrition and infectious diseases exist in a baleful synergy. Under-nutrition reduces immunological capacity to defend the body against diseases, and diseases deplete and deprive the body of essential nutrients (Ezzati *et al*., 2002). Under-nutrition and infectious diseases further exacerbate poverty through lost wages, increased health care costs, and most insidiously impaired intellectual development that can significantly reduce earning potential. Deficiencies of micronutrients are highly prevalent in low- and middle-income countries and are globally the most important risk factors for illness and death from infectious diseases by reducing immune and non-immune defences and by compromising normal physiology or development (Muller and Brugnara 2001). The adverse squeal of micronutrient deficiencies are profound and include premature death, poor health, blindness, growth stunting, mental retardation, learning disabilities, and low work capacity (Darnton-Hill *et al*., 2005).

People with SCA suffer from many nutrient deficiencies, but preliminary research on dietary habits shows that food and nutrient intake by sickle cell patients in general meets or exceeds recommendations and is not significantly different from healthy controls (Prasad, 1997). This suggests that the higher rate of nutrient deficiencies may be due to an increased need for many nutrients in sickle cell patients (Hsu and Muller 2001). The effectiveness of dietary interventions in supplying adequate nutrition to meet these higher demands needs to be continually examined.

So far, some researchers believe that the same antioxidants and anti-clotting foods that help prevent heart disease, stroke, and cancer also help reduce health problems caused by SCD (Hsu and Muller 2001). In SCDs, extra calories are needed. Recent research shows that children with sickle cell need about 20% more calories than other children to fuel their production of red blood cells to replace the damaged, sickled ones (Muller and Brugnara 2001). Not getting enough calories may lead to delays in growth and maturation. The average energy intake of sickle cell patients is typically below the suggested allowance for calories during the quiescent phase of the disease, and it drops to roughly half the recommended levels during times of illness requiring hospitalization (Prasad, 1997).

2.2.2 Micronutrient Deficiencies

Micronutrient deficiencies which are a constituent of malnutrition continue to be a major health burden in developing countries (Young *et al*., 2004). They are now recognised to be an important contributor to the global burden of diseases (UNICEF, 1999). Over a decade ago, the World Bank publicly announced that vitamin deficiencies deprive one billion people worldwide of their intellect, strength, and vitality. Of this population, pregnant women and children are often and unfortunately the most affected (Hale, 2008). Micronutrient deficiencies also occur in other special groups like those with Human Immuno Deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) patients as well as SCD patients primarily due to altered metabolisms and increased requirements (Patrick, 1999).

2.2.3 Epidemiology

Micronutrient deficiencies affect at least 2 billion people worldwide (Muller and Brugnara 2001). Globally, 740 million people are deficient in iodine (Underwood, 2003), including up to 300 million with goitre and 20 million with brain damage from maternal iodine deficiency during their foetal development. About 1 billion people are deficient in zinc (Prasad, 1998); 3.5 billion have iron-deficiency anaemia and Vitamin A deficiency affects 140- 250 million (Underwood, 2003) mainly young children and pregnant women in developing countries (Darnton-Hill *et al*., 2005).

Malnutrition is consequently the most important risk factor for the burden of diseases in developing countries. It is the direct cause of about 300 000 child deaths per year and is

indirectly responsible for about half of all deaths in young children (Muller and Brugnara 2001). Micronutrient deficiencies among pregnant women are widespread in low-income countries (Kapil, 2009).

2.2.4 Micronutrient Deficiencies and Sickle Cell Disease

Finding a widely available cure for SCD still remains a challenge one hundred years after its discovery as a genetically inherited disease, creating growing interest in seeking nutritional alternatives (Hyacinth *et al*., 2010). Not only do protein–energy malnutrition and micronutrient deficiencies overlap, but a lack of one micronutrient is typically associated with deficiencies of other micronutrients (e.g. anaemia due to iron deficiency often coexists with zinc deficiency) (Muller and Brugnara 2001). Deficiencies in micronutrients are associated with increased risks of morbidity and mortality (Muller and Brugnara 2001).

Micronutrient status in people with SCD may need correction. Blood levels of several vitamins and minerals are often low in individuals with SCD, including vitamin A, carotenoids (Gray *et al*., 1992), vitamin B6, vitamin C, magnesium, and zinc (Prasad, 1997). These deficiencies cause a significant depreciation in blood-antioxidant status in these patients, and the resulting oxidative stress may precipitate vaso-occlusion-related-crises (Romero *et al*., 2000). Studies indicate that vitamin-mineral supplements of certain nutrients (vitamins C and E, zinc, magnesium) or treatment with a combination of high-dose antioxidants can reduce the percentage of irreversibly sickled cells and hence the incidence of crises (De Franceschi, 1997).

2.3 Magnesium

Magnesium (Mg) is a mineral that is essential for proper functioning of the body. Magnesium is found inside every living cell in the body. It is alkaline and binds acids in the body, regulating acid-alkaline balance. Some of the major functions of magnesium in the body are: calcium, potassium, sodium and vitamin C utilization, bone, protein, and fatty acid formation, activation of B vitamins (Okochi and Okpuzor 2005). In addition, magnesium plays a role in efficient production of enzymes, cardiac function, relaxation of muscles (including the heart muscle-thus has some blood pressure lowering effects), making new cells and prevention of aging in the cells and organs (Rude, 1993).

2.3.1 Magnesium and sickle cell diseases

One of the distinguishing characteristics of SCD is the presence of dense erythrocytes (De Franchesci, 2009). These can be formed as a result of a reduction in erythrocyte magnesium which activates the K-Cl co-transporter, causing dehydration of erythrocytes characterised by an increase in the concentration of HbS (Joiner *et al*., 1998), lower HbF content, and a high percentage of irreversible sickle cells (ISCs) (De Franchesci, 2009). The dense dehydrated RBCs so formed are easily trapped in post capillary venules, promoting micro-vascular obstruction (Joiner *et al*., 1998).

Studies on K-Cl co-transport function have identified reduced cell magnesium (Mg^{2+}) content as one of the triggers of activation (Brugnara *et al*., 1993). Thus, prevention of red cell dehydration via magnesium supplementation represents an exciting possible new therapeutic strategy. In a study by De Franceschi *et al*., (1997), administration of oral magnesium (0.6mEq/kg/day of Magnesium Pidolate) caused a significant increase in sickle erythrocyte magnesium. This change was associated with a significant increase in potassium, a reduction in erythrocyte K-Cl co-transport activation, and a significant reduction in absolute reticulocyte count. A reduction in the K-Cl co-transport activation will reduce RBC dehydration (Brugnara *et al*., 1993). Reticulocyte count is usually high in SCA (Konotey-Ahulu, 1991), thus the significant reduction in absolute reticulocyte count caused by Mg supplementation in the study by De Franceschi *et al*., (1997) reversed the high reticulocyte count. Oladipo *et al*., (2005) also reported use of magnesium in clinical practice to reduce erythrocyte dehydration in SCD.

2.3.2 Other important uses of magnesium

Magnesium has been used primarily in the management of the following conditions: cardiac arrhythmias (Ford, 1999), congestive heart failure, diabetes (there is an increased loss of magnesium in urine due to hyperglycaemia) (American Diabetes Association, 1999), as adjunct in therapy of Attention Deficit Hyperactivity Disorder (ADHD), anaemia, angina, high blood pressure (Simopoulos, 1999) (especially for people taking potassium depleting diuretics).

2.3.3 Magnesium deficiency

Magnesium deficiency may be caused by diminished intake, reduced gastrointestinal absorption and increased losses (laxative abuse, chronic diarrhoea (Rude and Olerich 1996), small bowel resection or bypass, malabsorption syndromes-as seen in pancreatitis, and renal wasting (Rude, 1993). It is also seen in alcoholics (30-60%), diabetes mellitus, and in people with major burns (Elisaf *et al*., 1998). 80-90% of the United States population is magnesium deficient (Evers, 2002).

Deficiencies of magnesium that are serious enough to cause symptoms should be treated with supplements (Weisinger Bellorin-font 1998). Some of these symptoms include, fatigue, abnormal heart rhythms, muscle weakness and spasm, depression, loss of appetite, nausea, vomiting, listlessness, (Saris *et al*., 2000), muscle contractions, hypocalcaemia, and hypokalaemia (Shils, 1999).

2.3.4 Magnesium toxicity

Magnesium taken in from dietary sources is unlikely to present with any signs of toxicity. However, oral magnesium supplements when taken excessively can cause diarrhoea, abdominal cramping, changes in mental status, difficulty breathing (Jaing *et al*., 2002), and may compete with other minerals like calcium for absorption. Vitamin B6 increases the amount of magnesium absorbed by the cells and so can be taken together. Risk of magnesium toxicity increases with kidney failure, because magnesium is solely excreted by the kidneys (Xing and Soffer 2001).

2.3.5 Natural sources of magnesium

Many nutritionally oriented doctors recommend 250–350 mg per day of supplemental magnesium for adults. Some sources include: nuts, grains, beans, dark green vegetables, fish, meat, avocados, banana, cashews, enriched cereals, and spinach, (Okochi and Okpuzor 2005).

2.3.6 Available forms of magnesium

The amount of elemental magnesium in a compound and its bioavailability influence the effectiveness of the magnesium supplement. In a study that compared the bioavailability of different forms of magnesium preparations, magnesium oxide had the highest bioavailability.

This was followed by magnesium carbonate, magnesium hydroxide and magnesium citrate. Magnesium lactate and magnesium chloride followed with a similar bioavailability, while magnesium sulphate had the lowest bioavailability (Firoz and Graber 2001). In the same study, magnesium chloride was found to be the most readily absorbed form. This supports the belief that both the magnesium content of a dietary supplement and its bioavailability contribute to its ability to restore deficient levels of magnesium.

2.4 Zinc

Zinc is an essential trace mineral that can be derived from food. Next to iron, zinc is the most common trace mineral in the body and is found in every cell (Prasad, 1998).

2.4.1 Zinc and sickle cell disease

People who have SCD are often deficient in zinc. Prasad *et al*., in 1975, first reported that HbSS patients had decreased zinc levels in plasma, erythrocytes and hair associated with increased urinary excretion (hyperzincuria) compared with controls. He noted clinical similarities between patients with zinc deficiency and those with SCD. Subsequent findings indicated a combination of hyperzincuria, high protein turnover (due to increased haemolysis) and inadequate dietary intake as contributing to the significantly increased zinc requirement demonstrated in the HbSS patients (Prasad *et al*., 1999). 44% of children with SCD (Leonard *et al*., 1998), and 60-70% of adults with SCD (Prasad, 2002) suffer from zinc deficiency. In children with SCD, low plasma zinc may be due to poor nutritional status (Zemel *et al*., 2002). Studies suggest that taking zinc supplements may help reduce symptoms of the disease and children who took zinc showed improvements in height and weight, and had fewer sickle cell crises (Zemel *et al*., 2002). Trials have shown that zinc supplementation results in improved growth in children, lower rates of diarrhoea, malaria, and pneumonia, and reduced child mortality (Sazawal *et al*., 2001).

Serjeant *et al*., (1970) successfully demonstrated that oral zinc supplements improved epithelial wound healing in SCD patients in the West Indies three times faster than in controls. In 1981, Prasad *et al*. showed that zinc supplementation in patients with SCA seemed to prolong red cell life span and reduced vascular obstruction and also caused maturation of gonads. In a study by Konote-Ahulu (1991) in Ghana, correction of zinc deficiency was seen to produce weight gain and improvement of general well being in patients with SCD.

Prolonged zinc supplementation decreases the incidence of infections, number of hospitalizations and the number of vaso-occlusive pain crises in patients with SCD (Prasad *et al*., 1999). Infections are usually characterised by the presence of bacteria, increased white blood cells and fever-all of which are factors that enhance sickling. Thus, by increasing immunity against infection, zinc reduces sickling and frequency of crises (Saper and Rash 2008). In 2008, Bao *et al*., demonstrated that zinc supplementation to adult SCD patients decreased the incidences of infections and hospital admissions. In the same study, red blood cell, haemoglobin (Hb), hematocrit, plasma zinc, and antioxidant activity increased. Zinc has been shown to reduce the number of irreversibly sickled cells (ISCs) in the blood and to increase the survival of RBCs. It does this by antagonizing calcium binding to the red cell membrane (an important step in the formation of irreversibly sickled cells) (Brewer and Oelshlegel 1974). In SCA, there is increased oxidative stress and peroxidation as well as low antioxidant potential which predisposes the patients to vaso-occlusive crisis (Adelekan *et al*., 1989). Zinc exerts its antioxidant action by inhibition of lipid peroxidation which occurs in red blood cells and liver thereby stabilizing biomembranes and biostructures thus protecting the body against oxidative stress (Hasanato, 2006). These effects of zinc are believed to give zinc its ability to reduce vaso-occlusive crisis in SCA.

2.4.2 Other important uses of zinc: Zinc can prevent and palliate diarrhoea and pneumonia (Zinc Investigators' Collaborative Group, 1999) and also may reduce malaria morbidity in young children (Caulfield *et al*., 2004). Improvements in growth have been demonstrated (Brown *et al*., 2002), which may operate directly or indirectly through increased immune function and decreased infectious diseases. It has been used since ancient times to help heal wounds. It has antioxidant properties; therefore it helps protect cells in the body from damage caused by free radicals (Shankar and Prasad, 1998). It plays a central role in reproduction, growth, taste, vision, smell, blood clotting, proper insulin and thyroid function, and in the function of cells mediating unspecific immunity, including neutrophils and natural killer cells, and is needed for specific immune processes, such as balancing T helper cell functions (Prasad, 1998).

2.4.3 Zinc deficiency

The importance of zinc deficiency is being increasingly recognized. In total about 800,000 child deaths per year are attributable to zinc deficiency (Black, 2003). Globally, about 2 billion people

are deficient in zinc and zinc deficiency is the attributable cause of 1.9% of global burden of diseases (Muller and Brugnara 2001).

Zinc deficiency results from inadequate intakes (e.g., in populations whose primary diet is plant based, as plants are a poor source of dietary zinc) and to some extent, due to increased losses either through urine (in SCDs and during systemic infections) (Hambridge, 2000) or through faeces (in diarrhoea). In SCD, zinc deficiency has been attributed to reduced appetite (hence reduced intake) and haemolysis. During sickle cell crises, there is an increased demand and utilisation of zinc (Temiye *et al*., 2011). In addition, there is loss of zinc in the urine due to impaired renal concentration and hypoxanthinuria (Essien, 1995).

Consequences of zinc deficiency include loss of appetite, poor growth, weight loss, lack of taste or smell, poor wound healing, skin problems (such as acne, atopic dermatitis and psoriasis), hair loss, lack of menstrual period, night blindness, white spots on the fingernails, depression (Miyata, 2007), impaired immune function, hypogonadism, cognitive dysfunction, and susceptibility to infection (Shankar and Prasad 1998).

2.4.4 Zinc toxicity

Acute toxicity is fairly rare and is usually caused by food poisoning of extremely high intakes such as 225-450mg of zinc. Dietary zinc in large doses leads to copper deficiency by antagonizing copper absorption, resulting in hypocupraemia and iron deficiency anaemia. Thus, it is recommended that 2 mg of copper should be taken along with a zinc supplement. This effect is however reversed on discontinuation of the zinc (Edwin *et al*., 1991). Copper deficiency, iron-deficiency anaemia, neutropenia, deficient immune function, and undesirable changes in the ratio of low density lipoprotein (LDL) to high density lipoprotein (HDL) cholesterol have all occurred with increased zinc intake (Fosmaire, 1990).

2.4.5 Natural sources of zinc

The recommended daily allowance for adults is 8 - 11 mg (Hambridge, 2000), 20-40% of which the body absorbs from food. The best sources of zinc are oysters (richest source), red meats-especially beef, lamb and liver, poultry, cheese (ricotta, swiss, gouda), shrimp, crab, and other shellfish. Zinc is also found in legumes (especially lima beans, black-eyed peas, soybeans, and

peanuts), whole grains, tofu, brewer's yeast, cooked greens, mushrooms, green beans, tahini, pumpkin, almonds, blackcurrant, and sunflower seeds. Thus, as with iron deficiency, populations consuming a primarily plant-based diet are susceptible to having zinc deficiency.

2.4.6 Available Forms of zinc

Zinc is available in several forms. Zinc sulphate is the least expensive form, but it is the least easily absorbed and may cause stomach upset. More easily absorbed forms of zinc are zinc picolinate, zinc citrate, zinc acetate, zinc glycerate, and zinc monomethionine.

2.5 Copper

Copper (Cu) was established as an essential nutrient in the 1920s and 1930s (Danks, 1988). It is the third most abundant trace mineral in the body. It is involved in numerous biochemical reactions in human cells, and is a component of multiple enzymes, which contributes to the functions of many antioxidants in the body (Okochi and Okpuzor 2005). Copper is involved in the absorption, storage, and metabolism of iron (Fe). Via cearuloplasmin, Cu is able to oxidise Fe before being transported in the blood to all the tissues. Thus, deficiency of copper can lead to iron deficiency anaemia. Copper deficiency is unusual in people with an adequate diet but low levels in serum do occur (usually in association with Menke's syndrome) and can lead to severe anaemia (Romero *et al*., 2000).

2.5.1 Copper and sickle cell diseases

In SCA, zinc deficiency may be common, and since copper levels are commonly reciprocal to the zinc level, it is assumed that SCA patients may suffer from an excessive amount of copper (Hasanato, 2006). Akenami *et al*., (1999) in a study in Ibadan found significantly lower serum zinc concentration and significantly higher serum copper in haemoglobin S patients compared with controls (HbA).

Anaemia and shortened survival rates are the major manifestations of copper deficiency in both animals and human beings. The biochemical mechanism that results in the reduced life span of RBCs in copper deficiency anaemia is very complex (Jaing *et al*., 2002). At least in part the anaemia appears to be due to defective iron utilization. The shortened RBC survival is due to the fact that copper deficiency results in reduction of the enzyme superoxide dismutase in charge of

detoxifying free radicals, and lecithin-cholesterol acyl transferase which functions to esterify free cholesterol (Bettger *et al*., 1978). Thus there will be a resultant accumulation of free radicals leading to peroxidation of RBC-membrane lipids and accumulation of free cholesterol respectively. This resultant cumulative elevation in the amount of lipids leads to a decrease in membrane fluidity, and hence increased viscosity of the RBCs. This will impair passage of RBCs through narrow splenic sinusoids, reduce RBC survival, and contribute to the anaemia of copper deficiency (Stern *et al*., 2007). Thus, copper is essential for antioxidant effects, regulation of cholesterol, boosting iron absorption, and for red blood cell production.

2.5.2 Other important uses of copper

Medicinal use of copper compounds dates to Hippocrates in 400 B.C. Hospitals historically installed copper-alloy doorknobs and push-panels as a measure to prevent transmission of infectious diseases, because, bacterial growth is inhibited on copper's surface. Other uses include, the use of copper bracelets in the treatment of arthritis, childhood growth promotion, as an immune booster, as a supplement to treat infants who develop marasmus (malnutrition). It is also used in Menkes' kinky-hair disease (a rare disorder of copper transport/absorption), treatment or prevention of osteopenia and other abnormalities of bone development related to copper deficiency (Stern *et al*., 2007).

2.5.3 Copper deficiency

Copper deficiency can occur in infants fed on only cow-milk formulas (which are relatively low in copper content) and in premature/low-birth weight infants. It commonly occurs in prolonged or chronic diarrhoea, malnutrition, malabsorption syndromes(including celiac disease, sprue, or short bowel syndrome), high dose zinc supplements, high vitamin C intake, high use of antacids, burns, and kidney disease (Stern *et al*., 2007). Symptoms of copper deficiency include: immune system dysfunction, brittle bones in children, vascular diseases, high cholesterol levels, poorly pigmented skin, and anaemia (Valerie, 2011).

2.5.4 Copper toxicity

Copper levels are tightly regulated in the body, thus, its toxicity is rare in the general population (Stern *et al*., 2007). Wilson's disease is a genetic disorder in which the body cannot rid itself of

copper, resulting in deposition in organs and serious consequences such as liver failure and neurologic damage (Stern *et al*., 2007). Obstruction of bile flow, contamination of dialysis solution (in patients receiving haemodialysis for kidney failure), Indian childhood cirrhosis (presence of excess copper binding proteins in the liver), and idiopathic copper toxicosis are other rare causes of potentially dangerous excess copper levels. Such individuals should be closely monitored by a physician and nutritionist (Okochi and Okpuzor 2005). Copper toxicity presents with haemolytic anaemia, abdominal pain, nausea, vomiting, diarrhoea, dementia, muscular pain; and in severe cases liver damage, kidney failure, coma and death. Copper toxicity can be treated with dimercaptosuccinic acid (DMSA), penicillamine, or Ethylene Di-amine Tetra Acetic Acid (EDTA) (heavy metal detoxification), methionine (lowers serum copper), vitamin C, zinc and manganese (interfere with copper absorption), molybdenum (intake of molybdenum at doses as low as 0.54mg per day has been associated with an increased loss of copper in urine), and pyridoxine (50mg daily increases copper excretion) (Stern *et al*., 2007).

2.5.5 Natural sources of copper

Most diets contain enough copper (2-5mg daily) to prevent a deficiency and not enough to cause toxicity. The World Health Organization (WHO) suggests that 10-12mg per day may be the upper safe limit for consumption (Stern *et al*., 2007). Copper is a mineral that occurs naturally in many foods, including vegetables, legumes, nuts, grains, and fruits, as well as shellfish, avocado, and beef (organs such as liver). Because copper is found in the earth's crust, most of the world's surface water and ground water used for drinking purposes contains small amounts of copper (Okochi and Okpuzor 2005).

2.5.6 Available forms of copper

Some available forms of copper include: Copper bands, copper sulphate, copper carbonate, copper gluconate, cupric oxide, copper amino acid chelates.

2.6 Iron

Iron is the most common element on earth. Almost two-thirds of iron in the body is found in haemoglobin (Dallman, 1986).

2.6.1 Iron and sickle cell disease

Iron deficiency anaemia is uncommon in individuals with SCD because of availability of an adequate iron source potentially from increased red cell turnover and from blood transfusions. However, when present, it can often go unnoticed because the SCD patients are already anaemic (Mohanty *et al*., 2008). One third of the haemolysis in SCA is intravascular and the resulting urinary losses of iron may lead to iron deficiency. According to Mohanty *et al*., (2008), this may result in lowering the intracellular haemoglobin concentration and this may ameliorate sickling. The lability of serum iron during infection is well known. In view of the ubiquitous requirement for iron by microbes infecting humans (Tomkins, 2003), some investigators have hypothesized that deficiency protects against infectious disease or that iron supplementation increases infectious disease (Caulfield *et al*., 2004). The clinical improvement in SCA following the induction of iron deficient erythropoiesis by repeated phlebotomies or by erythrocytapheresis has been reported (Koduri, 2003). In the same study, the investigator concluded that there was no evidence of iron overload in SCA and iron deficiency may be more common than suspected (Koduri, 2003). Conversely, the human body has no effective physiological mechanism for excreting excess iron. Therefore, in conditions such as SCD, where transfusions are frequently indicated, exogenous iron can accumulate in circulation and enter tissues (such as the liver, endocrine organs, and heart). They may form reactive oxygen species (ROS) in these tissues/organs and result in end organ damage by ROS-mediated lipid peroxidation (Hershko *et al*., 2005). The ROS also cause severe membrane damage and worsening of haemolysis in HbSS patients (Hyacinth *et al*., 2010). Chelation therapy is routinely employed to prevent and treat iron overload in chronically transfused SCD patients. Deferoxamine was the first chelating agent to be introduced; it is poorly absorbed orally and so is administered parenterally. Oral alternatives include Deferasirox (an FDA approved oral iron chelator) and Deferiprone (an oral iron chelator licensed for use in Europe) (Ballas, 2001).

2.6.2 Other important uses of iron

Iron is essential for the production or function of haemoglobin, myoglobin, and various enzymes, binding and transport of oxygen, production of adenosine triphosphate (ATP), treatment and prevention of anaemia, as well as for the regulation of cell growth and differentiation (Beard 2001).

2.6.3 Iron deficiency

Iron deficiency is considered the leading cause of anaemia worldwide, especially in children and adult women (Kassim *et al*., 2012). The World Health Organisation (WHO) estimates that worldwide there are 2 billion individuals with anaemia and up to 5 billion who are iron deficient (Black, 2003). Common causes of iron deficiency include: poor diet, elevated needs (e.g., while pregnant, in early childhood), chronic loss from parasitic infection (e.g., hookworm, schistosomiasis, whipworm) (Muller and Brugnara 2001), insufficient absorption of iron or excess loss (Branca and Ferrari 2002). Consequences of iron deficiency include impaired physical growth, potentially permanent adverse effects on neurological functions involving cognition and emotional behaviour, lowered immunity resulting in increased susceptibility to infections, and most commonly anaemia (Darnton-Hill *et al*., 2005).

Iron deficiency anaemia can be prevented by taking foods rich in iron, preventing malaria, preventing parasitic infestations such as hookworm, taking iron supplements by those who have increased requirements e.g., pregnant women and children who are not breast feeding (Akpotuzor *et al*., 2007).

2.6.4 Iron toxicity/over load

There is considerable potential for iron toxicity because very little iron is excreted from the body. Thus, iron can accumulate in body tissues and organs such as the abdomen, heart and liver, when normal storage sites are full which leads to damages in these organs causing abdominal pain and disturbances, hematemesis, hepatic portal fibrosis, cirrhosis, liver failure, respiratory distress syndrome, metabolic acidosis, shock, coma, and even death (Cheney *et al*., 1995).

2.6.5 Natural sources of iron

Iron is present in both heme and non-heme forms in diet. Heme iron, the most bioavailable form, is found in the greatest quantities in animal sources such as red meat, kidney and liver. Non-heme iron (which is absorbed less efficiently than heme iron) is most abundant in other sources of iron, including eggs and all dark green vegetable leaves (e.g. spinach, parsley, broccoli), roots, seeds, millet, beans, pulses, nuts, dates, and fruits.

2.6.6 Available forms of iron

Iron supplementation is indicated when diet alone cannot restore deficient iron levels to normal within an acceptable timeframe. Supplemental iron is available in two forms: ferrous and ferric. Ferrous iron salts are the best absorbed forms and include ferrous fumarate, ferrous sulfate, and ferrous gluconate containing 33%, 20%, and 12% of elemental iron respectively (Porter, 2011).

2.7 Strategies to Overcome the Problem of Micronutrient Deficiencies

Malnutrition increases morbidity and mortality and affects physical growth and development, some of these effects resulting from specific micronutrient deficiencies (Bhan *et al*., 2001). While public health efforts must be targeted to improve general dietary intakes, there is a need for additional measures to increase the intake of certain micronutrients. Food-based approaches such as dietary diversification through home gardens and small livestock are regarded as the long-term strategy for improving nutrition. But for certain micronutrients, supplementation, be it to the general population or to high risk groups or as an adjunct to treatment must also be considered (Bhan *et al*., 2001). The fortification of salt with iodine has been a global success story, but other micronutrient supplementation schemes have yet to reach vulnerable populations sufficiently. Plant breeding to increase the content and bioavailability of iron and other micronutrients holds great promise (Graham and Welch, 1996). To be effective, all interventions require accompanying nutrition-education campaigns and health interventions. In order to achieve the hunger and malnutrition related Millennium Development Goals, which are clearly associated with the insecure supply of food and nutrition, poverty needs to be addressed. A major pitch to end this health crisis in Nigeria is the implementation of low-cost micronutrient programs including food fortification, dietary diversification, supplementation and education (Hale, 2008).

2.7.1 Food fortification

Food fortification by nature is the process of adding vitamins and minerals to a staple food eaten by a majority of the population. During the food processing stage, beneficial vitamins and minerals are added to the staple foods and distributed to the population; thus, it requires the active participation of the food industry. Recent studies in Southern and Western Africa show that adding vitamin A to sugar and vegetable oil can lower the risk and severity of maternal

mortality, anaemia, and long-term effects of HIV/AIDS (Howson *et al*., 1998). Just a teaspoon of oil or sugar enriched with vitamin A taken twice a day can boost a child's immune system and deliver about one-third of their daily needs for vitamin A potentially saving their lives (UNICEF, 2007). Field fortification strategies can also be used to increase the content of certain trace elements in cereal grains by applying fertilizers to the soil to increase its content of selenium, iodine, and zinc, or to the leaves to enhance their iron content. Alternatively, plant-breeding can be employed to produce new varieties of cereal grains, orange-fleshed sweet potatoes and cassava roots, with an increased content of iron, zinc, and/or beta-carotene (Gibson and Hotz 2001).

2.7.2 Dietary diversification

Dietary diversification and changes in meal composition require individuals, families, and communities to change eating behaviour in their unique cultural context. Dietary diversification/modification is an approach that aims to enhance the availability, access, and utilization of foods with a high content and bioavailability of micronutrients throughout the year. It involves changes in food production practices, food selection patterns, and traditional household methods for preparing and processing indigenous foods. To implement these strategies effectively, knowledge of the local dietary patterns, and food beliefs, preferences and taboos is required, as well as the ability to change attitudes and practices. Dietary diversification/modification may be more sustainable, economically feasible, and culturally acceptable than supplementation or fortification and can be used to alleviate several micronutrient deficiencies simultaneously without risk of antagonistic interactions (Gibson and Hotz 2001). Food fortification (with the exception of iodized salt) and dietary diversification are not appropriate as therapeutic measures, but can be successful as sustainable preventive strategies to control micronutrient malnutrition (Howson *et al*, 1998).

2.7.3 Food supplementation

Supplementation refers to the addition of pharmaceutical preparations of nutrients (capsules, tablets, or syrups) to the diet. Research has shown supplementation of adequate dosage and duration to be efficacious in treating, correcting, and preventing deficiencies of iron, vitamin A, and iodine for groups in which there are serious health problems (Howson *et al*, 1998). There has

been a growing consensus on the need to provide a multiple micronutrient supplement to women on a daily basis during pregnancy and on a weekly basis ahead of pregnancy; there is clear evidence that these nutrients are strongly related to safe motherhood (reducing anemia and other maternal health concerns) and pregnancy outcomes (Mason *et al*., 1999).

A major challenge will be to scale up supplementation to a program level that achieves adequacy in target group coverage, dosage, and frequency of dosing that assures effectiveness, representing the combined impact of efficacy and the process of implementation (Howson *et al*., 1998). Supplementation has traditionally been considered "short- term," although it may usefully continue until effective alternatives are in place (Howson *et al*., 1998). Another challenge is that they would need to be taken regularly and frequently and side effects may be a constraint. Other constraints could include compliance, distribution, counseling, and the simple difficulty of taking tablets every day (Mason *et al*., 1999).

Strengths of supplementation include its immediate impact on micronutrient status, health, and survival ability. It can achieve rapid coverage in at-risk populations and be linked to the health care delivery system. In addition, the cost of worker training is relatively low, compared, for example, with that for dietary modification (Howson *et al*., 1998). This has been seen in countries where supplementation to prevent subclinical vitamin A deficiency has also been shown to dramatically reduce maternal mortality (Mason *et al*., 1999). On the other hand, because of inadequate targeting or coverage, deficient individuals may not be identified or reached routinely, and many at-risk persons, particularly in rural settings, can be missed. In addition, periodic high coverage has often not been sustained over time for a variety of reasons, including lack of sustained financial or political support or other overriding priorities in a limited health infrastructure (Howson *et al*., 1998). Supplementation is the method of choice when therapeutic treatment is necessary to address severe micronutrient deficiency.

2.7.4 Education

Another strategy to overcoming the problem of micronutrient deficiencies is education. Nigerians or other potentially affected population should be educated to make small changes in their diets and eating habits to protect themselves and their children against deficiencies (Howson *et al*., 1998). For this task, the Ministry of Health in collaboration with private

organisations and companies hold a responsibility for educating people about the benefits of food fortification through media campaigns, public health messages, and nutrition education programmes through primary and secondary schools.

2.7.5 Agricultural interventions

The goals of these are to increase the concentration of micronutrients and/or the efficiency of their utilization in the crop, decrease the concentration of absorption inhibitors such as phytates and increase the amount of promoter compounds (for iron and zinc in particular) such as sulphur containing amino acids. The technologies span from the selection of naturally occurring genetic variants with relatively high metabolic efficiency in the use of elements such as zinc; genetic modification of plants; and the creation of new foods using biotechnology to micronutrient fortification of fertilizers (Brown, 1998). Although extensive use of phosphorous-containing fertilizers is necessary for high yields of cereals, it can also induce zinc deficiency in crops, especially in many of the new high yielding varieties, and may also increase the phytate content of the grain used for human consumption. Adding zinc sulphate to zinc deficient soils can increase crop yields substantially and also, to some extent, increase the zinc content of the edible portions of the plants. This is an example of an agricultural intervention where producers benefit while consumers may experience a substantial health benefit. However, there are many challenges. Will farmers be interested in crop cultivars that have higher micronutrient concentrations or utilization efficiency but little or no yield advantage? Will the target population have access to or accept these new foods? Moreover, strategies that increase the concentrations of some micronutrients may have a negative impact on the bioavailability of others, such antagonism may be observed between copper, zinc and manganese. There is wide agreement in support of food-based approaches as the long-term strategy for improving nutrition in general and micronutriture in particular.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Site/Study Area

The study was carried out in the Haematology Department of Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria. ABUTH is a tertiary institution that comprises various specialised units. In collaboration with other departments, the Haematology Department hosts referrals from within and outside Nigeria.

3.1.2 Study population

The study population comprised three groups: SCD patients in painful crises, SCD patients in steady state (not in crises), and healthy volunteers with genotype HbAA. In this study, SCD patients in painful crises are those subjects who had pains at the time of recruitment or within 48 hours before recruitment in any of their limbs (Temiye *et al*., 2011), while steady state were subjects who were apparently well without evidence of recent infection, bone pain, or other apparent disease processes for at least 4 weeks before recruitment (Juwah *et al*., 2004).

3.1.2.1 Inclusion criteria

Sickle cell patients who attended sickle cell clinic at the Department of Haematology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria and who:

i. Had not been on any magnesium, zinc, copper, or iron - containing medications.

ii. Had not been transfused with blood or blood products in the previous three months preceding the study.

iii. Did not have any symptoms or signs of infection such as fever (auxillary temp <□37.0°C), acute respiratory infection, diarrhoea, or malaria.

iv. Understood and signed the informed consent.

Additionally, controls were subjects with genotype HbAA and who:

i. Had no evidence of infection or chronic diseases (protein energy malnutrition)

ii. Had neither been transfused or been on any magnesium, zinc, copper, or iron - containing medications in the previous three months preceding study.

iii. Understood and signed the informed consent.

3.1.2.2 Exclusion criteria – subjects who did not consent to partake in the study and those who do not meet inclusion criteria were excluded.

3.1.3 Sample size

The sample size for the study was determined using standard formulae for the calculation of minimum sample size (Oyejide 1991).

$$\text{Sample size } n = \frac{(Zi - a)^2 (P)(1-P)}{d^2}$$

where, n = minimum sample size

Zi – a = value of the standard normal deviation which at 95% confidence level has been found to be 1.96

P = the best estimate of the population prevalence obtained from literature review = 3% (0.03) (Ugwu and Eke 2007)

1-P = 1 – 0.03 = 0.97

d = difference between the true population rate and sample that can be tolerated; that is, the absolute precision required (in percentage point) on either side of the population 0.05

Sample size n = $\frac{(1.96)^2 \times 0.03 \times 0.97}{0.05^2} = 44.7$

Therefore, 45 sickle cell disease patients in crises, 45 sickle cell disease patients in steady state, and 45 apparently healthy volunteers (a total of 135) subjects comprised the sample population.

3.1.4 Study design

The research was approved by the Research and Ethics Committee of ABUTH and was a retrospective and prospective case controlled study.

3.1.5 Reagents

i. Amino-methyl-propanol; Ethylene Glycol Tetra-acetic Acid (EGTA) (magnesium buffer)- 1mmol/L; 0.21mmol/L – CHEMELEX, SOUTH AFRICA - LKBSDTT28

ii. Calmagite (chromogen)- 0.30mmol/L - CHEMELEX, SOUTH AFRICA

iii. Magnesium aqueous primary calibrator (Magnesium CAL.) - 2mg/dL - CHEMELEX, SOUTH AFRICA

iv. Zinc buffer (pH-8.6)- 0.2mmol/L - CHEMELEX, SOUTH AFRICA – LKBSDTT40

v. 2-(5-Bromo-2-pyridiylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino] phenol, disodium salt, dehydrate (5-Br-PAPS) – Colour – 1.1 mmol/L- CHEMELEX, SOUTH AFRICA – Ref.: 30400

vi. Ascorbic acid powder (reducing agent) - CHEMELEX, SOUTH AFRICA

vii. Zinc primary standard calibrator (Zinc CAL.) - 200µg/dL - CHEMELEX, SOUTH AFRICA

viii. Acetate (Copper buffer-pH-4.7)- 1mmol/L - CHEMELEX, SOUTH AFRICA – LKBSDTT39

ix. 3, 5- [4-(3, 5-dibromo-2-pyridylazo)-N-ethyl-N.sulfo- propylaniline – (3,5- DiBr-PAESA) – colour - 0.4mmol/L - CHEMELEX, SOUTH AFRICA – Ref.: 30205

x. Copper aqueous primary solution (Copper CAL.)- 100µg/Dl - CHEMELEX, SOUTH AFRICA

xi. Acetate (Iron buffer- pH 4.9)- 100mmol/L - CHEMELEX, SOUTH AFRICA – LKBSDTT24

xii. Iron aqueous primary standard (Ferrozine)- 40mmol/L - CHEMELEX, SOUTH AFRICA

xiii. Bleach

xiv. Distilled water

3.2 Methods

This was a retrospective and prospective case controlled study at the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. The study, which was approved by the research and ethics committee of the hospital, lasted for a period of 12 months. Ninety patients attending sickle cell clinic in the haematology department of ABUTH, who met the research criteria were enrolled after consent (both verbally and by signing the consent forms). Of these, 45 were in crises and 45 were in steady state. The control group consisted of 45 apparently well volunteers whose haemoglobin genotype was AA. Both the SCD patients in steady state and the HbAA volunteers were selected such that they were matched for age and sex with the SCD patients in crises. A detailed history of each subject was obtained from the subjects, their care-givers, and from the patients' records/folders. The history included the age at diagnosis of each SCD patient, number of blood transfusions per year, co-morbid illness experienced by each subject, and a detailed drug use history of each subject in the study. Data generated for all subjects was analysed using Microsoft Excel 2007.

Five ml of blood was collected from each subject by venepuncture after appropriate skin preparations. Two ml of blood was transferred into a sodium ethylene-diamine-tetra-acetate (EDTA) bottle for determination of full blood count (platelet count, white blood cell count,

neutrophil count, lymphocyte count, and packed cell volume) using standard laboratory procedure. The remaining three ml was transferred into plain bottles previously washed clean of possible micronutrient contamination by leaving them soaked in 10% nitric acid for 24 hours and rinsed three times with deionised water. The blood was then centrifuged at 1500 rpm for 10 minutes. The plasma was removed with a micropipette prepared by previously washing with 10% nitric acid and thoroughly rinsed with deionised water as described for the bottles above and transferred into another plain bottle prepared as above. Residual RBCs were used to determine genotype using solubility test followed by haemoglobin electrophoresis; while, the plasma was stored at 2-8ºC pending analysis (within seven days). All haemolysed samples were discarded. Before the analysis, the plasma samples were allowed to come to room temperature and each mixed by gently inverting the tube four times. Plasma magnesium, zinc, copper and iron levels were determined using a Beckman Coulter DU 520 colorimeter.

3.2.1 Preparation of Reagents

3.2.1.1 Magnesium:

The working reagent (WR) was prepared by mixing equal volumes of magnesium buffer and calgamite. The WR is stable for 4 days when stored at 2-8ºC or for 24 hours at room temperature (15-25 ºC). Magnesium CAL (the standard) is stable when stored in a tightly closed bottle at 2-8 ºC; care was taken during its use due to its ease of contamination, and it was protected from light.

3.2.1.2 Zinc:

The WR was prepared by dissolving 5ml of ascorbic acid powder in 1ml of zinc buffer. It is stable after reconstitution for 30 days at 2-8ºC; when stored tightly closed. 5-Br-PAPS is ready to use and is stable for 90 days at 2-8 ºC. Zinc CAL (the standard) should be handled with care due to ease of contamination.

3.2.1.3 Copper:

The WR was prepared by dissolving 5ml of ascorbic acid in 1ml of the copper buffer. WR is stable up to 15 days after reconstitution when stored at 2-8ºC in a tightly closed container. The 3, 5-DiBr-PAESA is ready for use and is stable for up to 90 days after opening when stored at 2-

8ºC and recapped immediately after use. Copper CAL. (the standard) is also ready for use and should be used carefully cause of its ease of contamination.

3.2.1.4 Iron:

The WR was prepared by dissolving the contents of 1m of ascorbic acid in 5ml of iron buffer. WR is stable for three months at 2-8ºC or for one month at 15-25ºC. Iron CAL. (the standard) should be used with great care due to its ease of contamination. All the reagents should be stored tightly closed at 2-8ºC and protected from light.

3.2.2 Quantitative determination of the micronutrients:

Simple serum or plasma level assessment is what is usually feasible as a biochemical indicator in population based assessment of micronutrients (Bhan *et al*., 2001).

3.2.2.1 Magnesium

Theory: In alkaline solution, magnesium forms a purple coloured complex with calgamite. The intensity of the colour formed is proportional to the magnesium concentration in the sample.

Assay conditions: Wavelength – 520nm (500-550nm), Cuvette – 1.0 cm light path, Temperature - 37 ºC or 15-25 ºC

Method

The spectrophotometer was adjusted to zero with distilled water. 1ml of the WR was pipetted into each of three cuvettes (blank, standard, and sample). 10µL of magnesium CAL. was pipetted into the second cuvette (standard), while 10µL of the sample was pipetted into the third cuvette (sample). The content of each cuvette was mixed and incubated for 5 minutes at room temperature or 3 minutes at 37 ºC. The absorbance (A) of the sample and standard against the blank was read and recorded, and the concentration of magnesium in the sample in mg/dL was calculated using

$$\frac{\text{(A) sample}}{\text{(A) standard}} \times \text{2(calibrator concentration)}$$

3.2.2.2 Zinc

Theory: At pH 8.6 in buffered medium, zinc forms a stable coloured complex with specific complexant 5-Br-PAPS. The intensity of the colour formed is proportional to the amount of zinc present in the sample.

Assay conditions: Wavelength- 560 nm (550-580 nm)**,** Cuvette- 1cm light path**,** Temperature- 25/30/37 ºC

Method

The spectrophotometer was adjusted to the zero mark with distilled water. 1ml each of the working reagent was pipetted into each of 3 cuvettes – blank, standard, and sample. 50µL of distilled water, 50µL of zinc CAL. (standard), and 50µL of the sample was pipetted respectively into each of the 3 cuvettes, and their contents mixed. The absorbance (A_1) of the sample against the blank was read and recorded. 100µL each of 5-Br-PAPS was then pipetted respectively into each of the 3 cuvettes, and their contents mixed. The absorbance (A_2) of the sample and standard against the blank was read and recorded. The concentration of zinc in µg/dL was then calculated

$$\frac{(A_2\text{-}A_1)\ \text{sample}}{(A_2\text{-}A_1)\ \text{standard}} \times 200\ (\text{standard concentration})$$

3.2.2.3 Copper

Theory: At pH 4.7 in a buffered medium, copper is released from ceruloplasmine complex and forms a stable coloured complex with the specific complexant 3, 5-DiBr-PAESA; the colour intensity of which is proportional to the amount of copper present in the sample.

Assay conditions: Wavelength - 582 nm (570-590nm)**,** Cuvette - 1cm light path**,** Temperature - 37 ºC

Method

The reagents were allowed to reach working temperature and the spectrophotometer was adjusted to zero with distilled water. 3 cuvettes were used: one for the blank, the second for the standard (copper CAL.) and the third for the sample. 1ml of the WR was pipette into each of the 3 cuvettes, then, 5mls each of distilled water, the standard and the sample was pipette into each

of the 3 cuvettes respectively. The contents of each cuvette were mixed and the absorbance A_1 of the sample against the blank was read and recorded. 5mls of 3, 5-DiBr-PAESA was then pipetted and added into each of the 3 cuvettes. The contents were mixed and incubated at 37 ºC for 4-5 minutes. The absorbance A_2 of the sample and the standard against the blank was read and recorded.

The quantity of copper in the sample in µg/dL was then calculated using:

$$\frac{(A_2\text{-}A_1)\ \text{sample}}{(A_2\text{-}A_1)\ \text{standard}} \times 100$$

3.2.2.4 Iron

Theory: In a weakly acidic medium, iron is dissociated from transferring-iron complex. This liberated iron (Fe^{3+}) is reduced by ascorbic acid into bivalent ferrous ions (Fe^{2+}) which forms a coloured complex with ferrozine. The intensity of the colour formed is proportional to the iron concentration in the sample.

Assay conditions: Wave length – 562nm (530-590)**,** Cuvette – 1cm light path**,** Temperature - 37ºC

Method

The spectrophotometer was adjusted to the zero with distilled water. 1ml of the working reagent (WR) was pipetted into each of 4 cuvettes: WR blank, calibrator, sample blank, and sample. 1drop of ferrozine and 200µL of distilled water was pipetted into the first cuvette, 1 drop of R.3 and 200µL of iron CAL. (standard) was pipetted into the second cuvette, 200µL of the sample was pipetted into the third cuvette, and 1 drop of ferrozine and 200µL of the sample was added into the last cuvette. The contents of each cuvette was mixed and incubated for 5 minutes at 37ºC (or for 10 minutes at 15-25ºC).

The absorbance (A) of calibrator and sample against WR blank was read and recorded.

The quantity of iron in µg/dL will then be calculated using:

$$\frac{(A)\text{sample} - (A)\text{sample blank}}{(A)\text{standard}} \times 100\ (\text{calibrator concentration})$$

3.3 Data analysis

Data was generated and analysed using Microsoft Excel 2007. Frequency distributions were generated for all categorical variables. Statistical significance between multiple means was assessed using one-way analysis of variance (ANOVA), while Student *t*-test was used where there was two means. Differences between values were accepted as statistically significant where probability was less than 0.05. Data was presented as means ± standard error of means as well as in tables and charts.

CHAPTER 4

RESULTS

4.1 Relative Incidence of Sickle Cell Diseases, Biodata and Anthropological Findings of Study Group

The most common type of Sickle Cell Disease (SCD) in this study was the homozygous HbSS variant (70%), followed by the HbSS + F variant (23%), and the least common variant was the HbSC (7%) - (Figure 4.1). The study population was made up of 71(53%) males and 64(47%) females; 84(62%) were single and 51(38%) were married; while 82(60%) were Muslims and 53(40%) were Christians (Table 4.1).

The mean age of the sickle cell disease patients in crises was 23.44 ± 0.58 years; while the mean age of sickle cell disease patients in steady state was 23.17 ± 0.57years; and the mean age of the control group of individuals with genotype HbAA was 23.98 ± 0.44years. At $p < 0.05$; there was no statistically significant difference between the mean ages of all the groups (Table 4.2)

The mean weight of the sickle cell disease patients in crises was 51.59 ± 1.26kg; while the mean weight of sickle cell disease patients in steady state was 52.43 ± 1.34kg; and the mean weight of individuals with genotype HbAA was 62.27 ± 1.45kg. At $P < 0.05$; there was a statistically significant difference between the weights of both SCD groups compared with the HbAA group (Table 4.2).

Occupation, tribe, and educational level of the study group can be seen in Tables 4.3.

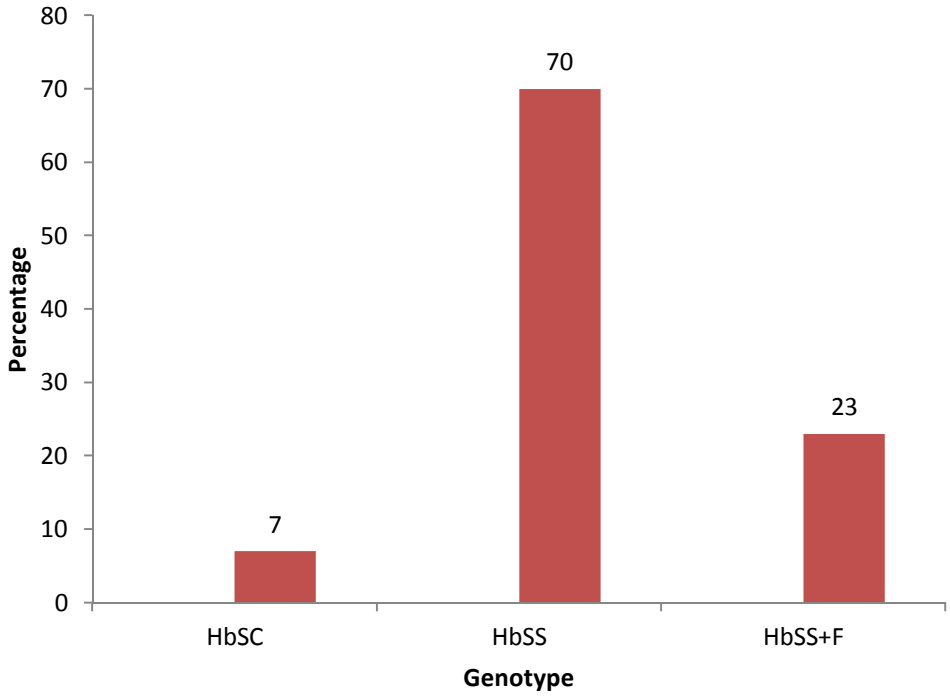


Figure 4.1: Variants of Sickle Cell Disease of Patients in the Study

Table 4.1: Sex, Marital Status and Religion of Individuals who participated in the Study

Parameter		Frequency (Percentage)
Sex	Male	71 (53)
	Female	64 (47)
Marital Status	Single	84 (62)
	Married	51 (38)
Religion	Islam	82 (60)
	Christianity	53 (40)

Table 4.2 Mean Ages and Weights of the Groups

Study Population	Age (Years)	Weight (Kg)
SCD in Crises	23.44± 0.58	*51.59± 1.26
SCD in Steady State	23.17± 0.57	*52.43± 1.34
HbAA Control	23.98± 0.44	62.27± 1.45

* Statistically significant difference at $p<0.05$ compared with HbAA

Table 4.3: Occupation, Highest Educational Level Attained and Tribe of the Study Population

Parameter		Frequency (Percentage)
Occupation	Civil Servant	27 (20)
	Student	50 (37)
	Homemaker	18 (13)
	Unemployed	9 (7)
	Business	27 (20)
	Others	4 (3)
Highest Educational Level	No Formal Education	17 (13)
	Primary School	15 (11)
	Secondary School	40 (30)
	Tertiary Education	63 (46)
Tribe	Hausa	53 (39)
	Yoruba	38 (28)
	Igbo	12 (9)
	Others	32 (24)

4.2 Clinical History of the Sickle Cell Disease Patients

The mean ages at diagnosis for the HbSC, HbSS, and HbSS + F were; 3.83 ± 0.54 years, 3.49± 0.28 years, and 4.26 ± 0.78 years respectively; however, at $p<0.05$, there was no statistically significant difference between them (Figure 4.2).

The common clinical features experienced by the sickle cell disease patients are seen in Figure 4.3; they are bone pains (74%), headache (51%), jaundice (46%), urinary tract infection (30%), anaemia (23%), septicaemia (20%), priapism (18%), acute chest syndrome (17%), hepatomegaly (14%), haematuria (14%), osteomyelitis (12%), avascular necrosis femoral head (10%), gnathopathy (10%), leg ulcers (8%), eye disorder (8%), frontal bossing (3%), and avascular necrosis humeral head (3%).

Among the SCD patients, 51% had an average of two episodes of crises per year, 23% had an average of one, 16% had an average of three, 8% had an average of less than one, while, only 2% had more than three crises episodes in a year (Table 4.4).

The following presentations were observed during crises episodes of the patients: vaso occlusive crises (63%), headache (11%), epigastric pain (8%), avascular necrosis (6%), priapism (6%), osteomyelitis (4%), and leg ulcers (2%) (Table 4.4).

There were 19% patients who had never been on hospital admission, 46% were admitted once in a year, 27% had been admitted two times in a year, 6% had been admitted three times in a year, and 2% had been admitted four times in a year (Table 4.5).

The common presentations which led to hospital admissions in the SCD patients are seen in Figure 4.4. They include, vaso occlusive crises (58%) , priapism (7%), osteomyelitis (6%), septicaemia (3%), urinary tract infection (3%), pneumonia (3%), leg ulcer (3%), chest pain (3%), avascular necrosis (3%), headache (2%), tuberculosis (1%), pregnancy (1%), nose bleeding (1%), miscarriage (1%), epigastric pain (1%), diarrhoea (1%), cholelithiasis (1%), cardiac decompensation (1%), and accident (1%).

Some patients had never been transfused (32%), 36% did not know if they had been transfused or not, 19% had been transfused once, 8% had been transfused twice, 3% had been transfused

three times, 1% had been transfused four times, and 1% had been transfused twelve times (Figure 4.5).

Co morbid illnesses were seen in 46 (51%) of the patients and 44(49%) had no co morbid illness. Of those with co morbid illnesses, 22% had peptic ulcer disease, 4% had high blood pressure, 3% had hepatitis and heart failure, while tuberculosis, erythroblastopenia, pyelonephritis, HIV/AIDS, myeloproliferative disorder, and asthma each had an occurrence of 1% (Figure 4.6).

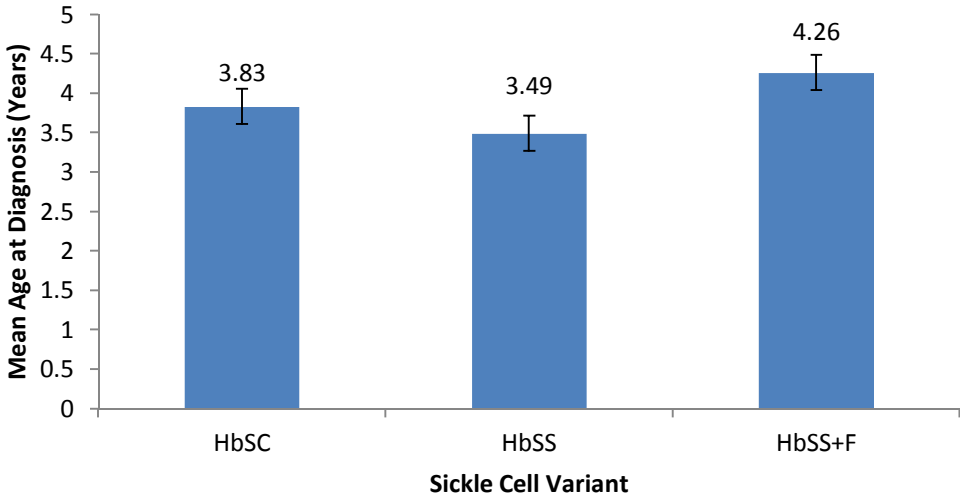


Figure 4.2: Ages at Diagnosis of Sickle Cell Disease

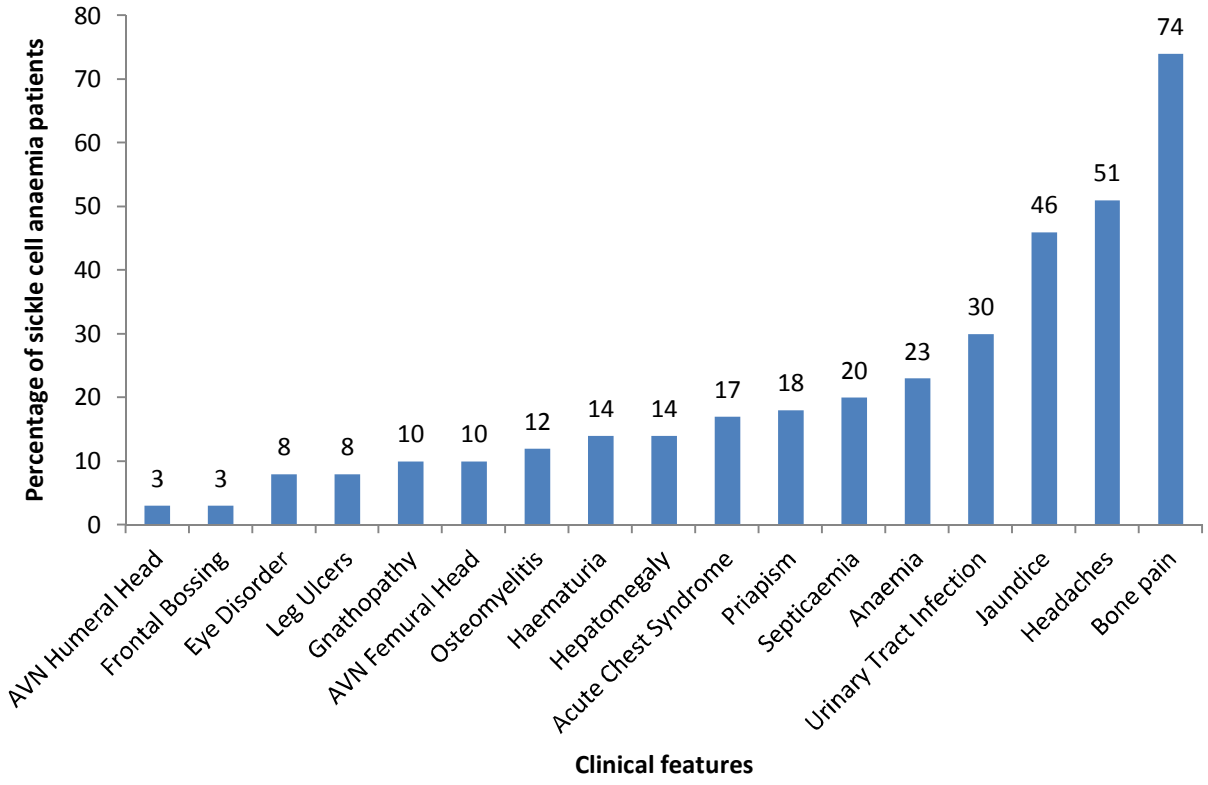


Figure 4.3: Clinical Features of Sickle Cell Disease Patients in the Study

Table 4.4 Average Number of Crises per year and Most Common Presentations of Sickle Cell Disease Patients in the Study

Parameter		Frequency(Percentage)
Average Number of Crises per Year	Less than 1	7 (8)
	1	21 (23)
	2	46 (51)
	3	14 (16)
	Greater than 3	2 (2)
Most Common Presentation during Crises	Leg Ulcer	2 (2)
	Osteomyelitis	4 (4)
	Priapism	5 (6)
	Avascular Necrosis	5 (6)
	Epigastric Pain	7 (8)
	Headache	10 (11)
	Vaso Occlusive Crises	57 (63)

Table 4.5: Average Number of Hospital Admissions per year

Number of Hospital Admissions	Frequency (Percentage)
0	17 (19)
1	42 (46)
2	24 (27)
3	5 (6)
4	2 (2)

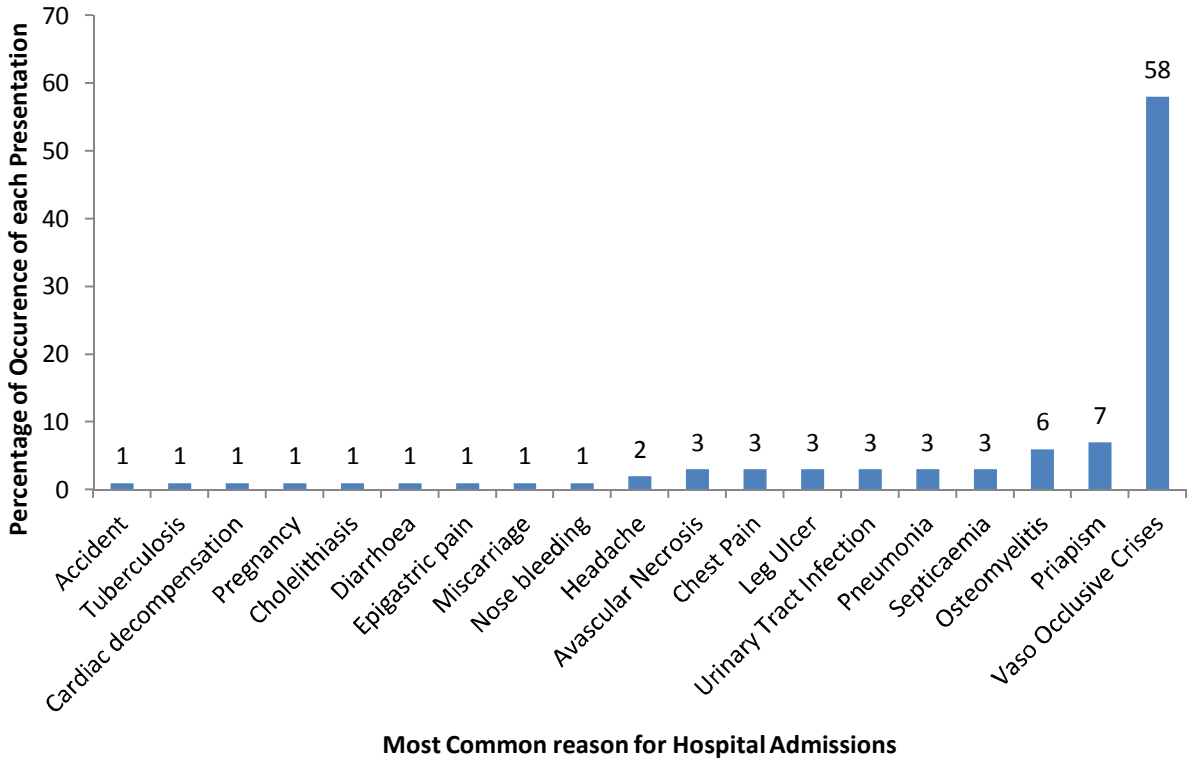


Figure 4.4: Most common Presentations during Hospital Admissions

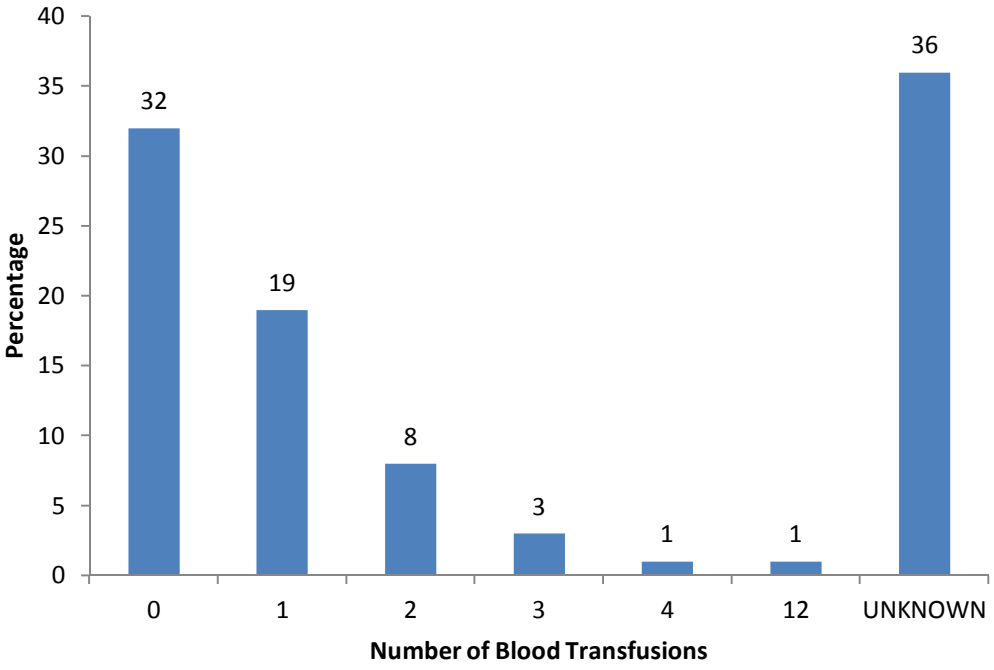


Figure 4.5: Number of Blood Transfusions had by Sickle Cell Disease Patients

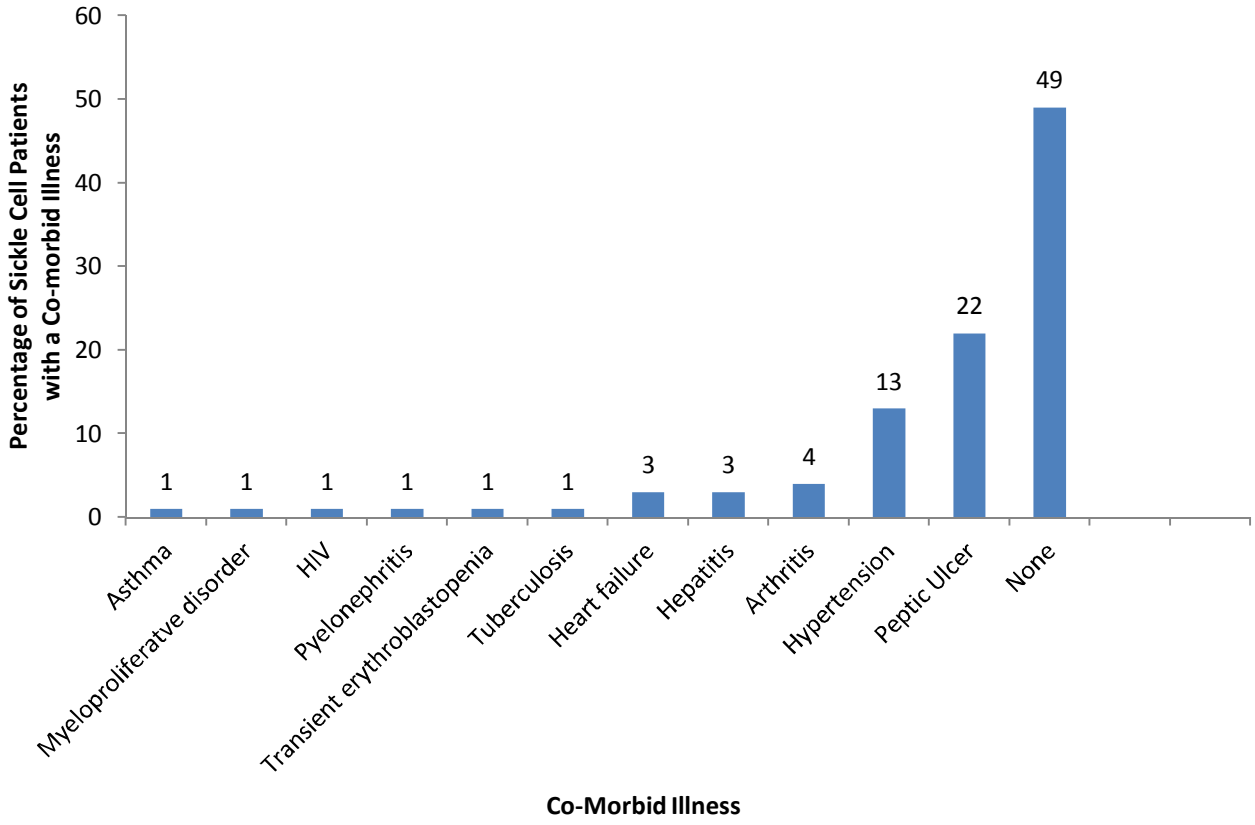


Figure 4.6: Co-Morbid Illness of Study Group

4.3 Medication History of Sickle Cell Disease Patients in the Study

The routine drugs/interventions, vitamins, and analgesics used by SCD patients in crises and SCD patients in steady state are seen in Figures 4.7, Figures 4.8, and Figures 4.9.

The utilisation of these medications by the different groups of SCD variants are seen in Figures 4.10, Figures 4.11, and Figures 4.12.

The white blood cell counts of SCD patients were compared to their amoxicillin requirements and utilisation; this was presented in table 4.13.

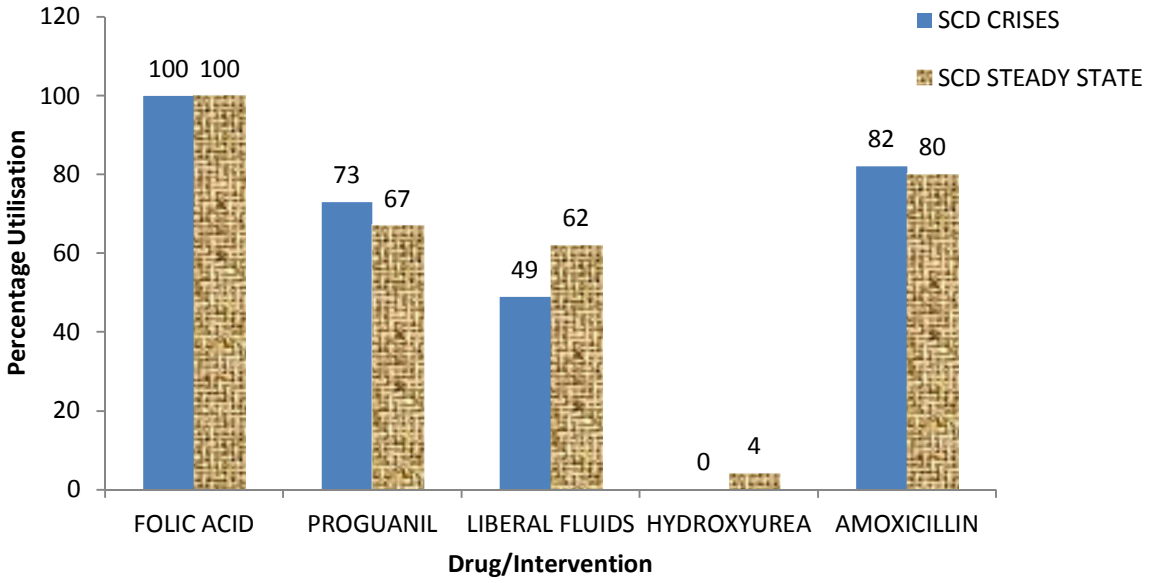


Figure 4.7: Routine Drugs/Interventions used in the Management of Sickle Cell Disease Patients in the Study

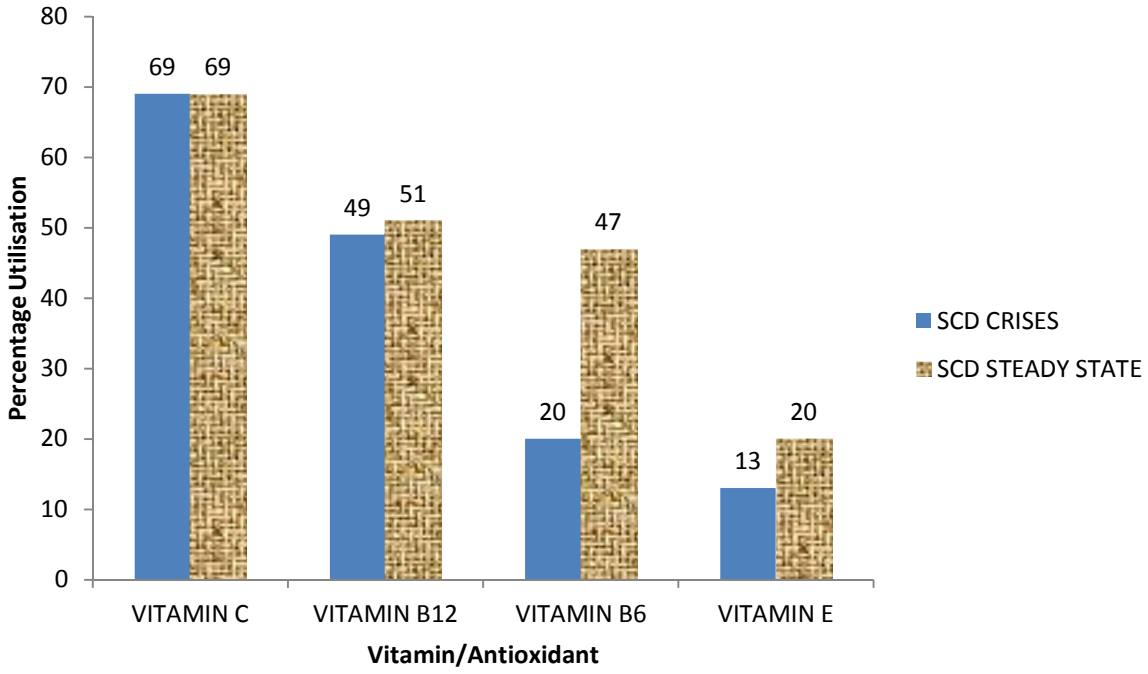


Figure 4.8: Vitamin/Antioxidant use of Sickle Cell Disease Patients in the Study

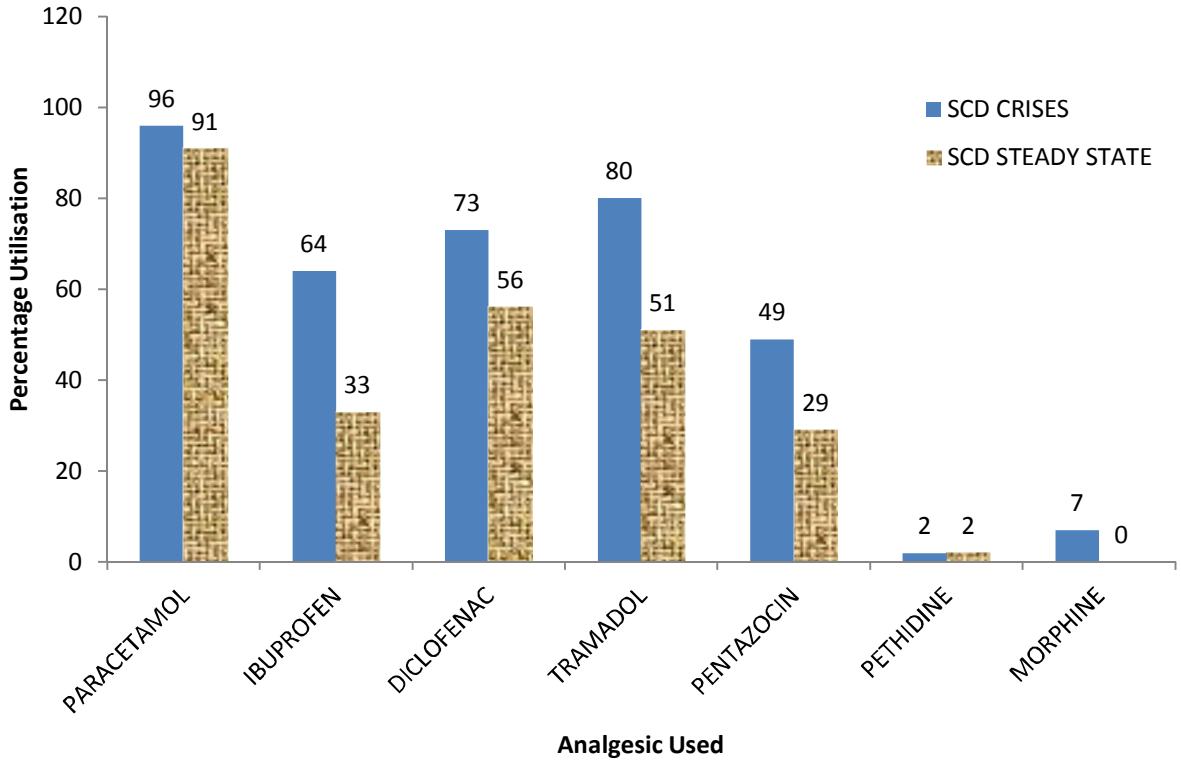


Figure 4.9: Analgesic use in Sickle Cell Disease Patients in the Study

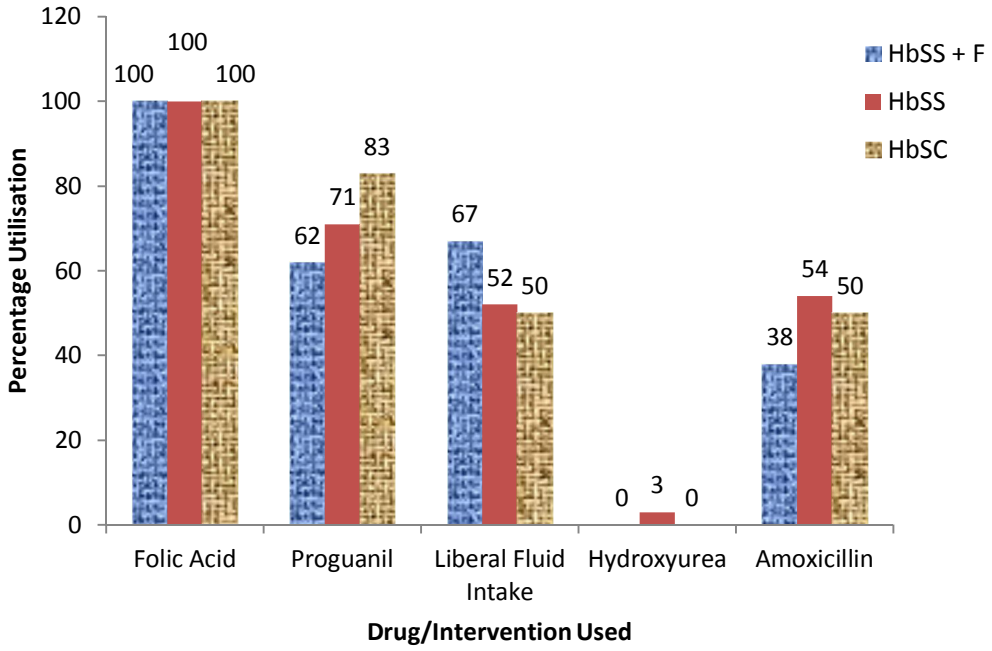


Figure 4.10: Routine Drug/Intervention Utilisation Pattern in Sickle Cell Disease patients Grouped by Haemoglobin Variant

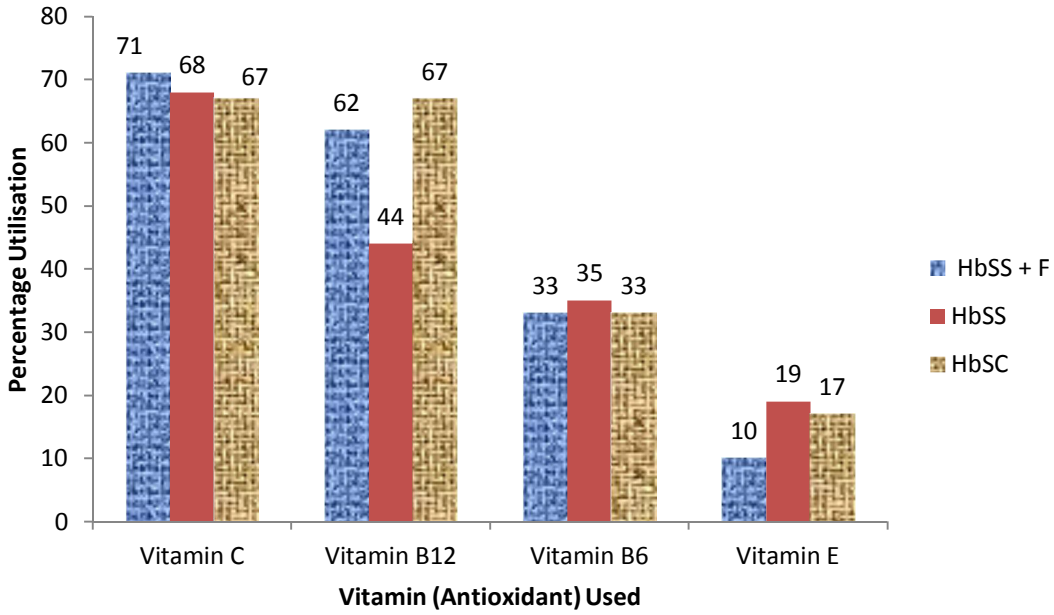


Figure 4.11: Vitamin/Antioxidant Utilisation Pattern in Sickle Cell Disease Patients Grouped by Haemoglobin Variant

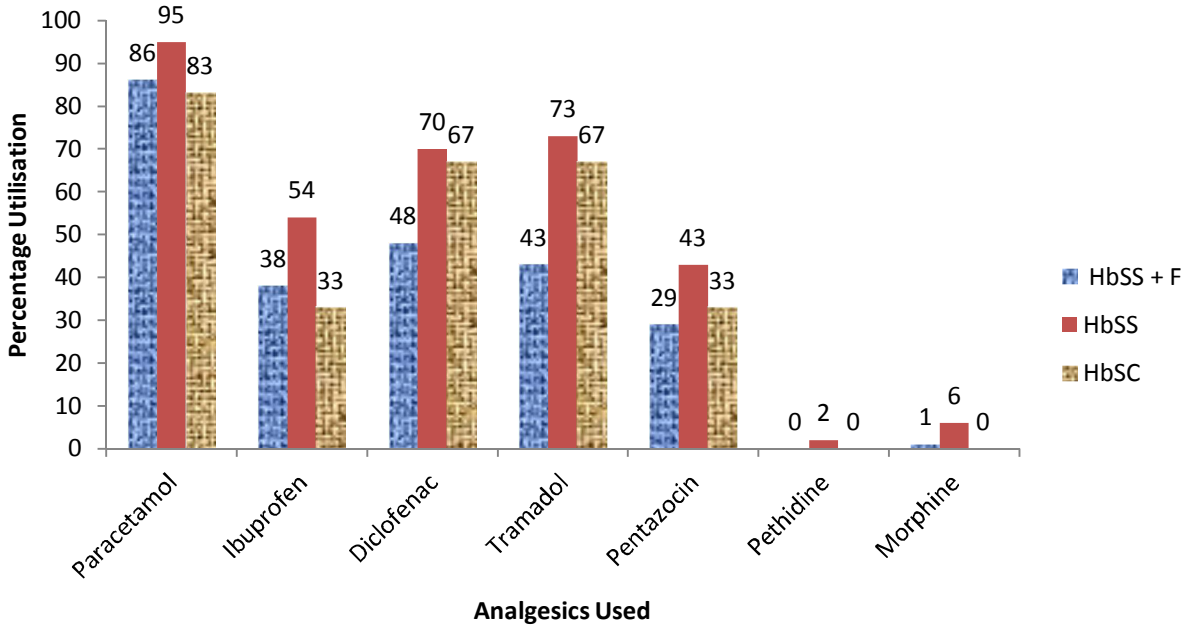
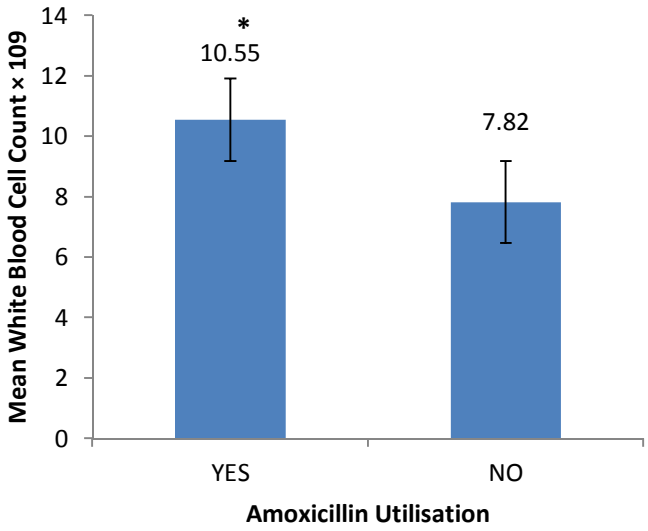


Figure 4.12: Analgesic Utilisation Pattern in Sickle Cell Disease Patients grouped by Haemoglobin Variant



* Statistically significant difference at $p < 0.05$ compared with patients who do not use amoxicillin.

Figure 4.13: Amoxicillin Utilisation in Sickle Cell Disease Patients compared with their White Blood Cell Counts

4.4 Full Blood Counts of the Groups

The mean platelet count of the SCD in crises and HbAA individuals were within normal range; while the mean platelet count of the SCD in steady state was above normal range. At $p < 0.05$, there was a statistically significant difference between the SCD in crises and HbAA; a statistically significant difference between the HbAA and SCD in steady state; and also a statistically significant difference between the SCD in crises and the SCD in steady state (Table 4.6).

Although, the mean WBC counts of each group fell within normal range, at $p < 0.05$, there was a statistically significant difference between the mean WBC counts of SCD patients in crises and HbAA volunteers; a statistically significant difference between the HbAA and SCD in steady state; and also a statistically significant difference between the SCD in crises and the SCD in steady state (Table 4.6).

The mean values of neutrophils all fell within normal range. At $p < 0.05$, there was a statistically significant difference between the SCD in crises and HbAA as well as in the SCD in steady state and HbAA, but no statistically significant difference between the SCD in crises and SCD in steady state (Table 4.6). The mean values of lymphocytes in all three groups fell within normal range and at $p < 0.05$ there was no statistically significant difference between them (Table 4.6).

The packed cell volume (PCV) of the two SCD groups fell below the normal range; while that of the HbAA individuals was within normal range. At $p < 0.05$, there was a statistically significant difference between the PCV values of the SCD in crises and HbAA as well as the HbAA and SCD in steady state. Although the PCV of the SCD in crises was lower than the PCV of SCD in steady state, there was no statistically significant difference between them (Table 4.6).

Table 4.6: Mean Haematological Parameters of all the Groups

Parameters	Suggested Normal Ranges	Genotype (Mean ± SEM)		
		HbAA Volunteers n = 45	SCD in Crises n = 45	SCD Steady State n = 45
Platelet Count (× 10^9)	150.00-450.00	259.04± 13.41	*347.81± 35.94^	*518.38± 36.99
WBC Count (× 10^9)	2.50-13.00	5.08± 0.30	*10.10± 0.48^	*8.26± 0.56
Neutrophils (%)	45.00-70.00	45.75± 1.39	*58.26± 1.41	*60.27± 1.36
Lymphocytes (%)	17.00-48.00	42.11± 1.09	40.67± 0.94	39.91± 1.19
PCV (%)	30.00 – 55.00	38.36± 0.79	*22.76± 0.52	*24.53± 0.76

***Statistically significant difference at p<0.05 when compared to HbAA**

^ Statistically significant difference at p<0.05 when compared with SCD in steady state

4.5 Result of the Micronutrient Assay

Micronutrient assays of magnesium, zinc, copper and iron showed the following results:

At $p < 0.05$, there was a statistically significant difference between the mean plasma magnesium levels of the SCD patients in crises and the HbAA group; a statistically significant difference between the plasma magnesium levels of the HbAA and the SCD in steady state; as well as a statistically significant difference between the plasma magnesium levels of both SCD groups (Table 4.7).

Although, at $p < 0.05$ there was no statistically significant difference between the plasma zinc levels of all three groups; the plasma zinc levels of the HbAA was the highest, followed by that of the SCD in steady state, and that of the SCD in crises was the lowest (Table 4.7). Similarly, there was no statistically significant difference between the plasma copper levels of all three groups. However, the plasma copper levels of the HbAA was the lowest, followed by that of the SCD in steady state, and that of the SCD in crises was the highest (Table 4.7).

Plasma iron levels of the SCD in crises was the highest, followed by SCD in steady state; with the HbAA having the lowest iron levels. At $p < 0.05$, there was a statistically significant difference between the plasma iron levels of the SCD in crises and the HbAA individuals; a statistically significant difference between the plasma iron levels of the HbAA and the SCD in steady state; but there was no statistically significant difference between the plasma iron levels of both SCD groups (Table 4.7).

Table 4.7: Mean Plasma Micronutrient Levels of all the Groups

Micronutrients	Genotype (Mean ± SEM)		
	HbAA Group	SCD in Crises	SCD in Steady State
	n = 45	n = 45	n = 45
Magnesium (mg/dL)	0.88 ± 0.06	*0.62 ± 0.02^	*0.74 ± 0.03
Zinc (µg/dL)	221.28 ± 20.87	175.33 ± 18.33	201.80 ± 17.60
Copper (µg/dL)	82.39 ± 4.14	94.39 ± 3.93	90.15 ± 4.44
Iron (µmol/dL)	14.15 ± 0.73	*19.09 ± 1.89	*17.60 ± 1.05

*** Statistically significant difference at p<0.05 when compared to HbAA Group**

^ Statistically significant difference at p<0.05 when compared to SCD in steady state

CHAPTER 5

DISCUSSIONS

5.1 Relative Incidence of Sickle Cell Diseases, Biodata and Anthropological Findings of Study Group

The findings in the present study showed that HbSS was the most common variant of SCD in ABUTH, Zaria, followed by the HbSS+F and HbSC. This is in line with other reports in Nigeria and sub Saharan Africa which shows that the highest frequencies of homozygous sickle cell disease (HbSS) in the world occur in sub-Saharan Africa (WHO, 2002). The pattern in Nigeria is similar to that in Jamaica with the HbSS variant being the most common, followed by HbSC (Oniyangi and Omari 2006). An incidence of 6% HbSC was reported in the Yoruba's in the Western States of Nigeria; while 0.07% incidence was reported in the Igbos of the Eastern States of Nigeria (Uzoegwu and Onwurah 2003). The high incidence of HbSC in the Yorubas can be attributed to their relative geographical proximity to Ghana where the incidence of HbSC is highest in the world (Akinyanju, 1989).

The mean age of adult SCD patients who attended adult haematology clinic in this study was 23.44 ± 0.58 years for those in crises and 23.17 ± 0.57 years for patients in steady state. This is in line with the study by Chijioke and Kolo (2009) who found a mean age of 23.00 ± 6.60 years in adult sickle cell patients in Ilorin, Nigeria. They concluded that since this value was in contrast with the mean age of the general healthy population, this may indicate that the life expectancy of sickle cell patients is reduced. However, such conclusion cannot be made here since sampling of controls in this study was age matched.

The mean weight of sickle cell patients in this study was significantly lower than that of the HbAA volunteers. Impaired growth is common in homozygous sickle cell disease; and there are early deficiencies in height and weight and delays in the onset of puberty, although the final height of patients with sickle cell disease appears to be normal (Thomas *et al*., 2000). In a study in India, Mukherjee and Gangakhedkar (2004) concluded that both males and female sickle cell disease children have lower weights and heights than controls. While in Ilorin, Nigeria, Chijioke and Kolo (2009) found that 47% of the sickle cell patients had weights below normal.

SCD is very widespread and occurs throughout Nigeria (Oniyangi and Omari 2006). HbSS is by far the most common and it is fairly evenly distributed throughout the country (Akinyanju, 1989). In contrast, HbSC is rare in northern and south-eastern Nigeria but common among the Yoruba people of south-western Nigeria in whom HbAC carrier rates of 5-7% have been reported. Majority of the study group were Hausas (39%) which was not unexpected as the hospital is located in a predominantly Hausa region, which may explain the results obtained.

Although 60% of the study group were Muslims while 40% were Christians, religion did not appear to have any effect on the course and severity of sickle cell crises in the patients. However, religion has been seen to have impact on the management of SCD where religious inclinations influence a patient's ability to accept treatment options such as blood transfusions and bone marrow transplant (Helton *et al*., 2001). The high percentage of Muslims in this study could possibly be due to the fact that Islam is the predominant religion in Northern Nigeria (Ejiofor, 1998). In addition, consanguineous marriages are more common among the Muslims and this could possibly be a contributing factor to the higher frequency of SCD among the Muslims in this study. The detrimental effects of inbreeding are the consequence of homozygosity of harmful genes such as HBSS (SCD); the distribution of different intensities of malaria infestation is matched with the frequency of human inbreeding (Denic and Nicholls 2007); possibly making the Hausa Muslims a double risk factor for SCD.

5.2 Clinical History of Sickle Cell Disease Patients

In Africa, children with sickle cell disease are usually first diagnosed following an acute disease and not by screening hence regrettably, diagnosis is often delayed (Afolayan and Jolayemi 2011). The findings in the present study are in line with this. The overall mean age at diagnosis of the sickle cell patients was 3.69 ± 0.27 years; this is close to the mean age of diagnosis of 3.8 years reported by Mamman and Durosinmi (2004) for SCD patients in Zaria. The mean ages of diagnosis for the HbSC, HbSS, and HbSS + F were; 3.83 ± 0.54 years, 3.49± 0.28 years, and 4.26 ± 0.78 years respectively; however, there was no statistically significant difference between them.

The early loss of splenic function in patients with SS disease renders them prone to overwhelming septicaemia especially in early childhood (Serjeant, 2005). In a study by

Aken'ova *et al*, (1998) in Ibadan, they found that 36.1% of sickle cell anaemia patients with fever had septicaemia. The present study showed a lower percentage of 20%, this could possibly be due to the fact that the variants of SCD in Lagos (Nigeria) vary from that in this study as reported by Akinyanju, (1989). Juwah *et al*., 2003 in Enugu reported an occurrence of septicaemia in 66.7% of sickle cell patients; showing a variability in frequency of occurrence of septicaemia in Nigeria.

Urinary tract infections (UTIs) are a significant cause of morbidity in childhood and individuals with sickle cell disease have been observed to be at increased risk (Brown *et al*., 2002). The present study showed that 30% of the patients had history of UTIs; this was close to results obtained in a study among sickle cell patients in University of Maiduguri Teaching Hospital which showed that urinary tract infections had a prevalence of 26% (Mava *et al*., 2012). A lower percentage of 22.2% was reported by Juwah *et al*., (2003) in Enugu.

Because of the short lifespan of the sickle red blood cells, the body is often unable to replace red blood cells as quickly as they are destroyed (haemolytic anaemia). Episodes of severe anaemia in sickle cell anaemia patients are major causes of morbidity and mortality (Juwah *et al*., 2003). The anaemia could be haemolytic, due to splenic sequestration, or aplastic anaemia (often triggered by a virus called human parvovirus B19). 23% of the sickle cell patients in the study had anaemia. The specific cause of anaemia in these patients was not determined.

An incidence of 29% of sickle cell patients with acute chest syndrome (ACS) was reported by the Cooperative Study of Sickle Cell Disease (CSSD) in America (Gladwin *et al*., 1999). The present study showed a 17% incidence of adult patients with ACS. ACS is a common complication of sickle cell anaemia, accounting for 25% of premature deaths (Gladwin *et al*., 1999). Although anecdotal series on ACS are available, there are no firm data on its incidence in Nigeria. It was present in 6% of adolescents and adults in a study conducted in Lagos, Nigeria (Fawibe, 2008).

Fourteen percent of sickle cell patients in ABUTH presented with haematuria. Patients with sickle cell anaemia are well known to show evidence of microscopic haematuria, but severe persistent haematuria is a rare complication (Davies *et al*., 2010). According to Abdu *et al*., (2011), haematuria occurs in about 27% of the SCD patients in Nigeria; however, Ugwu *et al*.,

(2007) reported 11% haematuria in sickle cell children in Port Harcourt; showing vast variability of presentations of SCD even within the same country.

Although often detected clinically, sickle cell gnathopathy can be evaluated radiographically simply and accurately by measurement of the palate-alveolar ridge angle. In the present study, 10% of the patients had clinically detectable gnathopathy. Afolayan and Jolayemi (2011) reported gnathopathy among sickle cell patients in Ilorin, Kwara State, Nigeria. However, they did not state the frequency of occurrence. Oredugba and Savage (2004) in a cross sectional hospital based study in Lagos found that some sickle cell patients presented with low weight, low height-for-age, gnathopathy, frontal bossing and reduced skin fold thickness. Chijioke and Kolo (2009) also in a study on sickle cell patients in Lagos reported bossing of skull bone being present in 6% of cases. About 3% of sickle cell disease patients in this study had frontal bossing which is lower than that reported by Chijioke and Kolo (2009) in Lagos.

A variation in occurrence of jaundice exists. Ibidapo and Akinyanju, (2000) reported that 71% of adolescents and adults with sickle cell anaemia who presented to the emergency unit of a Lagos hospital had jaundice. This value was significantly greater than that reported by Chijioke and Kolo (2009) who also in a study in Lagos found that 56% of sickle cell patients had jaundice. The present study found that 46% of sickle cell patients had jaundice. Differential diagnosis of severe jaundice in SCD is not always easy. A gradual or sudden increase in pallor and jaundice in the SCD patient is indicative of an infection (Konotey-Ahulu, 1991).

Twelve percent of patients in current study had osteomyelitis; this is similar to reports by Juwah *et al*., (2003) who found that 11.1% of sickle cell patients in Enugu had osteomyelitis; and the same with Almeida and Robert (2005) who found a prevalence of 12% in a French study which used SCD patients from four Parisian centres. Osteomyelitis in sickle cell disease has also been reported in association with tuberculosis (Almeida and Robert 2005). In the current study, only 1 patient (1%) presented with both tuberculosis and osteomyelitis.

Avascular necrosis (AVN) of the hip is a common cause of morbidity in sickle cell disease (SCD) (Adekile *et al*., 2001). A total of 26.7% of sickle cell disease patients in Kuwait were found to have AVN of the femoral head (Adekile *et al*., 2001). In the Guinea Savannah of Nigeria the incidence of symptoms of AVN of the femoral head among the SCD population is

about 3% (Iwegbu and Fleming 1985). In Ile-Ife, Nigeria, Akinyoola *et al*., (2007) reported a 15.6% incidence of AVN femoral head among SCD patients. Present study had a 10% occurrence of AVN in the femoral head of patients in ABUTH Shika, Zaria and a 3% occurrence of AVN humeral head. Avascular necrosis (AVN) of femoral and humeral heads is a frequent and debilitating complication in patients with sickle cell disease (SCD), its prevalence being highest in individuals with SCD-Hb SS and coincidental α-thalassemia (Ulug *et al*., 2009).

Chronic leg ulceration is the most common cutaneous manifestation of sickle cell disease. It is estimated that 8-10% of homozygous sicklers will develop leg ulceration between 10 and 50 years of age, but higher rates of more than 50% have been reported (Meshikhes *et al*., 1998). In present study 8% of the adult sickle cell disease patients had history of leg ulcers of which 3% had to be admitted due to the severity of the leg ulcer. In a study in Benin City, Nigeria; leg ulcers were found currently in 9.6% of the sickle cell disease patients, and were more prevalent in males than females (M:F=3:1) (Bazuaye *et al*., 2010). The aetiology of leg ulcers in SCD is not known but is thought to be due to micro-thrombi in the small capillaries of the legs resulting in ischaemia.

Headache has been described as a frequent symptom in SCD that is often attributable to anaemia or cerebrovascular disease. Sources of headaches in SCD patients may include acute painful episodes involving the head, headache secondary to central nervous system involvement, and comorbid migraine or tension headache (Palermo *et al*., 2005). In the SCD patient, it could also be a symptom of brain infarction, one of the most severe and most common complications of sickle cell disease, affecting 25% of patients with the disease. Bone infarction occurs in the medullary cavity, and patients present with acute, focal bone pain and headache (Saito *et al*., 2010). In the present study, 51% of the patients presented with headaches. Recurrent headaches are relatively frequent in adult Nigerians with sickle cell disease. In a study in SCD patients in Ilorin, a one year prevalence of 11.7% was seen among the male patients (Wahab *et al*., 2010). This value exceeds that in present study; perhaps this is as a result of a difference in the sex of the study groups.

Central retinal artery occlusion occurs in patients with SCD and could lead to permanent loss of vision (Isoa, 2009). Ocular manifestations can be severe and sudden (Akinsola and Kehinde 2004); and presence of ocular abnormalities varies in SCD patients. Majekodunmi and

Akinyanju (1978) reported no ocular findings among SCD patients in Nigeria. In a study carried out among adult SCD patients in Lagos University Teaching Hospital, 96% had normal vision, while 4% had impaired vision (Akinsola and Kehinde 2004). In another study in both adults and children SCD patients in Federal Capital Territory, 24% of them had SCD related posterior lesions indicative of sickle retinopathy (Babalola and Wambebe 2005). Present study found 8% of adult SCD patients presenting with ocular abnormalities which ranged from itching of the eyes and blurred vision to partial loss of vision.

The incidence of priapism among patients with sickle-cell anaemia is high (35%) (Adeyoju *et al*., 2002). Priapism has been found to be more common in HbSS patients than HbSC patients; with frequencies of 35-40% of adult male sickle cell disease patients being affected (Nolan *et al*., 2006). This was also found to be true in current study; although, generally there were more HbSS patients than HbSC patients among sickle cell disease patients of ABUTH Shika. 18% of the sickle cell patients (34% of the males) reported having had an episode of priapism.

Multiple hepatobiliary complications occur in patients with SCD due to the increased risk of hepatic injury from sickling, cholelithiasis, choledocholithiasis and acute hepatic failure (Meshikhes *et al*., 1998). Acute and transient hepatomegaly may occur during a painful crisis and may be secondary to sinusoidal dilatation by circulating sickle cells (Serjeant and Serjeant 2001). In a study by Olaniyi and Abjah (2007), hepatomegaly was found to be present in 59% of SCD patients in Ibadan, Nigeria. Percentage of SCD with hepatomegaly in the current study was lower (14%), perhaps this was due to the fact that this figure was obtained from documented patients history in their folders and not from physical examination of each patient.

Most of the sickle cell patients of Haematology Department, ABUTH (42%) had an average of 2 crises episodes per year that warranted hospital visits, 33% had an average of 1 episode per year, 16% had about 3 crises episodes per year warranting a hospital visit, 7% had less than 1 crises episode, and 2% had more than 3 crises episodes warranting a hospital visit.

Of the patients who had to visit the Haematology day care clinic as a result of crises, 63% were as a result of a vaso occlusive crises, 11% due to headache, 8% epigastric pain, 6% complained of avascular necrosis, 6% had priapism, 4% osteomyelitis, and about 2% had to visit the hospital day care as a result of leg ulcers.

19% of the sickle cell patients attending haematology clinic in ABUTH had never been admitted before, 46% had been admitted at least once, 27% had been admitted about two times in a year, 6% had been admitted about three times in a year, and only 2% claimed there were admitted an average of four times each year.

In a given year, about 60% of patients with sickle cell anaemia will have an episode of severe pain (Steinberg, 1999). The most common reason for hospital admission was vaso occlusive crises. Vaso occlusive crises of pain has been described by Mc Clish *et al*., (2009), as pain classically occurring in the back, chest, and extremities; however, they stated that this clinical observation is supported by limited empirical evidence. In the current study, 74% of the patients had a history of bone pains, 63% had to make a visit to the hospital as a result of vaso occlusive crises, while, 58% were admitted as a result of vaso occlusive crises; this is in line with a study by Ibidapo and Akinyanju, (2000) in Lagos who found that 68% of sickle cell patients presented with vaso occlusive crises.

Epigastric pain usually indicative of peptic ulcer was a cause for hospital visit in 8% of the patients. It is estimated that one third of patients with homozygous SCD with chronic recurrent epigastric pain have endoscopic evidence of peptic ulcer: with predominance of duodenal (27%) over gastric (8%) ulcer (Meshikhes *et al*., 1998). Patients with higher total and foetal haemoglobin level are at lesser risk of ulceration. Duodenal ulcer in sickle cell populations does not appear to be associated with high acid outputs; it is believed that the aetiology is related to decreased mucosal resistance as a result of repeated ischaemic infarcts secondary to sickling crises (Rao *et al*., 1985).

Transfusion is done using sickle negative blood to “dilute” out sickled RBCs, reduces ISCs count, precludes recurrent stroke if HbS is less than 30%, and reduces abnormal flow in cerebral vessels in children with sickle cell disease (Adams *et al*., 2000). Indications for transfusion include pregnant patients with anaemia, acute cerebral syndrome, priapism, acute chest syndrome, surgery, and anaemia (Steven *et al*., 2000). Thirty six percent of the SCD patients did not know whether they had been transfused or not. Thirty two percent said they had never been transfused; 17 (19%) had been transfused at least once; 7 (8%) had been transfused two times, 3 (3%) had been transfused three times, 1 (1%) had been transfused four times; and also 1 patient (1%) had been transfused 12 times.

In addition to SCD, 51% had a co-morbid illness while 49% did not have any. The most common co-morbid illness observed was peptic ulcer (22%); 13% of the patients were hypertensive; 4% had arthritis; hepatitis and heart failure was seen in 3% of the patients; while few patients (1% each) had asthma, myeloproliferative disorder, HIV/AIDS, pyelonephritis, and transient erythroblastopenia. Increased susceptibility to renal diseases calls for caution in the use of IVFs containing potassium, e.g. darrows solution.

Blood transfusions form an integral part of management of sickle cell disease. Blood transfusion is also established as a route of transmission of the Human Immunodeficiency Virus (HIV) and hepatitis; especially in developing nations that are lacking in properly organized blood transfusion services. 1.8% of patients in the Paediatric Haematology Clinic of A.B.U.T.H., Zaria, Nigeria were HIV-seropositive (Ogunrinde *et al*., 2005).

According to Adedoyin *et al*., (2010), prevalence of tuberculosis in SCD patients compared to the general population is quite low and unremarkable. Sickle cell patients are predisposed to infections due to depressed cell mediated immunity and functional asplenia; it is therefore thought that tuberculosis should occur commonly like in other immunosuppressive illness; however, it rarely does (Adedoyin *et al*., 2010). In present study, only 1% of the patients had tuberculosis.

5.3 Medication History of Sickle Cell Disease Patients in the Study

All the patients were prescribed "routine drugs" for maintenance of their general well being usually comprising: folic acid 5mg daily, proguanil 100mg daily, vitamin C 600mg daily in 3 divided doses, vitamin B12 50-150mcg daily, and vitamin B6 25-50mg daily. In addition to these, they were all advised (as a group; during "health talk" given by the nurses every clinic day, and individually by the physicians) to take fluids liberally. In cases of infection, amoxicillin was prescribed in accordance with the Standard Treatment Guidelines (2008). A full blood count is run for every patient that visits the haematology clinic in ABUTH before they are examined by the doctor and drugs are prescribed. The results of this laboratory tests are used together with the patients' presentations to determine use of other medication such as vitamin E, iron supplements, multivitamins, and hydroxyurea.

In the present study, medication utilisation of the SCD patients was grouped into two. The first grouping showed the medication utilisation patterns of the SCD patients in crises and the SCD patients in steady state. While, the second grouping showed the medication utilisation of the SCD patients based on their haemoglobin variants – HbSS, HbSS + F and HbSC variants.

Prophylactic folic acid (B9) use is the most popular vitamin in the management of HbSS. Its use in treatment is based on preventing deficiency from increased folate turnover (as in any chronic haemolytic anaemia), combined with limited reports of megaloblastic changes in HbSS responsive to folate supplementation. This practice remains a fundamental part of sickle cell treatment (Hyacinth *et al*., 2010). Folic acid is readily available and affordable. In the present study, folic acid utilisation of all the SCD patients across all the groups was the same (100%).

SCD is widely spread in the high malaria endemic areas of Nigeria (Uzoegwu and Onwurah 2003). Proguanil is therefore used for malaria prophylaxis. Malaria is the most common precipitating cause of crises in patients with SCD living in malaria endemic regions (Oniyangi and Omari 2006). Hence the lifelong malaria prophylaxis recommended for these patients. The widely accepted theory is that HbS offers selective protection against falciparum malaria probably because of induction of sickling even at physiological oxygen tension by *Plasmodium falciparum* followed by sequestration of parasitized red cells deep within reticulo-endothelial system where microenvironment is hostile for parasite growth (Desai and Dhanani 2004). Proguanil utilisation was lower than folic acid utilisation in all the sickle cell patients. Some patients complained it was more expensive and not as readily available as folic acid. Others mentioned that it has a bitter in taste. The SCD patients in crises had a higher proguanil utilisation (73%) than the SCD patients in steady state (67%). While, the HbSC had the highest proguanil utilisation (83%), followed by the HbSS (71%), and the HbSS+F had the lowest proguanil utilisation (62%).

Amoxicillin utilisation by the SCD patients in crises and the SCD patients in steady state was similar. The HbSS sickle cell patients had the highest amoxicillin utilisation (54%), followed by the HbSC (50%), while the HbSS+F had the lowest amoxicillin utilisation (38%). Further analysis showed that, the SCD patients on amoxicillin had a statistically significantly higher WBC count ($10.55 \pm 0.48 \times 10^9$) than the WBC count SCD patients that were not on amoxicillin ($7.82 \pm 0.52 \times 10^9$).

Vitamins B6 and B12 have been found to be deficient in SCD patients in addition they are antioxidants; and B6 acts as an essential cofactor for hundreds of enzymes. Vitamin C has been shown in many studies to modulate a wide variety of cellular functions which include bone formation, folic acid metabolism, enhancement of absorption of iron, formation and maturation of red blood cells and immune response mechanisms (Okochi and Okpuzor 2005). It has been proposed that antioxidants would be effective in alleviating the incidence and severity of crises in sickle cell patients (Okochi and Okpuzor 2005); thus they are commonly prescribed as routine drugs in the management of SCD.

Utilisation pattern of vitamin C and vitamin B12 was similar in the SCD patients in crises and in the SCD patients in steady state. Further analysis of utilisation patterns based on haemoglobin variant showed that the HbSS have the lowest utilisation of vitamin B12 (44%). The sickle cell HbSC (67%) and HbSS+F (62%) had similar utilisation patterns. Most patients who were on vitamin B12 used the single tablet combination containing 5 milligrams of folic acid and 5 micrograms of vitamin B12. Those who were not on vitamin B12 gave reason of its not being readily available as a single preparation and they were advised to obtain the combination preparation which is readily available at an affordable price. Vitamin C utilisation patterns across the haemoglobin variants were similar (HbSS+F, HbSS, and HbSC had utilisations of 71%, 68%, and 67% respectively).

Sickle erythrocytes are more susceptible to peroxidation than normal erythrocytes and this is due in part to decreased blood vitamin E levels. This peroxidative damage may accelerate or contribute to chronic haemolysis (Chiu *et al*., 1982). Vitamin E acts to neutralize free radical species produced during normal cellular metabolism, thus primarily functions as an antioxidant (Okochi and Okpuzor 2005). In the present study, vitamin E utilization by the SCD patients in crises was 13%, while the utilization by the SCD patients in steady state was 20%. Further analysis showed the utilization of vitamin by the HbSS and HbSC patients to be similar (19% and 17% respectively), while the HbSS+F had the lowest vitamin E utilization (10%).

The kidney in patients with sickle cell disease (SCD) exhibits numerous structural and functional abnormalities, changes that are seen along the entire length of the nephron. These abnormalities include hypostenuria, hematuria, acute renal failure, glomerular abnormalities and chronic renal failure. Patients with SCD should therefore be encouraged to drink liberal amounts of liquids in

order to compensate for the fluid loss that is brought on by hyposthenuria (Isoa, 2009). Eye disorders, priapism, bone pains, AVN, haematuria, are indicators of active vasoocclusion. Simultaneous to this, dehydration is an important indicator for vasoocclusion. Therefore, the SCD patient should be encouraged to take fluids. Patients presenting with VOC should as a first aid measure be given IVFs (except where contraindicated e.g. congestive heart failure). All patients were advised during each clinic visit to take liberal fluids, but only 49% of the SCD patients in crises and 62% of SCD patients in steady state took liberal fluids. A few patients said taking liberal fluids was inconvenient because it made them frequently pass urine and they were not always in a comfortable environment to do so.

Hydroxyurea was utilised by 4% of the SCD patients in the study. These SCD patients were all HbSS and during the study, they were in steady state. Hydroxurea is efficacious in children and adults with SCD, and has been proven to increase percentage of HbF, and reduce hospitalizations and pain crises (Segal *et al*., 2008).

Opiod and non opiod analgesics were used in management of pain in the haematology clinic of ABUTH, Zaria in accordance with the standard treatment guidelines (2008). Patients who presented to the haematology clinic in painful crises were immediately hydrated using five percent dextrose saline or if they were not too ill to drink adequate amounts of water, they were encouraged to do so. The patients were also assessed for the cause of pain and other possible complications, after which they were treated appropriately. Interview with the doctors revealed a deep concern for the SCD patient; who often experience pain severe enough to require treatment with analgesics. Analgesics are the foundation for the management of sickle cell pain, and their use should be tailored to the individual patient. However, in general, duration and frequency of pain episodes in the SCD patient is high, and this raises the risk of peptic ulcer and nephropathy with non steroidal anti-inflammatory analgesics (NSAIDS) and that of addiction with use of opioid analgesics. Both NSAIDs and opioid analgesics were used to manage pain in the SCD patients in the study, the WHO three-step ladder for the management of pain in cancer was used in making a choice of analgesic. In addition, the unique characteristic of each patient was taken into consideration with respect to previous adverse drug reactions (e.g. headache or insomnia with pentazocin, uncontrollable vomiting with tramadol especially when given intravenously, epigastric pain with ibuprofen) as well as sickle cell co morbid illnesses (mentioned above).

The HbSS patients generally had a higher analgesic utilisation pattern than the HbSS+F and the HbSC. Paracetamol utilisation across all the SCD patients was similar. Ibuprofen utilisation by the HbSS was the highest (54%), while HbSS+F had an ibuprofen utilisation of 38% and HbSC was 33%. Diclofenac utilisation by the HbSS and HbSC was similar (70% and 67% respectively), while the HbSS+F had a lower utilisation of 48%. Tramadol had a similar utilisation pattern with diclofenac (HbSS, HbSC and HbSS+F had utilisations of 73%, 67%, and 43% respectively). Pentazocin utilisation by the HbSS was highest (43%), followed by the HbSC (33%), while the HbSS+F had the lowest utilisation (29%). Utilisation of pethidine was 2% and the SCD patients that had used pethidine in the study were HbSS. Only 7% of all the SCD patients in the study had used morphine and they were in crises during the study. Morphine utilisation by the HbSS+F was 1%, while the utilisation by the HbSS was 6%.

5.4 Full Blood Count of the Groups

Recent evidence implicates the immune system and the clotting mechanisms in the pathophysiology of sickle cell disease (SCD) (Sarris *et al*., 2008). Platelet count of SCD patients in crises and HbAA volunteers were within normal range while that of SCD in steady state was above normal range. There was a statistically significant difference between the groups. Kenny *et al*. (1980) suggested that platelet hyperactivity of the sickle-cell steady state reflects an increased circulating population of young, metabolically active platelets resulting from previous autosplenectomy. They continued by saying that platelets are implicated in vaso occlusive crises, and may fall during crises compared with steady-state values (Kenny *et al*., 1980).

The most common cause of elevated platelet count is iron deficiency which was not the case in the present study. Perhaps the elevation found among the SCD patients in steady state in present study was due to the background inflammation in the SCD patient which could trigger a thrombocytic response. Therefore, to alleviate the vasodilatory effect, antiplatelet drugs such as aspirin and dipyridamole have a role. However, there is a need for caution in SCD patients with peptic ulcers and SC nephropathy.

High platelets and leucocyte counts are cautionary indicators in the SC patient undergoing surgery; especially as surgical and anaesthetic stress can trigger sudden intravascular stress with fatal outcomes. Therefore, nitric oxide (laughing gas) which is a vasodilator plus 50% oxygen

should be the hallmark of inhalation anaesthetics. When conditions permit, ketamine plus 50% oxygen can also be used.

Increasing evidence suggests that white blood cells (WBC), especially neutrophils, may be involved in the initiation and propagation of vaso-occlusive crisis in SCD (Lard *et al*., 1999). The WBC counts of all the groups fell within normal limits; SCD patients in crises had the highest WBC counts, followed by the SCD patients in steady state; with the HbAA volunteers having the lowest WBC counts. There was a statistically significant difference between the WBC counts of the groups. Elevated total WBC counts are common in SCD patients and WBC counts of more than 15×10^9/L are associated with an increased risk of early death in SCD (Lard *et al*., 1999). Elevated WBC counts are an indication of infection e.g. septicaemia or organ damage. Awogu (2000) in a study among sickle cell children in Lagos observed elevated WBC counts among the steady state patients and suggested that previously established criteria for the diagnosis of bacterial infections cannot be applied to sickle cell patients. High WBC and platelets play a predictive role in indicating the presence of infection and are routinely monitored in the SCD patients of ABUTH.

The neutrophil counts in all the groups had similar pattern as the WBC count. They all fell within normal limits; with the steady state group having the highest values (60.27%), followed by SCD patients in crises (58.26%); with the HbAA volunteers having the lowest neutrophil counts (45.75%). There was a statistically significant difference between the neutrophil counts of the SCD in crises and HbAA group; as well as the SCD in steady state and HbAA group. However, there was no statistically significant difference between the neutrophil counts in both SCD groups. Adhesion of (activated) neutrophils to endothelium in patients with SCD may lead to endothelial damage as described in other vascular diseases. Because the neutrophil is larger and more difficult to deform than the red cell, its attachment to the endothelium, particularly in the microcirculation, would impede passage of RBC and WBC, which could increase the risk for vaso-occlusive crises. Thus, one would assume that the neutrophil counts in the SCD in crises will be higher than that in SCD in steady state; however, there was no statistically significant difference between the two SCD groups in this study.

Lymphocytes which are believed to be responsible for the storage of immunologic memory are a primary source of viral defence and antibodies. The lymphocyte counts of all the groups were within normal limits and there was no statistically significant difference between them.

PCV is considered an integral part of a person's complete blood count results. It is the proportion of blood volume that is occupied by red blood cells. In the present study, PCV of the HbAA volunteers (38.36%) fell within normal range while that of the SCD in crises (22.76%) and SCD in steady state (24.53%) groups fell below normal range. In Ibadan (Nigeria), the average PCV of SCD patients was found to be 22.3% (Olaniyi and Abjah 2007); this was close to the average PCVs of SCD patients in the present study (23.65%). Transfusions are not needed for the usual anaemia or episodes of pain associated with sickle cell disease (Steinberg, 1999). Thus since SCD patients have a lower than normal PCV, blood transfusions in the SCD patient must be individualised and based on each patients steady state PCV.

5.5 Result of the Micronutrient Assay

Sickle erythrocytes are fragile and dehydrated; and they require a delicate balance of minerals and antioxidants to maintain hydration and membrane integrity (Okochi and Okpuzor 2005). Dehydration of sickle erythrocytes is mediated by the K-Cl co transport whose activity is triggered by low plasma magnesium (Mg) levels (Brugnara *et al*., 1993). When the internal Mg of the erythrocytes is increased, the activity of K-Cl co-transport is markedly diminished. Therefore, blockage of this pathway by intracellular Mg could result in decreased dehydration and sickling *in vivo* (Okochi and Okpuzor 2005). Among the many important functions of Mg is its involvement, along with calcium, in the organization of membranes. Both cations are known to act as bridges between the neighbouring carboxylate groups in lipoproteins and such bridges stiffen the cell membranes, thus reducing the tendency of sickle RBC to haemolyse (Okochi and Opkuzor 2005). Protection of red cell membranes from free radical-mediated oxidative stress is crucial to the successful management of the sickle cell crises (Aslan *et al*., 2000). Certain minerals, copper, iron, magnesium, selenium as well as some antioxidants and vitamins C, E, B6, B12, and folate, have been found to effectively relieve the oxidative stress that prevails in SCD (Hasanato, 2006). In addition, copper, iron and magnesium play important roles in haemoglobin synthesis.

Several studies have been done on magnesium concentration, but these studies have shown conflicting results. Reduced erythrocyte magnesium content and normal serum Mg was observed sickle cell disease patients in Lagos (Okochi and Okpuzor 2005). Other studies have measured normal circulating levels, while others are reported to be low (Hyacinth *et al*., 2010).

In present study, plasma magnesium levels of HbAA volunteers were highest (0.88 mg/dL). The levels of Mg in the SCD patients in steady state were lower (0.74 mg/dL), while the SCD patients in crises had the lowest plasma magnesium concentration (0.62 mg/dL). There was a statistically significant difference between the groups. This is in line with literature which suggests that low magnesium levels contribute to sickle cell crises by activating K-Cl co transport system, dehydrating the sickle red blood cell, increasing the fragility of the RBC and leaving the system exposed to reactive oxygen species. It has been shown that Magnesium (Mg) is effective in reducing not only the painful episode in SCD but also affects the hydration of RBC (Okochi and Okpuzor 2005). Oladipo *et al*., (2005) reported use of magnesium in clinical practice to reduce erythrocyte dehydration in SCD; thus, prevention of red cell dehydration using magnesium supplements represents an exciting possible new therapeutic strategy (DeFranchesci, 1997).

Zinc has been said to have important therapeutic potential in the management of sickle cell disease since 1974 (Brewer Oelshlegel Jr. 1974). Approximately 60-70% of adolescent and adult patients with SCD are zinc deficient (Prasad, 2002). In children with acute malaria infection, plasma zinc concentration has been found to be very low (Muller *et al*., 2001). A deficiency of zinc in patients with sickle cell disease results in growth retardation (dwarfism), hypogonadism in males, rough skin, poor appetite, mental lethargy and recurrent infections. Zn plays a very important role in iron metabolism (Prasad, 1999), prevents formation of irreversibly sickled cells and inhibits sickling (Okochi and Okpuzor 2005). It has been proposed that it produces its anti-sickling effect by antagonizing calcium. During sickling, there is an influx of calcium into the erythrocytes and this destroys the membrane leading to sickling; thus, zinc supplementation reverses this effect. In the present study, the plasma zinc levels of both SCD groups was lower than the HbAA controls, with the SCD crises group being lower than the SCD steady state group. This suggests that the SCD group could probably benefit from zinc supplementation; and the low

zinc levels could be a contributing factor to the crises being experienced by the SCD crises group, because, they had the lowest plasma zinc levels.

Plasma copper levels are reciprocal to plasma zinc levels. Deficiency of copper is known to cause anaemia and it is an important antioxidant (Okochi and Okpuzor 2005). There are reports of increased plasma copper levels in individuals with HbSS, and erythrocyte copper is either normal or increased. The clinical significance of this elevation in plasma copper is unclear, but it has been reported to occur in the event of decreased plasma zinc levels (Hyacinth *et al*., 2010). Plasma copper levels of all the groups were a reciprocal of the plasma zinc levels. The SCD patients in crises had the highest levels (94.39 µg/dL), while the SCD patients in steady state had a lower plasma copper concentration (90.15 µg/dL); with the HbAA volunteers having the lowest levels (82.39 µg/dL); however, there was no statistically significant difference between them. Since the copper levels were normal, it does not provide an avenue for therapeutic intervention.

Fairly recent reports from India (Mohanty *et al*., 2008) and Nigeria (Hyacinth *et al*., 2010), describe low iron stores in the bone marrow of 36%–67% of the patients studied (Hyacinth *et al*., 2010). In contrast, Vinchinsky *et al*., (1981) reported that only 16% of their non-transfused patients in the United States showed evidence of iron deficiency. In a study by Aken'ova *et al*., (1997) in Ibadan, all the adult sickle cell anaemia patients had serum iron levels within or above normal range; with serum iron levels being found to be deficient in only 6% of the patients. They concluded that sickle cell anaemia patients in the area had adequate iron levels and that iron supplements should only be given in cases of proven iron deficiency. Akpotuzor *et al*. (2007) found serum iron levels of 14.1-16.5 µmol/dL and 15.6-18.1 µmol/dL in Ekori and Calabar metropolis respectively in healthy adult volunteers of Cross River State Nigeria. In present study, plasma iron of the SCD patients in crises (19.09µmol/dL) and SCD patients in steady state (17.60µmol/dL) was higher than the HbAA volunteers (14.15 µmol/dL); with the SCD patients in crises having the highest iron levels. The values for the HbAA obtained were close to that of Akpotuzor *et al*. (2007). There was a statistically significant difference between plasma iron levels of the SCD patients in crises and the HbAA volunteers and the SCD patients in steady state and the HbAA volunteers; and no statistically significant difference between both SCD groups. This shows that since the plasma levels of the SCD groups are both above normal, iron

supplementation can only be used in these patients in cases of proven iron deficiency as was suggested by Aken'ova *et al*., 1997.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Sickle cell disease (SCD) is a common genetic disorder which represents a major medical problem in certain parts of the world. It is characterized by chronic haemolytic anaemia and vaso-occlusive crises, which can lead to widespread vascular occlusion by sickled red blood cells leading to multiple organ infarctions and damage. In this respect, SCD can be considered as a multisystem disease. Pain is a common mode of presentation in patients with SCD but there is considerable variability in the way SCD pain is managed. The standard treatment protocol for painful episodes has remained bed-rest, hydration and analgesia in spite of undesirable effects of the analgesics, some of which add to existing complications of SCD e.g. proteinuria and gastrointestinal ulceration.

Drug management of SCD in ABUTH complies with the standard treatment guidelines (2008). Magnesium and zinc was found to be deficient in SCD patients in this study. These levels were found to be lower than the plasma levels in apparently healthy HbAA volunteers who were age and sex matched. Plasma copper levels were reciprocal to the zinc levels. Plasma iron levels were however found to be higher in most of the SCD patients than in the apparently healthy HbAA volunteers. This emphasizes the fact that although iron deficiencies do occur in SCD patients, it must be confirmed by laboratory analysis before giving iron supplements - which was the practise observed by the clinicians in ABUTH.

The low plasma magnesium levels suggest that magnesium deficiency exists in the SCD patients. The SCD crises group had lower plasma magnesium levels than the SCD steady state group suggesting that sickle cell crises may be associated with magnesium deficiency. This shows that the SCD patients would probably benefit from magnesium rich diets or magnesium supplementation as an intervention method to preventing or reducing the frequency of occurrence of sickle cell crises. Zinc supplementation can also be considered because of its numerous benefits to the SCD patient including antioxidant activity and boosting immunity against infections. Certain minerals, copper, iron, magnesium, selenium as well as some antioxidants and vitamins C, E, B12, B6, and folate (B9), have been found to effectively relieve

the oxidative stress that prevails in SCD. It has been proposed that a cocktail of antioxidants would be effective in alleviating the incidence and severity of crisis in sickle cell patients; we now have evidence for the use of at least two of these (magnesium and zinc).

Deficiencies in some groups of people at special risk require supplementation, but the most effective way to meet community health needs safely is by population based approaches involving food fortification. These complementary methods, along with food security, education, and monitoring, are challenges for public health and for clinical medicine.

6.2 Recommendations

Barriers to care and to compliance with treatment of sickle cell disease should be studied, including economic factors such as cost of care and insurance coverage.

Patient education on importance of compliance with drug management strategies, availability, and accessibility to medication should be improved.

The positive impact of alleviating micronutrient malnutrition on physical activity, education and productivity, and hence on national economies suggests that there is also an urgent need for increased effort to demonstrate the cost of these deficiencies, as well as the benefits of addressing them, especially compared with other health and nutrition interventions.

Assessment of quality of life (QOL) is necessary to fully understand the needs of adults and children with sickle cell disease; and health care professionals should be able to use effective measures to evaluate QOL in the management of sickle cell disease.

Stem cell transplant is another area that should be looked into. It was successfully performed on the first Nigerian and was the fourth to be successfully performed in Africa on 14th October, 2011. He was presented as being of HbAA in January 2012; this feat rekindled the hope of sickle cell patients and others that require regenerative medicine for a cure. However, the cost of the transplant is put at between N2.5 million and N5million for a patient locally and N25 million overseas while the cost of the drugs as reported by University of Benin Teaching Hospital for the first beneficiary was put at a whooping N2.1 million. It is sad that a procedure that has been in existence for some years has only just been performed for the first time in Nigeria in 2011; in addition, the cost relative to the average income of a Nigerian is phenomenal.

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APPENDIX I: ETHICAL APPROVAL

POSTGRADUATE OFFICE

FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY TEACHING HOSPITAL
SHIKA - ZARIA, NIGERIA

P.M.B. 06 Samaru Zaria
Phone: 069 -551399
Fax: 069 - 555001
E-mail: abuthpg@yahoo.com

Your Ref:

Our Ref: ABUTH/PGO/COMM 9

Date: 11th November, 2010

Olorukooba Bashirat Abimbola
Dept. of Pharm
ABU
Zaria

Dear Bashirat

ETHICAL CERTIFICATE

Your research proposal titled **ROLE OF MICRONUTRIENTS IN PATIENTS WITH SICKLE CELL ANAEMIA AT AHMADU BELLO UNIVERSITY TEACHING HOSPIAL ZARIA** refers

This is to convey ethical committee's approval for commencement of the research.

You are required to keep this office informed of the progress being made on the research.

Yours sincerely,

DR. A J RANDAWA
Co-Ordinator Residency and ethics

APPENDIX II: Consent / Patients' Data Form

Study Title: DRUG MANAGEMENT AND MICRONUTRIENT LEVELS IN SICKLE CELL ANAEMIA PATIENTS OF AHMADU BELLO UNIVERSITY TEACHING HOSPITAL (ABUTH), SHIKA.

Researcher: Olorukooba, Bashirat Abimbola

Supervisors: Dr. A.U. Zezi, Dr. (Mrs) B.B. Maiha, and Prof. (Mrs) A.I. Mamman.

Summary: This research work titled 'Drug Management and Micronutrient Levels in Patients with Sickle Cell Disease in ABUTH is being carried out by Olorukooba, Bashirat Abimbola; following approval from the Ethical Health Committee of Ahmadu Bello University Teaching Hospital, Shika. Plasma magnesium, zinc, copper, and iron levels in patients with Sickle Cell Disease has been shown to differ from that of normal population and may need correction. The purpose of the study is to determine the levels of magnesium, zinc, copper, and iron in patients with sickle cell disease with a view to contributing positively to the care of the patients; for this purpose, 5mls of blood will be required. All information obtained; either retrospectively or prospectively will be treated confidentially and be utilised in only scientific interactions. The data will be reported as a thesis for an MSc degree in Pharmacology. In case you need the results they will be given to you.

Risks:

- The study involves collecting and storing medical information and blood samples, with no physical risks to the patient.
- All information obtained will be treated with utmost confidentiality.

Benefits:

- The information obtained from the research of medical information and blood samples may be useful in the care of the study population as well as in other sickle cell disease patients.

If you decide to participate in this research, fill the consent form below.

Consent Form:

_____ _____

Name of patient Hospital number

I have read/have been read to/ translated to and understood the information for the research on Drug Management and Micronutrient Levels in Sickle Cell Disease Patients in ABUTH by Olorukooba Bashirat Abimbola. I agree to allow my medical information and blood samples to be used for the research.

_____ _____

Signature of patient Date

Contact information of patient (Address and phone number) _____

_____

_____

_____ _____ _____

Name of witness Date Signature/Thumb print

_____ _____ _____

Name of researcher Date Signature

Contact information of researcher (Address and phone number): No 28 Queen Elizabeth road, G.R.A., Zaria. 08054584128

Patients' Data Form

Personal Data

1. Name:_____
2. Unit number: _____
3. Sex: _____
4. Age (years): _____
5. Weight (Kg) : _____
6. Height (m) : _____
7. Marital status : Single ( ), Married ( )
8. Occupation : Civil servant ( ), Student ( ), Home maker ( ), Unemployed ( ), Business ( ), Others ( )
9. Highest educational level : No formal education ( ), Primary school ( ), Secondary school ( ), Tertiary education ( )

Family, Clinical, and Social History

10. Age at diagnosis : _____
11. Number of siblings : _____
12. Number of siblings with sickle cell anaemia : _____
13. Average number of crises per year : _____
14. Most common presentation during crises : _____
15. Average number of hospital admissions per year : _____
16. Most common clinical presentation for admission : _____
17. Number of blood transfusions : _____
18. Co-morbid illness apart from sickle cell anaemia : yes ( ) / no ( ), if yes, specify: hypertension ( ), diabetes ( ), others_____
19. Drug use :
    a. Folic acid ( ), Proguanil ( ), Vitamin C ( ), Vitamin B12 ( ), Vitamin B6 ( ), Vitamin E ( ), Liberal Fluid Intake ( )
    b. Analgesics: Paracetamol ( ), Ibuprofen ( ), Dicofenac ( ), Tramadol ( ), Pentazocin ( ), Pethidine ( ), Morphine ( )

c. Hydroxyurea ( )

20. History of: septicaemia ( ), anaemia ( ), acute chest syndrome ( ), haematuria ( ), gnathopathy ( ), bossing of skull bones ( ), jaundice ( ), osteomyelitis ( ), avascular necrosis – humeral head ( ) / femoral head ( ), leg ulcers ( ), frequent headaches ( ), eye disorder ( ), priapism ( ), hepatomegaly ( ).

Diagnosis and Features (to be completed by researcher)

21. Laboratory tests to be carried out :
    a. Full blood count
        i. Platelet count
        ii. White blood cell count
        iii. Packed cell volume (PCV)
        iv. Reticulocyte count
    b. Film
    c. Haemoglobin electrophoresis
    d. Assay of micronutrients:

MICRONUTRIENT	PLASMA LEVEL (µg/dL)
Magnesium (Mg^{2+})	
Zinc (Zn^{2+})	
Copper (Cu^{2+})	
Iron (Fe^{2+})	

APPENDIX III: ANOVA: Single Factor: Mean Age (Years) of the Groups

SUMMARY

Groups	Count	Sum	Average (Years)	Variance
HbSS Crises	45.00	1055.00	23.44	15.39
HbAA	45.00	1079.00	23.98	8.75
HbSS Steady State	45.00	1043.00	23.18	14.42

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	14.93	2.00	7.47	0.58	0.56	3.06
Within Groups	1696.67	132.00	12.85			
Total	1711.60	134.00				

Since F (0.58) is less than F critical (3.06); it implies that there is no statistically significant difference between the mean ages of the three groups.

APPENDIX IV: ANOVA: Single Factor: Mean Weights (Kg) of the Groups

SUMMARY

Groups	Count	Sum	Average (Kg)	Variance
Crises	45.00	2321.50	51.59	71.15
HbAA	45.00	2802.00	62.27	94.88
Steady State	45.00	2359.40	52.43	80.23

ANOVA

Source of Variation	SS	df	MS	F	p-value	F critical
Between Groups	3171.94	2.00	1585.97	19.32	4.37E-08	3.06
Within Groups	10835.74	132.00	82.09			
Total	14007.68	134.00				

Since F (19.32) is greater than F critical (3.06); it implies that there is a statistically significant difference between the weights of SCD patients in crises, SCD patients in steady state, and HbAA individuals.

APPENDIX V: t-Test: Mean Weights (Kg) of SCD Patients in Crises and HbAA Group; SCD Patients in Steady State and HbAA Group; and SCD Patients in Crises and SCD Patients in Steady State.

Parameters	Crises	HbAA	Steady State	HbAA	Crises	Steady State
Mean (Kg)	51.59	62.26	52.43	62.26	51.59	52.43
Variance	71.15	94.88	80.23	94.88	71.15	80.23
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00		0.00		0.00	
df	86.00		87.00		88.00	
t Stat	-5.56		-4.99		-0.46	
P(T<=t) one-tail	1.49E-07		1.56E-06		0.32	
t Critical one-tail	1.66		1.66		1.66	
P(T<=t) two-tail	2.99E-07		3.12E-06		0.65	
t Critical two-tail	1.99		1.99		1.99	

Since t Stat (5.56) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the weights of SCD patients in steady state and healthy HbAA volunteers.

Since t Stat (4.99) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the weights of HbAA individuals and SCD patients in steady state.

Since t Stat (0.46) is less than t Critical two-tail (1.99); it implies that there is no statistically significant difference between the mean weights of both SCD groups.

APPENDIX VI: ANOVA: Single Factor: Mean Age (Years) at Diagnosis of the Groups

SUMMARY

Groups	Count	Sum	Average (Years)	Variance
HbSC	6.00	23.00	3.83	1.77
HbSS	63.00	219.60	3.49	5.02
HbSS+F	21.00	89.50	4.26	12.92

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	9.62	2.00	4.81	0.72	0.49	3.10
Within Groups	578.16	87.00	6.65			
Total	587.78	89.00				

Since F (0.72) is less than F critical (3.10); it implies that there is no statistically significant difference between the mean ages at diagnosis of the different sickle cell disease variants.

APPENDIX VII: t-Test: Two Sample assuming Unequal Variance: Showing White Blood Cell Counts (× 10^9) of SCD Patients as compared with their Amoxicillin Utilisation

	WBC Count of Patients on Amoxicillin	WBC Count of Patients not on Amoxicillin
Mean (× 10^9)	10.55	7.82
Variance	10.42	12.23
Observations	45.00	45.00
Hypothesized Mean Difference	0.00	
df	87.00	
t Stat	3.85	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.66	
P(T<=t) two-tail	0.00	
t Critical two-tail	1.99	

APPENDIX VIII: ANOVA: Single Factor: Platelet Count (× 10^9) of the Groups

SUMMARY

Groups	Count	Sum	Average (	Variance
Crises	16.00	5565.00	347.81	20665.50
HbAA	27.00	6994.00	259.04	4852.04
Steady	8.00	4147.00	518.37	10949.41

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	424621.20	2.00	212310.60	19.87	5.15E-07	3.19
Within Groups	512781.30	48.00	10682.94			
Total	937402.50	50.00				

Since F (19.87) is greater than F critical (3.19); it implies that there is a statistically significant difference between platelet counts of the groups.

APPENDIX IX: t-Test: Two Sample assuming Unequal Variance: Showing Platelet Count ($\times 10^9$) of SCD Patients in Crises and HbAA Group, SCD Patients in Steady State and HbAA Group; and SCD Patients in Crises and SCD Patients in Steady State.

	Crises	HbAA	Steady State	HbAA	Crises	Steady state
Mean ($\times 10^9$)	347.81	259.04	518.38	259.04	347.81	518.38
Variance	20665.50	4852.04	10949.41	4852.04	20665.50	10949.41
Observations	16.00	27.00	8.00	27.00	16.00	8.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	19.00			9.00	19.00	
t Stat	2.31			-6.59	-3.31	
P(T<=t) one-tail	0.02			5.02E-05	0.00	
t Critical one-tail	1.73			1.83	1.73	
P(T<=t) two-tail	0.03			0.00	0.00	
t Critical two-tail	2.09			2.26	2.09	

Since t Stat (2.31) is greater than t Critical two-tail (2.09); it implies that there is a statistically significant difference between the platelet count in SCD patients in crises and HbAA individuals.

Sine t Stat (6.59) is greater than t Critical two-tail (2.26); it implies that there is a statistically significant difference between the platelet count of HbAA individuals and SCD patients in steady state.

Sine t Stat (3.31) is greater than t Critical two-tail (2.09); it implies that there is a statistically significant difference between the platelet count of SCD patients in crises and SCD patients in steady state.

APPENDIX X: ANOVA: Single Factor: White Blood Cell (WBC) Count (× 10^9)

Groups	Count	Sum	Average	Variance
Crises	45.00	454.70	10.10	10.58
HbAA	45.00	228.60	5.08	4.15
Steady state	45.00	371.90	8.26	14.13

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	581.56	2.00	290.78	30.21	1.57E-11	3.06
Within Groups	1270.37	132.00	9.62			
Total	1851.94	134.00				

Since F (30.21) is greater than F critical (3.06), it implies that there is a statistically significant difference between the mean WBCs of the groups.

APPENDIX XI: t- Test: Two Sample Assuming Unequal Variance: showing WBC Count ($\times 10^9$) of SCD Patients in Crises and HbAA Group; SCD Patients in Steady State and HbAA Group; and SCD Patients in Crises and SCD Patients in Steady State.

Parameters	Crises	HbAA	Steady State	HbAA	Crises	Steady state
Mean ($\times 10^9$)	10.10	5.08	8.26	5.08	10.10	8.26
Variance	10.58	41.5	14.13	4.15	10.58	14.13
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	74.00			68.0	86.0	
t Stat	8.78			-4.99	2.48	
P(T<=t) one-tail	2.15E-13			2.17E-06	0.00	
t Critical one-tail	1.66			1.66	1.66	
P(T<=t) two-tail	4.31E-13			4.35E-06	0.01	
t Critical two-tail	1.99			1.99	1.98	

Since t-stat (8.78) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the WBCs of patients in crises and HbAA individuals.

Since t stat (-4.99) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the WBCs of HbAA individuals and SCD patients in steady state.

Since t-stat (2.48) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the WBCs of SCD patients in crises and SCD patients in steady state.

APPENDIX XII: ANOVA: Single Factor: Neutrophil Count (%)

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	2622.00	58.26	89.65
HbAA	45.00	2059.00	45.75	87.05
Steady State	45.00	2712.00	60.26	82.92

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	5566.50	2.00	2783.25	32.15	4.19E-12	3.06
Within Groups	11423.91	132.00	86.54			
Total	16990.41	134.00				

Since F (32.15) is greater than F Critical (3.06); it implies that there is a statistically significant difference between the neutrophil counts of the groups.

APPENDIX XIII: t- Test: Two Sample Assuming Unequal Variance: showing Neutrophil Count (%) in SCD Patients in Crises and HbAA individuals; SCD Patients in Steady State and HbAA Group; and SCD Patients in Crises and SCD Patients in Steady State.

	Crises	HbAA	Steady state	HbAA	Steady state	Crises
Mean (%)	58.26	45.75	60.26	45.75	60.27	58.26
Variance	89.65	87.05	82.92	87.05	82.92	89.65
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	88.00			88.00	88.00	
t Stat	6.31			-7.46	1.02	
P(T<=t) one-tail	5.38E-09			2.81E-11	0.15	
t Critical one-tail	1.66			1.66	1.66	
P(T<=t) two-tail	1.07E-08			5.62E-11	0.31	
t Critical two-tail	1.99			1.99	1.99	

Since t stat (6.31) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the neutrophil count in the SCD patients with crises and the HbAA group.

Since t Stat (-7.46) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the neutrophil counts of HbAA individuals and SCD Patients in Steady State.

Since t Stat (1.02) is less than t Critical two-tail (1.99); it implies that there is no statistically significant difference between the neutrophil counts of the two SCD groups.

APPENDIX XIV: ANOVA: Single Factor: Lymphocyte Count (%)

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	1830.00	40.67	40.18
HbAA	45.00	1895.00	42.11	53.46
Steady State	45.00	1791.00	39.80	60.94

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	122.68	2.00	61.34	1.19	0.31	3.06
Within Groups	6801.64	132.00	51.53			
Total	6924.33	134.00				

Since F (1.19) is less than F Critical (3.06); it implies that there is no statistically significant difference between the lymphocyte counts of all the groups.

APPENDIX XV: ANOVA: Single Factor: PCV Levels (%)

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	1024.00	22.75	12.09
HbAA	45.00	1726.00	38.35	28.51
Steady state	45.00	1104.00	24.53	25.84

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	6563.62	2.00	3281.81	148.16	1.82E-34	3.06
Within Groups	2923.82	132.00	22.15			
Total	9487.44	134.00				

Since F (148.16) is greater than F Critical (3.06); it implies that there is a statistically significant difference between the PCVs of the three groups.

APPENDIX XVI: t-Test: Two Sample Assuming Unequal Variance: showing PCVs (%) of SCD Patients in Crises and HbAA Individuals; PCVs of HbAA individuals and SCD Patients in Steady State; and PCVs of SCD Patients in Crises and SCD Patients in Steady State

	Crises	HbAA	Steady state	HbAA	Crises	Steady state
Mean (%)	22.75	38.35	24.53	38.35	22.75	24.53
Variance	12.09	28.51	25.85	28.51	12.09	25.85
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	76.00			88.00	78.00	
t Stat	-16.42			12.57	-1.94	
P(T<=t) one-tail	5.14E-27			1.25E-21	0.03	
t Critical one-tail	1.67			1.66	1.66	
P(T<=t) two-tail	1.03E-26			2.31E-21	0.06	
t Critical two-tail	1.99			1.99	1.99	

Since t Stat (16.42) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the PCVs of SCD patients in crises and HbAA individuals.

Since t Stat (12.57) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the PCVs of HbAA individuals and SCD patients in steady state.

Since t Stat (1.94) is less than t Critical two-tail (1.99); it implies that there is no statistically significant difference between the PCVs of SCD patients in Crises and SCD patients in steady state.

APPENDIX XVII: ANOVA: Single Factor: Plasma Magnesium Levels (mg/dL)

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	28.12	0.62	0.02
HbAA	45.00	39.62	0.88	0.14
Steady state	45.00	33.48	0.74	0.03

ANOVA

Source of Variation	SS	df	MS	F	P-value	F Critical
Between Groups	1.47	2.00	0.74	11.20	3.20E-05	3.06
Within Groups	8.67	132.00	0.06			
Total	10.14	134.00				

Since F (11.20) is greater than F Critical (3.06); it implies that there is a statistically significant difference between the plasma magnesium levels of the groups.

APPENDIX XVIII: t-Test: Showing Plasma Magnesium Levels (mg/dL) in SCD Patients in Crises and HbAA Individuals; SCD Patients in Steady State and HbAA Individuals; and SCD Patients in Crises and SCD Patients in Steady State

	Crises	HbAA	Steady state	HbAA	Crises	Steady state
Mean (mg/dL)	0.62	0.88	0.74	0.88	0.62	0.74
Variance	0.02	0.14	0.03	0.14	0.02	0.03
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	59.00			65.00	86.00	
t Stat	-4.25			2.21	-3.28	
P(T<=t) one-tail	3.82E-05			0.02	0.00	
t Critical one-tail	1.67			1.67	1.66	
P(T<=t) two-tail	7.63E-05			0.03	0.00	
t Critical two-tail	2.00			1.99	1.99	

Since t Stat (4.25) is greater than t Critical two-tail (2.00); it implies that there is a statistically significant difference between the plasma magnesium levels of the SCD patients in crises and HbAA individuals.

Since t Stat (2.21) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the plasma magnesium levels in the HbAA individuals and SCD patients in steady state.

Since t Stat (3.28) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between the plasma magnesium levels in both SCD patient groups.

APPENDIX XIX: ANOVA: Single Factor: Plasma Zinc Levels (µg/dL) in all the Groups

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.0	7889.80	175.33	15124.13
HbAA	45.0	9957.50	221.27	19599.12
Steady State	45.0	9080.90	201.79	13938.72

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	47870.59	2.00	23935.30	1.48	0.23	3.06
Within Groups	2141127.00	132.00	16220.66			
Total	2188998.00	134.00				

Since F (1.48) is less than F Critical (3.06); it implies that there is no statistically significant difference between plasma zinc levels in SCD patients and HbAA individuals.

APPENDIX XX: ANOVA: Single Factor: Plasma Copper Levels (µg/dL) in all the Groups

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	4247.60	94.39	694.13
HbAA	45.00	3707.50	82.38	770.97
Steady State	45.00	4056.90	90.15	886.80

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	3334.48	2.00	1667.24	2.13	0.12	3.06
Within Groups	103483.60	132.00	783.97			
Total	106818.00	134.00				

Since F (2.13) is less than F Critical (3.06); it implies that there is no statistically significant difference between plasma copper levels in SCD patients and HbAA individuals.

APPENDIX XXI: ANOVA: Single Factor: Plasma Iron Levels (µmol/dL) in all the Groups

SUMMARY

Groups	Count	Sum	Average	Variance
Crises	45.00	859.20	19.09	161.44
HbAA	45.00	636.70	14.15	24.05
Steady state	45.00	792.10	17.60	49.69

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	578.95	2.00	289.47	3.69	0.03	3.06
Within Groups	10348.43	132.00	78.39			
Total	10927.38	134.00				

Since F (3.69) is greater than F Critical (3.06); it implies that there is a statistically significant difference between the mean plasma iron levels of the groups.

APPENDIX XXII: t-Test showing Plasma Iron levels (µmol/dL) in SCD Patients in Crises and HbAA Individuals; SCD Patients in Steady State and HbAA Individuals; and SCD Patients in Crises and SCD Patients in Steady State

	Crises	HbAA	Steady state	HbAA	Crises	Steady state
Mean (µmol/dL)	19.09	14.15	17.60	14.15	19.09	17.60
Variance	161.44	24.06	49.69	24.06	161.44	49.69
Observations	45.00	45.00	45.00	45.00	45.00	45.00
Hypothesized Mean Difference	0.00			0.00	0.00	
df	57.00			79.00	69.00	
t Stat	2.44			-2.69	0.69	
P(T<=t) one-tail	0.00			0.00	0.25	
t Critical one-tail	1.67			1.66	1.67	
P(T<=t) two-tail	0.02			0.00	0.49	
t Critical two-tail	2.00			1.99	1.99	

Since t Stat (2.44) is greater than t Critical two-tail (2.00); it implies that there is a statistically significant difference between the plasma iron levels of SCD patients in crises and the HbAA individuals.

Since t Stat (2.69) is greater than t Critical two-tail (1.99); it implies that there is a statistically significant difference between mean plasma iron levels of HbAA individuals and SCD patients in steady state.

Since t Stat (0.69) is less than t Critical two-tail (1.99); it implies that there is no statistically significant difference between the iron levels in SCD patients in crises and SCD patients in steady state.