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Nutritional Assessment of Children with Sickle Cell Diseases at the Komfo Anokye Teaching Hospital

By

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis.

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ABSTRACT

Sickle cell disease (SCD) is a chronic haemolytic disease mostly associated with impaired growth, delayed maturation and poor nutrition status. It is also one of the major contributing factors for childhood mortality. The study aimed to assess the nutritional status of children with sickle cell diseases. This was done using dietary intakes, anthropometric measurements and biochemical markers. A cross sectional study was conducted on 100 children with sickle cell diseases aged 3- 12 years at the Komfo Anokye Teaching Hospital. Multiple 24-hr dietary recall and food frequency questionnaire were used to assess dietary intake. Serum protein, albumin and ferritin, as well as full blood count were used to assess biochemical status. Weight, height and Mid-Upper-Arm-Circumference were used to calculate Body Mass Index (BMI), weight-for-age (percentile), height-for-age (percentile), BMI-for-age (percentile) and MUAC-for-age (percentile). The mean intake of iron was 5.9±3.0 mg/d, zinc was 5.1±3.0 mg/d, and vitamin A was 107 ± 112.4 µg/d, while vitamin E was 4.2±2.9 mg/d for the children with SCD. Energy consumed were 852 ± 342.3 kcal while proteins were 25.0 ± 10.7 g/d. Low BMI-for-age, MUAC-for-age, weight-for-age and height-for-age were observed in 40%, 37%, 22%, and 69% of the children, respectively. There was significant association ($p = 0.00$, $r = 0.64$) between vitamin B_{12} and the Red Blood Cell count. The study observed inadequate nutritional intake of the children who were assessed.

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LISTS OF ABBREVIATIONS

CVD	Cerebrovascular Disease
Hb	Haemoglobin
HCT	Haematocrit
MCV	Mean Corpuscular Volume
PLT	Platelet
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
SCA	Sickle Cell Anaemia
SCD	Sickle Cell Disease
VOC	Vaso- Occlusion Crisis
WBC	White Blood Cell
WHO	World Health Organisation

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CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND OF THE STUDY

Malnutrition, especially undernutrition, is highly prevalent in developing countries, despite economic growth (Daboné *et al.*, 2011). Child undernutrition is one of the major worldwide health challenges (Chikhungu and Madise, 2014). Annually, undernutrition is implicated in 50% of child death in developing countries (Chikhungu and Madise, 2014). One of the major contributors of childhood malnutrition is sickle cell disease (Hyacinth *et al.*, 2010). Malnutrition has been considered as an important modifiable risk factor for sickle cell disease mortality and morbidity in Africa, where dietary intakes are mostly inadequate (Cox *et al.*, 2011).

Sickle cell disease (SCD), is a group of inherited genetic defect that results in abnormal structure of one of the globin chains of the haemoglobin molecules (Rees *et al.,* 2010). Common types of sickle cell diseases are; sickle cell anemia (HbSS), hemoglobin SC disease and Beta thalassemia (HbSB) minor and major (Hyacinth *et al.,* 2013). The most common is sickle cell anaemia (HbSS) (Jacob, 2011) which occurs as a result of the exchange of amino acids in the codon of the *beta* globin chain of haemoglobin gene, creating another haemoglobin (S) gene. The expression of the disease is mainly the presence of haemoglobin (S) in which there is replacement of glutamic acid with valine, resulting in hydrophobic interaction which triggers polymerization. When there is low oxygenation, red blood cells assume longer and inflexible shape mostly termed as sickle cell-shaped red blood cell (Oliveira *et al.,* 2015). SCD is associated with increased metabolism and may cause shortage of substrate for normal growth (Archer *et al.,* 2008). According to Barden *et al.* (2000), SCD is a long term haemolytic disease that is mostly associated with impaired growth, delayed maturation and poor nutritional status. The disease is mostly linked with conditions such as

inflammation, ischaemia, localised infarction and reduced red blood cell survival in their patients. Patients with sickle cell diseases may have increased metabolism of about 20 percent higher than a healthy person (de Oliveira *et al.,* 2015).

Sickle cell disease (SCD) affects many people throughout the world and it is highly common in sub-Saharan Africa, South American, Cuba, Central America, Saudi Arabia, India and Mediterranean. In the United States, the population affected by sickle cell disease each year is about 89,079 of which most are in the black race (Brousseau *et al., 2*010). In Africa, sickle cell disease contributes to about 50 to 90 percent childhood mortality (Grosse *et al.,* 2011). It also affect over 200,000 children each year in Africa (Ansong *et al.,* 2013). The prevalence of SCD is highest in sub-Saharan Africa, especially in West Africa. In Nigeria about 130,000 children are born with the disease yearly (Hyacinth *et al.,* 2013).

In Ghana sickle cell disease (SCD) is a major cause of childhood morbidity and mortality (Magalhaes and Clements, 2011). It affects 2 percent of all babies born in Ghana and 95 percent of the children with SCD die before they are five years old (Dennis-Antwi *et al.,* 2008). Research shows that children with sickle cell disease may have high risk of developing malnutrition due to reduced appetite levels, poor dietary intake and infectious complications (Mandese *et al.,* 2016). Some nutritional deficiencies found in sickle cell disease include Iron, Zinc, Folic acid, B Vitamins, Vitamin C, Vitamin D and Vitamin E (Behera *et al.,* 2012; Hasanato, 2006; Jackson *et al.,* 2012). Micronutrients play a major role in the development of children and their deficiency may result in poor growth, reduced motor and brain development and increased illness and death (De-Regil *et al.,* 2011). Children with sickle cell disease may develop vitamin D deficiency, as a result of impairment in the absorption of UV light which may be a major source of vitamin D (Hasanato, 2006). Calcium and vitamin D are required for bone mass formation, hence low levels may lead to growth retardation (Oliveira *et al.,* 2015). Zinc deficiency has also been attributed to inadequate

dietary intake and impaired intestinal absorption (Lesprit and Lesprit, 2004). Other nutrients such as vitamin B_{12} and folic acid deficiency have been reported to affect the production of red blood cells (Kennedy *et al.,* 2001). Iron deficiency anaemia in children with SCD is major problem in developing countries due to low socioeconomic status (Olivieri, 2001). Many studies have associated low Body Mass Index (BMI) and weight in children with SCD with micronutrient deficiency owing to low dietary intake (Hasanato, 2006; Jacob, 2011; Mohamed, 2015).

Macronutrient deficiency has been associated with elevated energy expenditure and inadequate caloric intake (Singhal *et al.,* 2002). Some studies have linked growth retardation in sickle cell children to protein and energy deficiency (Hyacinth *et al.,* 2010). Malnutrition affects the disease process in SCD (Katona and Katona-Apte, 2008) and influences disease severity (Mandese *et al.,* 2016). Therefore, for children with sickle cell disease to benefit from comprehensive treatment, it is important that treatment goes in conjunction with good nutrition (Kawchak *et al.,* 2007).

1.2. PROBLEM STATEMENT

Undernutrition is a critical feature of sickle cell disease. Although efforts have been made by clinicians in improving care to reduce severity of problems, undernutrition which is a serious complication of the disease has been given less attention as part of the treatment protocol (Hyacinth *et al.,* 2010). Studies have shown stunting, poor anthropometric measures such as low weight and poor growth percentiles in height and weight among children with sickle cell disease and these are nutrient-related risk factors which need critical attention. Currently, there are no dietary recommendations for micro and macro nutrient requirements for sickle cell disease patients, although many researchers suggest an increase in dietary intake because of hyper metabolism (Zemel *et al.,* 2007). Despite supplementation of some micronutrients,

malnutrition continues to be a major problem in children with sickle cell disease (Digban *et al.,* 2016). It is therefore, important to assess the nutritional status of sickle cell disease children (3-12year) in order to provide useful information for the administration of nutritional interventions, to avert certain deficiencies associated with this condition. This will be a step in the right direction, especially for nutritional status of children with sickle cell diseases in Ghana, where fewer studies have been done.

1.3. RESEARCH QUESTIONS

- What are the levels of macro and micronutrient intakes in children with children with SCD?
- What are the anthropometric and biochemical markers of nutrition in children with SCD?
- What is the association among nutritional intake, anthropometric and biochemical parameters in children aged 3-12 years with SCD?

1.4. GENERAL OBJECTIVE

The study assessed the nutritional status of sickle cell disease children aged 3-12 years at the Komfo Anokye Teaching Hospital.

1.5. SPECIFIC OBJECTIVES

- To assess the nutrient intake of SCD children aged 3-12 years visiting a sickle cell clinic at the Komfo Anokye Teaching Hospital.
- To determine the anthropometric indices of SCD children aged 3-12 years.
- To determine the correlations between biochemical and nutritional parameters as well as biochemical and anthropometric parameters.

1.6. RESEARCH HYPOTHESIS

Undernutrition is prevalent in children with sickle cell disease.

1.7. JUSTIFICATION

Assessing the nutritional status of SCD children (3-12 years) will help identify nutrient deficiencies and aid in the recommendation of appropriate nutritional interventions for such children. This research intends to fill knowledge gaps and stimulate further research of the role of nutrition in sickle cell disease. The outcome of this study will severe as evidence-based information for dieticians, clinicians, policy makers and the government to develop appropriate nutritional intervention as part of the management of sickle cell disease.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. HISTORICAL BACKGROUND OF SICKLE CELL DISEASES

Sickle cell disease (SCD) was known in Africa before the twentieth century, as a disease that causes acute pains or it refers to children destined to die and be reborn (Ansong *et al.,* 2013). Africa and Asia were considered as the origin of the sickle cell gene mutation gene and spread to Americans through slave trade (Adewoyin, 2015). In the United States, sickle cell disease is relatively prevalent in the black American population (Piel *et al.,* 2014). The four chromosomal haplotypes linked with Beta-S mutation of the haemoglobin were named after the regions where they had the highest frequency (Ansong *et al.,* 2013). Most severe sickle phenotype was associated with the Bantu (Central African region) haplotype, while Asian haplotype, was linked with the mild sickle phenotype (Yawn *et al.,* 2014). Although SCD is popularly found among regions which have high prevalence of malaria, individuals with sickle cell trait are resistant to *Plasmodium falciparum* (Marsh *et al.,* 2011).

In the Ashanti region of Ghana, 23.9% of infants had SCD gene in a new born screening programme and 1 in every 55 babies born had SCD (Marsh *et al.,* 2011). Currently SCD affects millions of populations worldwide and this poses physical, emotional and financial burden on patients affected, their families and the community as a whole (Alapan *et al.,* 2016). However, early diagnoses and improved health care have reduced mortality and morbidity caused by SCD in developed countries, while in developing countries, early mortality due to SCD is still strikingly high (Tewari *et al.,* 2015).

2.2. PATHOPHYSIOLOGY OF SICKLE CELL DISEASES

SCD is a genetic disorder, resulting from structural changes in the arrangement of the amino acid on the beta globin chain of the haemoglobin (de Oliveira *et al.,* 2015). The changes in

the DNA causes a single base change from adenine to thymine, causing the substitution of valine for glutamate in the 6th amino acid of the beta globin chain (Ballas *et al.,* 2012). This abnormality causes polymerization of the haemoglobin under low oxygen tension in the red blood cells, causing the red blood cells to assume sickle shape (Pakbaz and Wun, 2014). On the sickle haemoglobin, the glutamate amino acid molecule which is hydrophilic, polar and negatively charged is substituted with valine which is non polar or hydrophobic and has no charge (McGann *et al.,* 2013). Under low oxygen tension, the abnormal valine causes hydrophobic interaction within the red blood cell of the sickling haemoglobin molecule, leading to precipitation and formation of polymers or gelation (McGann *et al.,* 2013). Polymerization causes rigidity of the red blood cell under low oxygen tension (Hyacinth *et al.,* 2013). Sickling cell resumes its normal shape when there is reoxygenation. However, repeated sickling under changes in oxygen tension causes the damage of the red blood cell membrane (Taylor *et al.,* 2013).

Many factors affect the rate and degree of the sickling of the red blood cells. Dehydration of the red blood cells increases the Mean Cell Haemoglobin Concentration (MCHC) and this can stimulate sickling. Another factor is the presence of other non-sickling haemoglobin which is the foetal haemoglobin. Foetal haemoglobin's affinity for oxygen is substantially greater than that of adult haemoglobin, hence high foetal haemoglobin lowers sickling (Akinsheye *et al.,* 2011). Hydroxyurea is given to SCD patients to promote the production of foetal haemoglobin (Goodman *et al.,* 2016).

Intracellular pH is another factor that affects sickling. Under acidic conditions, haemoglobin molecules give off oxygen easily and sickling occurs (Hyacinth *et al.,* 2013). Average lifespan of a sickled red blood cell is between 16-20 days, as compared to 100-120 days in the normal state and this increases disease severity (de Oliveira *et al.,* 2015).

2.3. TYPES OF SICKLE CELL DISEASES

Haemoglobin SS disease is the most common type of sickle cell disease. It constitutes 70% of cases of SCD in people of African origin (Hirst and Williamson, 2001). It occurs due to the inheritance of two beta globin S (βs) alleles from both parents, thus commonly known as haemoglobin SS (HbSS) (Steinberg, 2005). It is the most severe form of SCD and patients with this genotype suffer the worst symptoms at a higher rate and reduced life expectancy (Tsang *et al.,* 2014).

Haemoglobin SC disease is the second most common type of SCD and it affect 30% of the population in Africa (Steinberg, 2009). It occurs as a result of the inheritance of HbS gene from one parent and HbC from another parent. Individuals with this type of SCD experience similar symptoms as patient with sickle cell anaemia; however, anaemia is less severe in them (Steinberg, 2009).

Thalassaemia is characterized by imbalanced alpha and beta synthesis and anaemia. In beta thalassaemia, there is excess production of free α-globin chains, the excess unpaired a-globin denatures and precipitate and impair the maturation of erythroid precursors, causing ineffective synthesis of haemoglobin (Ngo and Steinberg, 2015). This plays a major role in the causation of anaemia (Mettananda *et al.,* 2015). The reduced amount ($S\beta^{+}$) or the absence ($S\beta^{0}$) of the beta globin chain plays a major role in the precipitation of the erythroid precursors, leading to the ineffective erythropoiesis (Ngo and Steinberg, 2015). Alpha thalassaemia is another type of thalasaemia caused by under-production of alpha chain of haemoglobin which causes erythrocyte damage and shortens cell survival (Vichinsky, 2013). Individuals with α-thalassaemia may be clinically normal but may have minimal anaemia and reduced MCV and MCH (Mettananda *et al.,* 2015). Other genotypes may be haemoglobin SE, haemoglobin SO and many others.

2.3.1. Clinical Features and Complication of Sickle Cell Diseases

2.3.1.1. Vaso-Occlusion Crisis

Vaso-occlusion episode is the clinical hallmark of SCD and it is mostly associated with pains (McGann *et al.,* 2013). Pain is also linked with severity of the disease and often accompanies acute chest syndrome (Iughetti *et al.,* 2016). Several factors may trigger the induction of vaso-occlusion crisis (VOC) but the two main pathophysiological processess underlining vaso-occlusion crisis include chronic haemolysis and increased viscosity of the red blood cells (Potoka and Gladwin, 2015; Yuditskaya *et al.,* 2010).

As a result of polymerization of deoxy HbS, red blood cells become rigid and inflexible and this causes sluggish flow of blood in various vascular beds. The inability of the inflexible sickle cells to move through capillaries and microvascular blood vessels causes occlusion. Adhesion of the sickling cell to the endothelium has also been reported to promote vaso-occlussion (Sparkenbaugh and Pawlinski, 2013).

Another cause of vascular occlusion in SCD is the scavenging of nitric oxide (NO), as a result of chronic haemolysis (Yuditskaya *et al.,* 2010). Nitric oxide is a vasodilator, produced by the endothelium, which promotes regulation of the normal vascular function (Kato *et al.,* 2007). In SCD, the life span of nitric oxide is short, due to decompartmentalised haemoglobin which scavenges nitric oxide, a vasodilator, blocking its role in the endothelium of the blood vessel (Halphen *et al.,* 2014). Nitric oxide (NO) is protected through a barrier created by haemoglobin compartmentalization within the erythrocytes, hence, preventing this reaction from occurring (McGann *et al.,* 2013). The continuous release of haemoglobin, however, destroys the barrier leading to the formation of methemoglobin and nitrite.

Again, high haemoglobin levels in the plasma as a result of intravascular haemolysis reduces the bioavailability as well as circulating levels of NO and this promotes endothelial dysfunction (Kato *et al.,* 2007; Owusu-Ansah *et al.,* 2016). Low circulating levels of NO

contributes to vaso- constriction, pulmonary hypertension and proliferative vasculopathy (Halphen *et al.,* 2014).

2.3.1.2 Pains

Pains may be abdominal pains or bone pains. Acute abdominal pains may be due to vaso-occlusion in the mesenteric vessels causing ischaemic pains (Belfer *et al.,* 2014). Bone pains in SCD are as a result of ischaemic bone activation of nociceptive afferent nerves endings which cause pains (Taylor *et al.,* 2013). Affected sites may include the femur, humerus, vertebrae, pelvis, ribs and sternum. Bone pain crisis occurs mostly in homozygous sickle cell disease or in patients with low foetal haemoglobin (Sauntharajah *et al.,* 2003). Some environmental conditions have been shown to influence the intensity of the disease. These may include exposure to cold temperatures and dehydration. Infections such as malaria affect the intensity of cerebrovascular disease (CVD) which causes pains (Tewari *et al.,* 2015). Diseases such as gastroenteritis, appendicitis or cystitis which are not associated to SCD may also cause pains (Adewoyin, 2015).

2.3.1.3. Cerebrovascular Disease

Development of cerebrovascular diseases (CVD) is one of the major problems in SCD (Rees *et al.,* 2010). Cerebrovascular disease or stroke is a neurologic disorder from vascular origin, lasting more than 24 hours (Iughetti *et al.,* 2016). Stroke can either be ischaemic or haemorrhagic. It affect 25% of patients with SCD and the most occurring type of stroke in SCD is ischaemic stroke (Yuditskaya *et al.,* 2010). Ischaemic stroke is often associated with occlusion of the blood vessels (Potoka and Gladwin, 2015) which may be the distal internal carotid, proximal middle cerebral and the anterior cerebral arteries. Overt stroke is prevalent among children between 2 to 5 years with a prevalence rate of 10% (Iughetti *et al.,* 2016).

Patient may have temporary history of transient ischaemic attack which may gradually graduate into overt stroke, characterized by hemiparesis, speech or visual impairment or coma (Switzer *et al.,* 2006).

2.3.1.4. Other Complications

Other complications may be acute chest syndrome which is caused by vaso-occlusion of the pulmonary vessels and implicated microbes such as *pneumoccus, Haemophilus influenza* and respiratory viruses (Vichinsky *et al.,* 2000). Another example of complications is priapism which is as a result of painful erection that is not associated with sexual arousal and is normally caused by penile ischaemia (Yawn *et al.,* 2014). Immunological deficiency, leading to infection is another complication, associated with suboptimal immunity as a result of disruption of the spleen (Gladwin and Vichinsky, 2008).

2.4. MANAGEMENT OF SICKLE CELL DISEASES

2.4.1. Hydroxyurea (HU)

Management of sickle cell disease may include the use of hydroxyurea (HU) which is normally recommended for patients above 24 months. Hydroxyurea is a molecule that stops the synthesis of DNA by inhibiting ribonucleotides reductase which stops the sickling phase. Hydroxyurea, however, stimulates the production of foetal haemoglobin which has higher affinity for oxygen and prevents sickling of the red blood cells (Davies and Gilmore, 2003). HU is known to increase steady state haemoglobin levels and mean cell volume (MCV), reduce leucocytes and platelet counts. It improves cell hydration, limits interaction of the sickle cells with the vascular endothelium, and acts as a nitric oxide donor which is a potent vasodilator (Yawn *et al.,* 2014). HU is of benefit to patients with moderate to severe sickle

cell disease and recurrent VOC. Minimum time interval for evaluation of therapeutic efficacy is 6 to 9 months. The use of HU has been reported to reduce mortality in SCD (Platt, 2008).

2.4.2. Haematopoietic Stem Cell Transplantation

Haematopoietic stem cell transplantation is considered the only available treatment with a curative intent. It is used to correct the basic genetic defect by replanting genes that are essential for normal haematopoiesis (Lucarelli *et al.,* 2012). Stem cell transplant considered applicable when there is availability of matched sibling donor (Shenoy, 2013). This therapy is recommended for those above 17 years and for patients with severe complication or patients having difficulties in maintaining transfusion therapy due to difficulties in getting compatible unit (Fitzhugh *et al.,* 2014). According to Frenette and Atweh (2007), this procedure is considered too risky since mortality associated with this procedure is 20%.

2.4.3. Transfusion of Red Blood Cells

Transfusion of red blood cells to SCD patients is considered as a baseline correction to anaemia and it prevents clumping of the red blood cells and vaso-occlusion. Blood transfusion from a normal donor contains a normal haemoglobin (HbA) which decreases the percentage of circulating erythrocytes that contain sickled hemoglobin (HbS) (Rees *et al.,* 2010). Sickled cells possess abnormal properties which induce vascular changes and promote vaso-occlusion. However, transfusion from a normal donor is used to reduce these effects (Ohene-Frempong, 2001). Some complications that are associated with transfusion are hyperviscosity, haemolysis, iron overload and alloimunization (Yawn *et al.,* 2014). Alloimunization occurs when the donor erythrocyte have different antigen from that of recipient's own erythrocytes; thus an immunological response is generated against the foreign antigen (Yazdanbakhsh *et al.,* 2012). Iron overload may also occur due to accumulation of

iron outside its normal pathway regulation and thus lead to overload. This excess iron can be removed by chelation (Wanko and Telen, 2005).

2.4.4. Pharmacological Management

Paracetamol and non-steroidal anti-inflammatory drugs are used to control pains. (Rees *et al.,* 2003). Antibiotic penicillin prophlylaxis is used to control infections and non-steroidal anti-inflammatory drugs such as opioids are used to manage more severe pains (Dunlop and Bennett, 2014). Opioids reduce the perception of pains at the level of the central nervous system and they may have adverse effect such as vomiting, nausea, itching and others (Ballas *et al.,* 2012).

2.5. SICKLE CELLS DISEASE AND NUTRITION

Micro and macronutrient deficiency have been associated with immunologic, nutritional and growth defect in SCD patients (Hyacinth *et al.,* 2013). Some studies have suggested that dietary intake is sufficient, but this does not meet the high demand of nutrients imposed by the disease (Manci *et al.,* 2014). Increase in nutrient demand has been attributed to high catabolism, leading to hypermetabolism (Cox *et al.,* 2011). Hyper-metabolism has been linked with high production of pro-inflammatory cytokines and myocardial energy demand (Hyacinth *et al.,* 2013). Furthermore, increased haemolysis and protein turnover place high demand on caloric intake (Barden *et al.,* 2000). Reduced dietary intake has been associated with undernutrition which affects the immunoglobin levels (Hyacinth *et al.,* 2013).

2.5.1. Zinc

Zinc is an essential micronutrient required in various biochemical and physiological functions, such as growth and development and sexual maturation. It has the potential of

improving mental health, and the immune system. Zinc also plays an important role in the metabolism of iron (Okochi and Okpuzor, 2005), synthesis of collagen, DNA and RNA (MacDonald, 2000). It has the ability to function as an antioxidant, protecting the cell membrane and reducing inflammation in SCD patients (Bao *et al.,* 2008). Zinc deficiency is common to sickle cell patients because of chronic breakdown of erythrocytes (Prasad *et al.,* 1975). High protein turnover, renal impairment and mal-absorption in SCD also lead to Zinc deficiency. Availability of Zinc is reduced by phytate, an anti-nutrient which is present in staple foods such as cereals (Prasad, 2008). Zinc deficiency has been related with short stature, low body weight poor growth and poor appetite in children with SCD (Booth *et al.,* 2010). Depression, anxiety and attention deficit hyperactivity disorder has also been associated with Zinc deficiency (Prasad, 2008).

2.5.2. Iron

Iron deficiency is the most common nutritional deficiency affecting over 2 billion individuals globally (Care, 2006). It is the leading cause of anaemia in children, especially those in developing countries. Children with sickle cell disease may be susceptible to iron deficiency, due to increased requirement of iron for growth, diet with low iron bioavailability and chronic haemolysis (Care, 2006). Frequent breakdown of red blood cell increases the absorption of iron from the gastrointestinal tract but the provision of blood transfusion makes iron deficiency rare (Harmatz *et al.*, 2000; Kohgo *et al.*, 2008). Mohanty *et al.* (2008) have, however, reported iron deficiency, despite all these mechanisms to maintain iron balance. The identification of iron deficiency is important since its deficiency may lead to long-term negative impact on the growth and development and cognitive function of children. Iron is needed for the synthesis of haemoglobin, hence its deficiency leads to reduced oxygen

carrying capacity and this affects immunity (Black, 2003). Iron deficiency has been reported more in SCD patients than iron overload (Care, 2006; Don and Kaysen, 2004).

2.5.3. Antioxidants

Antioxidants are compounds which are able to inhibit or delay the oxidation of substrates, *in vivo*, that may lead to oxidative stress. Oxidative stress in sickle cell disease can generate chronic diseases such as stroke and therefore, antioxidant nutrients act as radical scavengers to reduce potential damage. In SCD, denaturation of haemoglobin releases free haem (iron) which undergoes Fenton reaction to form hydroxyl radicals, a damaging of reactive oxygen species (ROS). Production of ROS results in protein oxidation, lipid peroxidation, damage to the DNA and plays a major role in the pathogenesis of vaso-occlussion and organ damage (Tayo *et al.,* 2014). Antioxidants protect dangerous effect of ROS, through cellular and extracellular enzymes such as catalase, superoxide dismutase and free radical scavengers like vitamins C, E and A (Tayo *et al.,* 2014). Catalase is a haem containing enzyme which speeds up the breakdown of hydrogen peroxide to water and oxygen molecule, under physiological conditions. Superoxide dismutases are zinc and copper containing enzymes which convert superoxide radicals to hydrogen peroxides and are later broken down out by catalase.

Aside the role vitamin A plays as an antioxidant, it is needed for immune function, growth, reproduction and vision (Duester, 2000). It aids in protein synthesis and its deficiency promotes infections and affects vision. Its active forms may include retinol, retinal and retinoic acid, collectively known as retinoid. They are readily absorbed as retinol in the intestines. Foods derived from animal sources provide retinyl esters, while those from plant sources provide carotenoids (Rolfes *et al.,* 2014).

Another nutrient which functions as antioxidant is vitamin E. Vitamin E inhibits the action of γ-tocopherol which scavenges Nitric Oxide (NO), thus, protecting the endothelial lining and

preventing inflammation (Walter *et al.,* 2006). Its ability to scavenge free radicals helps in the maintenance of cell membrane (Marwah *et al.,* 2002). It prevents the oxidation of polyunsaturated fatty acids and so protects lipids and other related compounds. Vitamin E deficiency may be associated with fat mal-absorption and this leads to erythrocyte haemolysis, impaired vision and speech and loss of muscle coordination (Rolfes *et al.,* 2014). Vitamin C promotes iron absorption by protecting it from oxidation. It also serves as a co-factor in the synthesis of collagen which helps in wound healing. Patient with SCD may have increased requirement of vitamin C, due to infections and chronic haemolysis. Its deficiency may lead to scurvy, impairment in the formation of collagen, muscle degeneration and neurodegeneration (Covarrubias-Pinto *et al.,* 2015).

2.5.4. B Vitamins

Children with sickle cell diseases have higher risk of B_{12}, folate and vitamin B_6 deficiency because of increased rate of haemolysis and production of red blood cells. Low levels of these vitamins have been reported to increase homocysteine levels which cause stroke in SCD (Aslan *et al.,* 2000). Vitamin B_6 (pyridoxine) in its active form is Pyridoxal-5-phosphate (PLP) and plays a key role as a co-enzymes for protein and urea metabolism (Nelson *et al.,* 2002). The conversion of amino acid tryptophan to niacin is dependent on PLP and it aids in haem synthesis and its deficiency has been shown to cause anaemia. In sickle cell disease, PLP has been reported to prevent gelation of deoxyhemoglobin S, hence inhibiting erythrocyte sickling (Tayo *et al.,* 2014) and also reduces reticulocyte counts in patients (Hyacinth *et al.,* 2010).

Folate and Vitamin B_{12} depend on each other for activation. Vitamin B_{12} removes a methyl group to activate folate co-enzyme and as folate gives up its methyl group for vitamin B_{12} co-enzymes become activated (Rolfes *et al.,* 2014). Vitamin B_{12} and folate are needed for the

formation of red blood cells. Increased breakdown of red blood cells puts high demand on their intake and suboptimal intake lead to their deficiency (Enwonwu, 1988). Deficiency of both vitamin B_{12} and folate leads to poor synthesis of DNA and RNA which eventually lead to anaemia. Vitamin B_{12} deficiency is difficult to diagnose especially if it interferes with iron deficiency. Iron deficiency also masks macrocytosis (Morris *et al.,* 2007). Vitamin B_{12} deficiency may be as a result of reduced absorption from the terminal ileum, reduced production of intrinsic factor that enables absorption or reduced levels of transcobalamin (Kennedy *et al.*, 2001).

2.5.5. Protein

Protein deficiency in sickle cell patients has been attributed to high resting metabolic rate, thus, increasing the energy demands and lowering dietary intake leads to its deficiency (Singhal *et al.,* 2002). Protein is needed for growth and muscle development (Manci *et al.,* 2014). Studies have shown decreased inflammation with normal protein levels in Sickle cell diseases (SCD) patients (Capers *et al.,* 2015). A study done by Zemel *et al.* (2007) associated poor growth and maturation in SCD patients with protein deficiency. Reduction of protein leads to oxidative stress which contributes to the pathogenesis of pulmonary hypertension in SCD patients. L–arginine is an amino acid that is supplied through high protein diet, and needed for protein synthesis, urea and Nitric Oxide (NO) production (Rees and Gibson, 2012). It donates nitrogen for the synthesis of NO which is a potent vasodilator during crisis, thus, preventing vaso-occlusive crisis. Protein is required for the formation of new tissues and the repair of worn-out tissues (Morris *et al.,* 2003).

2.5.6. Dietary Supplementation in SCD

Dietary supplementations of zinc, vitamin A, and magnesium have been shown to improve growth in patients with sickle cell disease (SCD). Micronutrient supplementation has led to the improvement in cognition, reduced inflammation and hospital visit. Anti-oxidant supplementation has reduced oxidative stress and improved anaemia status (Bao *et al.,* 2008). Vitamins A, C and E reduce arterial blood pressure, oxidative stress and increases haemoglobin levels (Tayo *et al.,* 2014). Combination of nutrient (macro and micronutrients) has been reported to yield better results than single supplementation (Hyacinth *et al.,* 2013). Currently, nutritional supplementations which are given to sickle cell disease patients as a first line management are folic acid, zincovite and hydroxyurea (Hyacinth *et al.,* 2010). Dietary supplementation of protein has been shown to reduce infections and improve weight in children with SCD, while zinc is known to offer antibacterial protection and improves weight and growth (Dekker *et al.,* 2012) as well as haemoglobin levels (Muskiet *et al.,* 1991). A study done by Hyacinth *et al.* (2010) reported improved growth after supplementation of protein and calories, in addition to their regular diet. Folate supplementation with B_{12} have been reported to mask the deficiency of vitamin B_{12} (Hyacinth *et al.,* 2010).

2.6. DIETARY ASSESSMENT

Dietary assessment among children is important to nutritional monitoring, clinical research and interventional studies (McPherson *et al.,* 2000). Accurate assessment of a child's dietary intake is an essential factor in determining the nutritional adequacy of an individual child's diet (Livingstone *et al.,* 2004). Valid and reliable methods of dietary assessment frequently used may include 24-hour dietary recall, food frequency questionnaire and weighed food record (McPherson *et al.,* 2000).

2.6.1. Twenty-Four Hour Dietary Recall

Twenty-four hour dietary recall involves an interview with participants that enable them to recall and describe precise food intake and their quantity or any dietary intakes for the previous 24 hours. This method assesses portion size as well as quantities of foods consumed through the use of food aids and models (Thompson and Subar, 2008). It is useful in measuring dietary intakes in groups where mean intakes are to be assessed. This method is useful for the assessment of nutrient intake of children with SCD since it enables food consumed to be quantified for the estimation of nutrients (Jonnalagadda *et al.*, 2000). However, reports of intakes totally depend on the memory of respondent. This may lead to over or under-estimation (Thompson and Subar, 2008).

2.6.2. Food Frequency Questionnaire

Food frequency questionnaire method focuses on dietary patterns of respondents. It sometimes requires information on portion size and cooking methods. Food frequency questionnaire helps in the gathering of habitual intakes of certain type of foods and it is a useful tool for examining long term food intakes (Hu *et al.,* 1999). This method allows for the assessment of usual dietary patterns of the children with SCD and it can capture past dietary intake (Carithers *et al.,* 2009).

2.6.3. Weighed Food Record Intakes

Weighed food record intakes involve the weighing and recording of all foods and beverages consumed on daily basis. Dietary intake is usually recorded over a period of 3-7 days. Weighed food record intake is a useful tool in the assessment of dietary intakes of children with SCD since it allows precise quantities of measured food intakes; however food eaten

outside the home cannot be estimated. Thus, weighed food record intake involves a lot of skills and is time consuming (Thompson and Subar, 2008).

2.7. IMPACT OF SICKLE CELL DISEASES ON GROWTH

Impaired growth is a recognized condition in children with sickle cell diseases and some studies have attributed this condition to marrow hyperplasia (Aguilar *et al.,* 2005). Marrow hyperplasia is a marrow disorder which causes ischaemia of the central portion of the vertebral growth plate, leading to disturbances of vertebral growth (Fitzhugh *et al.,* 2014). Others have associated this to shift from tissue build-up to compensation in SCD, due to chronic haemolysis (Hyacinth *et al.,* 2010). Increase in the demand of nutrients to replenish red blood cells and inadequate nutrients intake leads to poor growth. Children with SCD have shown significant delayed skeletal maturation and puberty growth (Adewoyin, 2015). They are deficit in z-score for weight-for-height and mid upper arm circumference which is an indicator of muscle wasting and low protein (Barden *et al.,* 2002). They have also shown short stature, wasting and delayed maturation and menarche as compared to healthy children (Fitzhugh *et al.,* 2014).

2.7.1. Anthropometric Assessment

Anthropometric assessment is a simple method, where measuring instruments are used to describe human form (Mukhopadhyay *et al.,* 2005). Anthropometric assessment can be done either by applying the measurement directly or through the use of additional calculations. It is an essential part of nutritional assessment to evaluate underweight or obesity determine body composition. Anthropometric assessment in children serves as a sensitive indicator of health, growth and development and thus aids in the classification of malnutrition (Boye *et al.,* 2002; Mukhopadhyay *et al.,* 2005).

2.7.2 Body Mass Index

Body Mass Index (BMI) is weight in kilograms divided by square of height in meters. It is a good indicator of body fat; however, BMI-for-age (percentile) is a good indicator for malnutrition. It involves the use of growth chart that uses the BMI, age and gender to produce a percentile. BMI-for-age above the 85^{th} percentile is classified as overweight while below 5^{th} percentile is underweight and in between 5^{th} and 85^{th} percentile is classified as normal. Using BMI–z score as a growth measure, Kawchak *et al.* (2007) reported poor growth in children with SCD.

2.7.3. Height-For-Age

Height-for-age is used to describe a condition in which children fail to gain height for their age. Stunting is an extreme low height-for-age score. It is mostly associated with chronic malnutrition or frequent illness which causes malnutrition. It is a good indicator of growth failure and this makes it useful in the long term planning of policies and intervention programs (Onis *et al.,* 2000).

2.7.4. Weight-For-Age

Weight-for-age is used to describe a situation where a child weighs less than expected considering a given age. Thus, underweight reflects extremely low weight-for-age. It is also a useful tool for monitoring growth and assessing malnutrition. However, it does not differentiate well between temporary and more permanent malnutrition (Rice *et al.,* 2000).

2.7.5. Mid Upper Arm Circumference

Mid upper arm circumference is a simple, low cost and objective method of assessing nutritional status. MUAC has been recommended by WHO standards as an independent

anthropometric tool for screening children 6-59 months (Goossens *et al*., 2012). It is a good anthropometric measure that predicts malnutrition in community-based studies. It is also a good indicator for protein-energy malnutrition and severe wasting (Berkley *et al.,* 2005). Arm and sub-cutaneous fat are major determinant of MUAC and they are both essential factors of survival in starvation. MUAC is the best predictor of death. MUAC less than 115mm is an indicator of malnutrition (Briend *et al*., 2012).

2.8.BIOCHEMICAL MARKERS OF CHILDREN WITH SICKLE CELL DISEASE

2.8.1. Full Blood Count

Full blood count measures haemoglobin levels, Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), red cell distribution and other haematological indices (Akinbami *et al.,* 2012) and is ordered as part of routine testing. Full blood count test helps to detect illness such as anaemia and with management of sickle cell disease (SCD) patients (Okpala, 2004). Consistent haemolysis and short survival durations of the erythrocytes can cause low levels of haemoglobin and haematocrit in steady state and vaso-occlusive crisis (Omoti, 2005). Low haemoglobin and high reticulocyte count have been related with crisis. Patients with SCD are known to have significantly higher mean of total white blood cell count which have been associated with infections (Okpala, 2004). Low mean corpuscular volume has been linked with reduced clinical severity and MCHC is the main determinant of the red cell membrane changes which indicate sickling. High platelets count greater than 450 counts have been linked with inflammation in sickle cell patients (Akinbami *et al.,* 2012). Hydroxyurea treatment has been reported to improve haemoglobin concentration, MCV and blood volume. It influences reduction of white blood cell count and reticulocyte count (Zimmerman *et al.,* 2004). Blood transfusion also improves haemoglobin

levels (Wanko and Telen, 2005). Tables 2.1 and 2.2 show the reference ranges of full blood count (Behera and Bulliyya, 2016) and haemoglobin levels (g/dl).

Table 2.1. Reference ranges adapted for haematological parameters (Behera and Bulliyya, 2016)

Haematological parameters	Age group	Reference values
Red Blood Count (million/mm^3)	2-6 years	3.9-5.3
	6-12 years	4.0-5.2
Haematocrit (%)	2-6 years	34-40
	6-12 years	35-45
Mean Corpuscular Volume(Fl)	2-6years	75-87
	6-12 years	23-31
Mean Corpuscular Haemoglobin (pg)	2-6years	24-30
	6-12 years	25-33
Mean Corpuscular Haemoglobin (g/dl)	2 years and over	32.3-35.7
White Blood Counts (thousand/mm^3)	2-4 years	6.0-15.5
	4-6 years	6.0-15.5
	6-12 years	4.5-13.5

Table 2.2. Reference range for diagnosing anaemia using haemoglobin according to WHO/UNICEF/UNU (2001) standards

ANAEMIA MEASURED BY HAEMOGLOBIN (d/l)				
	Anaemia	Mild	Moderate	Severe
Children 6-59 months	< 11.0	10-10.9	7.0-9.9	< 7.0
Children 5-11 years	< 11.5	10-11.4	7.0-9.9	< 7.0
Children 12-14 years	< 12.0	10-11.9	7.0-9.9	< 7.0

2.8.2. Serum ferritin

Ferritin is a high molecular iron storage protein that has approximately 20% iron (Brownell *et al.,* 1986). Ferritin exists in almost all tissues of the body especially in the hepatocytes and reticulo-endothelial cells. It is used to estimate body, iron store and exists in serum in small quantities but appears to reflect iron status in a healthy individual (Brownell *et al.,* 1986). Ferritin plays an important role in the absorption and storage of iron so levels below 15 ng/ml is classified as iron deficiency (Care, 2006). Ferritin remains in the body tissues until it releases iron for the synthesis of haemoglobin (Brittenham *et al.,* 1993). Iron molecules are

then released form apoferritin shell and attach itself to transferrin, a circulating plasma protein that transport iron to the erythropioetic cell. Transferrin binds iron strongly at a physiological pH (Booth *et al.,* 2010). Ferritin is an acute phase reactant which increases in the presence of infection and liver disorder associated with sickle cell disease (SCD) patients in crisis. Aside serum ferritin, transferrin serum iron and total iron binding capacity are also indicators of iron status in human (Mast *et al*., 1998). Blood transfusion raises the oxygen carrying capacity of the blood by replenishing abnormal red cells with normal ones, hence affecting the iron status (Walter *et al.,* 2006). Serum ferritin has been proven to be as a valid tool for the measurement of the iron status of sickle cell patients in the steady state (King *et al.,* 2005) and it provides additional information where there is infection (Mast *et al.,* 1998).

2.8.3. Albumin

Albumin is a soluble protein in human plasma or serum. It is produced solely by the liver and it helps in the regulation of fluid between the plasma and tissues, through its effect on colloid osmotic pressure (Don and Kaysen, 2004). It also transports metals, fatty acids, cholesterol, bile pigment, drugs and serve as antioxidant in the plasma (Roche *et al.,* 2008). Its half-life is 20 days and a decrease in the serum albumin level indicates reduced protein synthesis by liver or kidney impairment which causes the loss of protein. Other causes of low albumin level may be protein malabsorption, inadequate dietary protein or increased demand of protein due to infection and inflammation (Orhue *et al.,* 2005). Serum albumin is seen as a nutritional marker in clinical studies (Arrieta *et al.,* 2010). Malnutrition is related with low albumin levels which are often seen in SCD patient (Hyacinth *et al.,* 2013).

2.9. SYSTEMATIC REVIEW ON NUTRITIONAL IMPACT ON SICKLE CELL DISEASE

Nutritional deficiencies in children with sickle cell diseases (SCDs) have been associated with adverse disease outcomes (Nelson *et al.*, 2002). Micronutrients such as minerals and vitamins have been shown to improve growth, reduce pain, infection and decrease hospital emergency visit (Schall *et al.*, 2004). Children with SCD may, however, have high risk of some micronutrient deficiencies such as Iron, Zinc, B_6, Folate, Vitamin A, Vitamin D and macronutrients such as protein, due to increased haemolysis (Barden *et al.*, 2000). A search was conducted to evaluate the impact of nutrition on sickle cell diseases. The search considered all studies done on macro and micronutrient intakes, supplementation and other anthropometric indices which reveal nutritional status of children with SCD.

2.9.1. Method

This section describes the methods used in the extraction of data for the systematic review.

2.9.1.1.Sources of Data and Eligibility Criteria

A search was conducted on PubMed, Plos One, Cochrane and Google scholar to gather all studies done on the effect of macro and micronutrients intakes on sickle cell diseases (SCDs). Papers that considered nutrient intakes (macro, micronutrients, dietary intakes and supplement intakes) and effect of nutritional deficiency on disease severity in sickle cell diseases were included. Studies that considered other nutritional markers such as anthropometric measures were also added to the search. Studies done in animals and cells were excluded.

2.9.1.2. Data Extraction

An ordered process was used to extract data from eligible studies. A single reviewer abstracted data was reviewed by a second reviewer for accuracy and completeness. Disparities were resolved by another review and discussion. The study considered 8 prospective cohort studies, 12 cross-sectional studies, and 1 retrospective study. About 75% of the studies assessed micronutrient levels in children with SCD; that is, fifteen studies were considered for micronutrient assessment in children with SCD, three looked at macronutrient and two focused on nutritional intake which comprised both macronutrient and micronutrient. Majority of the studies were conducted in black Americans and Africans and most of the studies assessed dietary intake, anthropometric measure and biochemical parameters as part of the assessment of nutritional status in children with SCD. A detailed description of the extraction of data is presented in Table 2.3.

2.9.1.3. Quality Assessment

A scoring system was used to assess the quality of the observational studies as proposed by Sanderson *et al.* (2007). Quality assessment scale was based on type of studies, method of diagnosing malnutrition in children with sickle cell diseases (which included dietary intakes, biochemical parameters and anthropometric measures) and the appropriate use of statistical analysis for primary data.

2.9.1.4. Data Synthesis

As shown in Figure 2.1, a total of 38,102 references were extracted from PubMed, Plos one, Cochrane and Google scholar. After reviewing the titles, 37,783 of the papers were excluded. Further, two hundred and ninety-nine studies were excluded, based on the abstract with the remaining 20 studies being reviewed entirely for the research.

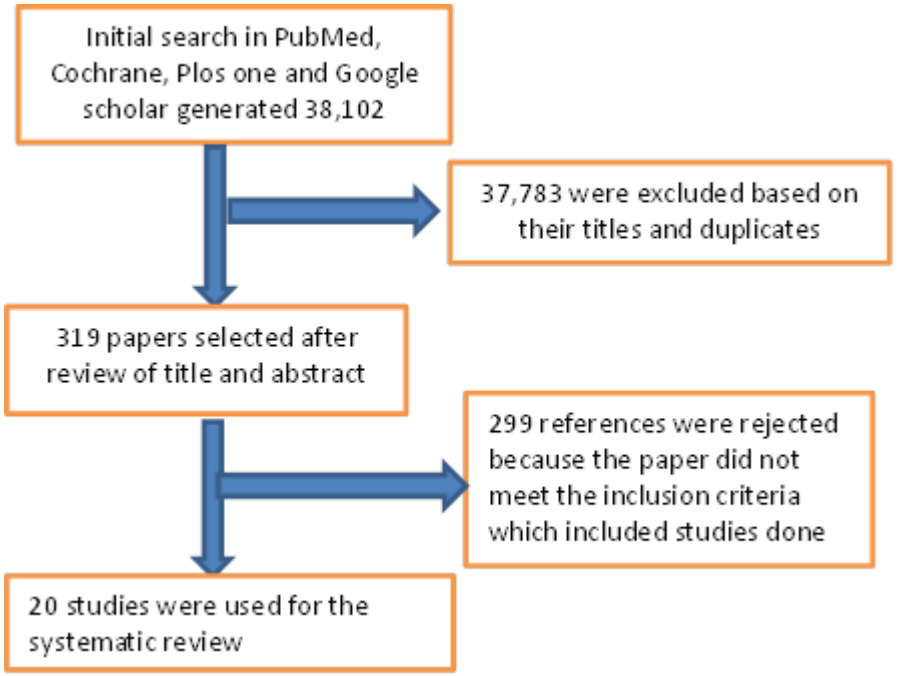

Figure 2.1. Selection process for articles

2.9.2. Results

2.9.2.1. Micronutrient and Sickle Cell Diseases (SCD)

Most of the studies focused on the assessment of micronutrient levels in patients with SCD and their impact on growth or on diseases. Low levels of micronutrients in children with SCD have been shown to affect them in several ways (Kennedy *et al.*, 2001). Increased hospitalization and overall poor growth in children with SCD were attributed to low vitamin A levels. Vitamin A deficiency showed significant inverse relation between serum retinol and hospitalization ($p<0.001$, $r = -0.49$). Serum retinol concentration was suboptimal in children with SCD with mean levels falling below 30µg/dl, thus influencing overall growth, particularly for weight and BMI (Schall *et al.*, 2004). Another study related low vitamin A in children with SCD with high breakdown of red blood cells (Behera *et al.*, 2012).

Barden *et al.* (2000) associated micronutrient intake insufficiency to poor growth, increased disease severity and hospital admissions. A longitudinal study conducted by Kawchak *et al.* (2007) revealed that inadequate dietary intakes in children with SCD contributed to

suboptimal growth (mean height z score -0.5±1.0 cm, weight z score – 0.8 ±1.2 kg, BMI z score -0.7±1.2 kg/m^2) and haemoglobin level of 7.8 ±1.1 g/dl. A research done by Kennedy *et al*. (2001), showed that children with SCD had low red blood folate (57%) and vitamin B_{12} deficiencies despite adequate caloric, protein and supplementation intakes. There was no relationship between vitamin B_{12} and growth but there was increased pattern towards high haematocrit levels with higher serum vitamin B_{12} status. Low levels of vitamin B_{12} and folate has been associated with high levels of homocysteine which increases the susceptibility to cerebrovascular disease like stroke in SCD patients.

Another study reported positive correlation between vitamin B_6 and anthropometric measures (z-score for weight ($p = 0.0037$), BMI ($p = 0.003$) and mid arm circumference ($p = 0.027$), such that, as vitamin B_6 increased, the anthropometric parameters also increased (Nelson *et al.,* 2002). Growth rate was poor in children with SCD and it was associated with poor nutrition (Zemel *et al.,* 2007). Malinauskas *et al.* (2000) reported an inverse correlation ($p = 0.001$, $r = -0.54$) of macro and micronutrient intakes and number of admissions such that inadequate intake increases hospital admissions.

Inadequate intake of micro and macronutrient also showed significant effect on the number and days of hospital admissions. Results of weight using analysis of variance-weight was directly related to the mean haemoglobin values and thus, to disease severity. Overall, suboptimal growth was found in children with SCD with mean height z-score of 0.5± 1.0 cm, weight z score of -0.8±1.2 kg, BMI z score of -0.7±1.2 kg/m^2 (Mandese *et al.,* 2016). Vitamin E deficiency was linked to oxidative damage in children with SCD, while vitamin D and calcium were linked to poor bone development and growth (Kawchak *et al.,* 2007). Studies have shown that children with sickle cell diseases have poor vitamin D status, as compared to healthy children (Chapelon *et al.,* 2009; Rovner *et al.,* 2008).

Low levels of antioxidants (vitamins A, C, E) in patients with sickle cell anaemia were associated ($p< 0.0001$) with increased infection and haemolysis, while zinc deficiency was associated with bacterial infection, vaso-occlusion crises, frequent hospital admissions and growth retardation (Hasanato, 2006). Another nutritional analysis conducted in 91 children with respect to vitamins D, B_6, B_{12} and zinc reported that half of the children had multiple nutrient insufficiencies and this was associated with increased disease severity (Malinauskas *et al.,* 2000). Zinc deficiency was associated with increased pain crises (Mohamed, 2015) and elevated copper levels (Mahyar *et al.,* 2011). Vitamin D deficiency in sickle cell patients leads to poor growth and bone development (Martyres *et al.,* 2016). Evaluation of some micronutrient such as iron in children with SCD shows low iron levels (86.66 ± 10.84 µg/dl), as compared to healthy controls (172.22 ± 2.54 µg/dl) (Digban *et al.,* 2016).

2.9.2.2. Macronutrient and Sickle Cell Diseases

Three studies were identified on macronutrient and sickle cells and most of these papers related macronutrient levels in sickle cell patient to their resting metabolic rate or total energy expenditure. A cross sectional study that looked at the intake of 41 children with SCD and 31 healthy children noticed that their resting metabolic rates (RMR) were significantly higher in the children with SCD (5.9 + 1.3 MJ/d) than in healthy children (5.47 + 0.93 MJ/d). Although intake of energy, protein, carbohydrate and fat did not differ between the two, energy intake rating to resting metabolic rate was lower in children with sickle cell diseases. Energy expenditure in SCD-SS have been shown to be higher than healthy controls (Singhal *et al.,* 2002). Slow growth rate in children with SCD was independently associated with decreased Hb concentration and increased total energy expenditure (Rhodes *et al.,* 2009). Low fat mass and stunting was associated with energy deficiency in children with sickle cell disease. Energy deficiency was attributed to high rate of protein synthesis and catabolism as well as

energy imbalance; however, there was no significant association between disease severity and energy deficiency (Barden *et al.,* 2000).

2.9.3. Discussion

Findings from the studies show that nutrition has an impact on disease severity and anthropometric measures such as body weight, height and BMI. Nutrition has been identified as one of the modifiable risk factors that has great impact on patients with SCD. Most research attributed poor growth, delayed maturation and underweight in children with SCD to poor nutrition (Barden *et al.,* 2002; Martyres *et al.,* 2016). Growth deficit begins in childhood and worsens with age through adolescence. The most researched nutrients were antioxidants (vitamins A, C and E), vitamins D, B_{12}, $B_{6,}$ folate and minerals, such as, zinc, iron, magnesium and copper. These nutrients have been shown to play a major role in children with sickle cell. Magnesium prevents sickle cell dehydration and therefore suboptimal intake of magnesium may contribute to erythrocytes dehydration that may increase illness episodes. Vitamin A deficiency causes impairment of epithelial integrity and systematic immunity (Hyacinth *et al.,* 2013). According to Behera *et al.* (2012) vitamin A deficiency increases the rate and severity of infections in sickle cell children.

Zinc has received much attention in the context of sickle cell disease, compared to other nutrients. Zinc helps in immune system function, wound healing, maturation and growth. Its deficiency has been associated with poor growth and delayed sexual maturation (Mohamed, 2015). A study done by Stevens *et al.* (1986) reported poor growth in and delayed pubertal growth in children with sickle cell disease, compared to normal children and this was attributed to multiple factors such as chronic anaemia, high erythropoietic turnover and suboptimal nutrition.

Vitamin B_{12} and folate deficiency were associated with low production and maturation of red blood cells (Klee, 2000). Poor development of bone and growth in patients with sickle cell disease were attributed to vitamin D deficiency (Chapelon *et al.,* 2009). Again, from the review, vitamin D deficiency was observed to contribute to increased of risk of pulmonary function (Jackson *et al.,* 2012). Patients with SCD may have impaired antioxidant demand, as sickle erythrocyte may form clumps with healthy RBC, damaging them and increasing circulating reactive oxygen species such as superoxide and hydroxyl radicals, and low levels of antioxidant has been shown to increase susceptibility to infection and haemolysis (Wright *et al.,* 2014). Iron deficiency is the most common nutritional deficiency in patients with SCD, especially, children. Iron deficiency leads to developmental delays and behavioral disorders in children with SCD (Baker and Greer, 2010). In developed countries patients with SCD do not show iron deficiency due to chronic blood transfusion, while in developing countries iron deficiency in SCD patient has been associated with insufficient dietary intake (Hyacinth *et al.,* 2010).

Appropriate calorie intake, proteins and some fatty acids such as n-3 fatty acids have been shown to play significant reduction in inflammation, oxidative stress, red cell density and pains episodes and were related to improved micro vascular function (Tomer *et al.,* 2001). High nutritional demand of SCD patients has been associated with hyper metabolism, organ defect (renal excretion of some nutrients such as protein and intestinal malabsorption) and chronic haemolysis. These factors have been shown to cause malnutrition in children with SCD. However, cultural choice of food and food intolerance may limit the intake of certain nutrients which may result in dietary inadequacy (Kawchak *et al.,* 2007).

2.9.4. Conclusion

Evidence presented above shows that nutrition cannot be overlooked in patients with sickle cell disease, especially in children, since it increases disease severity and influences poor growth. Major adverse health effect of poor nutrition in children poses threat to the quality of life. Nutritional assessment is, therefore, important in the care of patients with SCD, especially children, as it influences health outcomes.

Table 2.3. Extracted data on studies done on nutrition and sickle cell disease

Authors	Study design	Number of study participants	Nutrient studied	Aim of the studies	Findings /conclusion	Quality assessment score
Kennedy *et al*. (2001)	Prospective cohort	70	Vitamin B_{12}/folate	To determine red blood cell folate levels in children with SCD.	Low levels of folate and vitamin B_{12} in children with SCD due to inadequate intake.	9 (High)
Nelson *et al.* (2002)	Cross-sectional	109	Vitamin B_6	To determine vitamin B_6 status of children with SCD.	Low vitamin B_6 was associated with low dietary intake and increased haemolysis	7 (Medium)
Mandese *et al.* (2016)	Cross-sectional	29	Macronutrient (protein, carbohydrate and lipids) Micronutrients (calcium, iron, vitamins A,C,B_2	To assess the impact of growth and nutrition on severity in children with SCD	Inadequate nutritional intake, low weight and BMI have a significant impact on SCD disease severity.	9 (High)
Kawchak *et al.* (2007)	Prospective cohort	97	Macronutrient (protein),micronutrient (calcium ,vitamin C)	To assess dietary adequacy of children with SCD.	Energy and micronutrient intake in children with SCD is low.	6 (medium)
Malinauskas *et al.* (2000)	Cross-sectional	102	Dietary intake	To assess changes in the growth, nutritional intake, body composition during crisis and steady state	Children with SCD appear to be nutritionally at risk during illness, due to inadequate nutritional intake.	7 (medium)
Rhodes *et al.* (2009)	Prospective cohort	33	Energy intake	To correlate growth with metabolic and haematological factors during puberty.	Decreased growth in SCD children is independently associated with Hb concentration and Total Energy Expenditure	9 (High)

Table 2.3. Extracted Data (Cont'd)

Authors	Study design	Number of study participants	Nutrient studied	Aim of the studies	Findings /conclusion	Quality assessment score
Schall *et al.* (2004)	Prospective cohort	66	Vitamin A	To find the relationship between serum vitamin A levels and growth as well as nutritional and haematological and frequency of admissions	Sub-optimal intake lead to increased hopitalization.	9 (high)
Martyres *et al.* (2016)	Retrospective cohort	91	Vitamins D, B_6, B_{12} folate	To determine nutritional implication on disease severity	Disease severity was related to malnutrition	10 (High)
Digban *et al.*, (2016)	Cross-sectional	100	Iron, zinc ,cobalt	To evaluate some micronutrients in sickle cell patients	Low micronutrients in sickle cell patients.	6 (medium)
Mohamed *et al.* (2015)	Cross-sectional	120	Zinc	To determine zin status in relation to growth and maturation in children with SCD	Poor zinc levels lead to poor growth.	9 (High)
Mahyar *et al.* (2011)	Cross-sectional	40	Zinc and copper	To assess the serum zinc and copper levels in children with beta-thalassaemia	Low zinc levels in thalassaemia patients but no copper deficiency.	6 (medium)
Barden *et al.*, (2000)	Cross-sectional	36	Energy intake	To investigate energy balance in children with SCD	Poor growth in children with SCD is related high TEE	5 (medium)
Rovner *et al.* (2008)	Cross-sectional	61	Vitamin D	To compare the vitamin D status of children living SCD to healthy ones.	Children with SCD have lower vitamin D as compared with healthy ones.	9 (high)

Table 2.3. Extracted Data (Cont'd)

Authors	Study design	Number of study participants	Nutrient studied	Aim of the studies	Findings /conclusion	Quality assessment score
Chapelon *et al.* (2009)	Cross-sectional	61	Vitamin D	To assess vitamin D status in children with SCD and its impact on disease severity.	Decrease in vitamin D levels was unrelated to disease severity.	7 (medium)
Hasanato *et al.* (2012)	Cross-sectional	25	Zinc and copper	To evaluate the plasma levels of antioxidant and zinc levels in SCD patients	Low antioxidant and zinc levels are associated with elevated copper	6 (medium)
Jackson *et al.* (2012)	Prospective cohort	139	Vitamin D	To measure the risk of vitamin D deficiency in association with SCD	Patient with SCD have increased risk of vitamin D deficiency which is associated with pulmonary function	7 (medium)
Behera *et al.* (2012)	Prospective cohort	80	Vitamin A	To evaluate vitamin A status and haematological parameters in children	Vitamin A deficiency was mostly with haemolysis	8 (high)
Singhal *et al.* (2002)	Cross-sectional	72	Energy intake	To compare the energy intake in children with SCD	Energy deficiency in children SCD.	6 (medium)
Zemel *et al.* (2004)	Prospective cohort	148	Dietary intakes	To assess the effect of nutritional status on disease severity	Growth failure and malnutrition were related	8 (high)

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY DESIGN

A cross-sectional survey was used to assess the nutritional status of children with sickle cell disease attending a clinic as out-patients at the Komfo Anokye Teaching Hospital (KATH).

3.2. STUDY SITE

Komfo Anokye Teaching Hospital was selected as the study site. It is the second largest hospital in Ghana and it does screening for sickle cell disease for over 6000 new born babies per year and manages all cases associated with blood disorders. It is the main hospital for sickle cell clinic in Ashanti region and neighbouring regions.

3.3. ETHICAL CONSIDERATIONS

The Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST gave approval for the study. All participants who took part in the study signed an informed consent, form in accordance to the CHRPE regulations before participating in the study.

3.4. STUDY POPULATION

The study population was out-patient children with sickle cell disease who periodically visited sickle cell clinic. Children between the ages of 3 to 12 years who mostly depended on homemade foods and/school diet to meet their nutritional requirement were recruited for the studies.

3.5. SAMPLE SIZE CALCULATION

The sample size was determined by adopting the following statistical formula for minimum sample size calculation (Yamane, 1967).

$$n = \frac{N}{1 + N(e)^2}$$

Where n= minimum sample size,

N = 130 (average number of children with sickle cell disease who attend sickle cell clinic monthly)

e = 5% (the margin of error)

$$n = \frac{130}{1 + 130(0.05)^2}$$

n=130/1.325

n=98.11

Therefore the estimated minimum sample size used was 98.

3.6. INCLUSION CRITERIA

All children aged 3-12 years with sickle cell disease who did not have any clinical complications and in steady state were included in the study.

3.7. EXCLUSION CRITERIA

Participant who did not give consent to the study and those in crisis were excluded from the study.

3.8. SAMPLING TECHNIQUE

Children with sickle cell diseases were selected for the study as they reported for routine check-ups at the sickle cell unit. Parents/guardians attending clinic with children were educated, interviewed and SCD children who fell within the inclusion criteria were recruited after parents had given their consent.

3.8.1. Data Collection Techniques and Tools

3.8.1.1. Questionnaire

Pre-tested structured questionnaires were administered to respondents as they reported to the sickle cell clinic. The questionnaires were used to gather demographic information which included ages of children, religion and educational status of care givers. Information on health, dietary pattern and lifestyle was also collected.

3.8.1.2. Dietary Assessment

Food frequency questionnaires containing foods that are rich sources of Iron, Zinc, Folate, proteins, and vitamins A, $B_{12,}$ $B_{6,}$ C, and E were used to assess dietary intake for three months. Frequency of intakes was categorised as daily, weekly, monthly, and occasionally.

Twenty-four-hour dietary recall was done on one weekend and one weekday was used to assess their current dietary intake of some macro and micronutrients such as protein, iron, folic acid, vitamin B_{12} and vitamin C. In the interview, participants were asked to recall all foods and snacks intake the previous 24-hours. Household handy measures were used to help estimate the amount of foods or snacks consumed in the previous 24-hours. Conversion table was used to estimate the quantity of foods consumed by participants into grams. West African Food Composition Tables was used to determine the nutrient content of the food consumed.

3.8.1.3. Anthropometric Measurement

Patients were weighed with light clothes and without shoes (to within 0.1 kg) and weights were expressed in z-scores and compared to standards for normal growth (De Onis, *et al.*, 2006). Heights were taken (to within 0.1 cm) using an upright scale without shoes and children were made to stand with heels and back in contact with a height stadiometer. The height and weight were used to calculate BMI-for-age (Turconi *et al.*, 2006).

Mid-upper-arm-circumference of children was taken as well, with the children standing upright and bending their elbow to 90 degree angle with palms facing up. While the elbow was facing up, the acromion and the pointed part of the elbow were located. The tape measure was put with the zero mark just under the acromion and extended to the pointed part of the elbow. The length of the tape was noted and the midpoint marked on the arm. The child's hand was then relaxed and the marked mid-point was measured and recorded to the nearest 0.1 cm. All anthropometric data were taken by trained personnel and all measurements done in duplicates.

3.9. BIOCHEMICAL ANALYSIS

About 6 mL of venous blood sample was collected from the antecubital fossa of the study participants. Two (2) mL of the venous blood sample was dispensed into EDTA anticoagulated tube and the other four (4) mL into vacutainer plain tubes. To obtain serum samples, blood samples in plane tubes were centrifuged at 4000 rpm for 5 minutes. The serum was separated by pipetting it into an eppendorf tube and stored at -20 ^{o}C until assay was performed. Parameters measured included: serum ferritin, full blood counts, total proteins and albumin. Serum ferritin analysis was performed on Mindray® microplate reader MR 96 A (Shenzhen Mindray Bio-medical Electronics Co., Ltd, China). The full blood count

was determined using the Sysmex XP 300 haematology autoanalyzer (Sysmex Corporation, North America) to run a full blood count, using whole blood mode and serum.

3.9.1. Full Blood Count Determination

Each 2 mL EDTA anticoagulated blood was thoroughly mixed using Stuart Scientific Blood Tube Rotator SB-1 for approximately three (3) minutes. Each sample number was coded on the analyser. Well mixed sample tubes containing blood samples in sample tubes were aspirated using the probe of the three parts haematology autoanalyser (Sysmex Corporation, North America). Haemoglobin (Hb) concentration, Red Blood Cell (RBC) count, Mean Cell Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC) and White Blood Cell (WBC) count were displayed after 45 seconds. The parameters were then recorded.

3.9.2. Total Protein (Biuret Method)

A solution of 1.0 mL of Biuret reagent was pipetted into test tubes labelled blank (B), standard (S), and test (T). A solution of 50 µL of total protein standard was added to test tube labelled S. Another 50µL of test sample was added to test tube labelled T. Samples were mixed well and incubated at 37 ^{0}C for 5 minutes. Absorbance of standards (S) and test (T) was read against blank at 555 nm.

$$\textbf{Total protein (g/dl)} = \frac{\textbf{Absorbance of test}}{\textbf{Absorbance of standard}} \times \textbf{5.5 (concentration of standard)}$$

3.9.3. Albumin (Bromocresol Green)

A standard and blank were prepared to ensure accuracy of results. In the albumin determination, 0.1 mL of BCG reagent was pipetted into test tubes labelled blank (B), standard (S) and test (T). A standard (conc. 3.5 g/l) concentration of 10 µL was added to test

tube labelled S and 10 µL of test sample was pipetted into test tube labelled T. Samples were mixed well and absorbance of standard (S) and test (T) read against blank at 630 nm.

$$\textbf{Albumin (g/dl)} = \frac{\textbf{Absorbance of test}}{\textbf{Absorbance of standard}} \times \textbf{3.5 (concentration of standard)}$$

3.9.4. Serum Ferritin Assay

In determining serum ferritin, 20 µL of standard, specimen and controls were dispensed into appropriate wells and 100 µL of enzyme conjugate reagent was added into the well. They were thoroughly mixed for 30 seconds and incubated at room temperature (2O-25^{0}C) for 60 minutes. The incubated mixture was then removed by flicking plate content into a waste container. The microtiter wells were rinsed and flicked 5 times with washing buffer (1x). All residue water droplets were absorbed by paper towel. A 100 µL substrate of 3,3', 5,5'-tetramethylbenzidine was dispensed into each well and gently mixed for 5 seconds. Each well was then incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100 µL of stop solution to each well. A blue colour change to yellow was measured spectrophotometrically at 450 nm, using Mindray microplate reader MR-96A (Shenzhen Mindray Bio-medical Electronics Co., Ltd, China). The concentration of Ferritin was directly proportional to the colour intensity of the test sample.

3.10. DATA ANALYSIS

Data was entered and analysed with Statistical Package for the Social Sciences, version 20. Results were presented in tables and reported as frequencies, means and percentages. Continuous variables were summarized as means with their standard deviations. Independent-Student t-test was used to compare means between male and female variables at a confidence

interval of 95%. Pearson correlation test was used to determine relationships between nutritional intakes and biochemical variables, as well as anthropometric indices.

CHAPTER FOUR

4.0. RESULTS

The results of socio-demographic data, anthropometric data, dietary intakes, using 24-hour dietary recall and food frequency questionnaire and biochemical data which included complete blood count, serum ferritin, albumin and total proteins are presented in Tables **4.1**-**4.8**.

4.1. BASIC SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

The demographic characteristics of children who participated in the study are presented in Table **4.1.** A total number of 100 children with SCD participated in the study with a mean age of 7± 2.7 years. The minimum age was 3 years and maximum age was 12 years. The male participants had the larger prevalence (57%) compared to females (43%). Children were grouped according to age and those who fell between ages 3 to 5 years were 35%, 6 to 8 years recorded 37% and 9 to 12 years were 28%. According to the study, genotype SS (65%) recorded the highest prevalence, followed by SC (28%). Most of the children were in school (98%), with 46% in lower primary. From Table 4.1, a higher percentage of care-takers were Christians (77%), compared to Muslims (22%). A lower percentage of the caretakers 24% did not have formal education, while most of them had junior high school education (44%). Majority of the caretakers (60%) had nutritional education, while 38% had no nutritional education.

Table 4.1. Demographic Characteristics of study children with SCD and their caretakers

Parameter	Frequency (%)
Age	
3-5 years	35(35)
6-8 years	37(37)
9-12 years	28(28)
Gender	
Male	57(57)
Female	43(43)
Educational Status of Caretakers	
JHS	44(44)
SHS	19(19)
Tertiary	13(13)
None	24(24)
Religion	
Christians	77(77)
Muslims	22(22)
Traditionalist	1(1)
Educational Level of Children	
Nursery and kindergarten	35(35)
Lower primary	46(46)
Upper primary	14(14)
JHS	3(3)
Non schooling	2(2)
Type of SCD	
SCD-SS	65(65)
SCD-SC	28(28)
SCD-Sβ^0	6(6)
SCD-Sβ^+	1(1)
Family trait of SCD	40 (40)
Nutritional knowledge of caretakers	
Yes	62(62)
No	38(38)

Grouped data are presented as frequency with their corresponding percentage in bracket. SCD: sickle cell diseases.

4.2. NUTRIENTS INTAKE OF CHILDREN WITH SCD

Dietary intake of study participants was obtained using a multiple 24-hour dietary recall. This is presented in Table **4.2.** Micro and macronutrients were compared with age specific Recommended Dietary Allowance (RDA) of normal children and expressed as a percentage of RDA. The overall mean caloric intake of children was 882 ± 320.4 kcal and the rest of the nutrients were as follows: proteins recorded 24.7± 10.5 g/day, iron was 5.9 ± 3.0 mg/day, zinc was 5.1 ± 10.6 mg/day, vitamin C was 52.1 ± 42.6 mg/day, vitamin B_6 was 2.6 ± 17.1, folate was 179.6 ± 147.8 µg/day, vitamin A was 107.1 ± 112.4 µg/day and vitamin E was 4.2 ± 2.9 mg/day. Calories and protein intake for age group, 9-12 years were respectively 1300 kcal and 34 dg/day. Children aged 3 years and 4-8 years, respectively, recorded intake of zinc with 3.9 ± 2.6 mg/d and 3.8 ± 1.9 mg/d. The mean dietary intake of folate for children aged 3 years was 215 ± 217 µg/day, while that for the 4-8 was 177 ± 143 µg/day. Mean intake of vitamin B_{12} for the same group recorded 92% of RDA for the 3 years and 83% for the 4-8 years.

Table 4.2 Dietary intake of children with SCD compared with age-specific Recommended Dietary Allowance

Nutrients	RDA For Children			NUTRIENT INTAKES			OVER ALL MEAN	PERCENTAGE OF RDA MET			INTERPRETATION		
	3 years	4-8 years	9-12 years	3 years	4-8 Years	9-12 years		3 years	4-8 years	9-12 years	3 years	4-8 years	9-12 years
CALORIES	500	800	1300	964.2±262.3	883.7±321.1	852.0±342.3	882±320.4	192	110	66	Excess	Excess	Deficit
PROTEIN	13g/d	19g/d	34dg/d	23.4±9.4	24.7±10.7	25.0±10.7	24.7±10.5	176	126	70	Excess	Excess	Deficit
IRON	7mg/d	10mg/d	8mg/d	5.9±3.2	5.7±2.6	6.3±3.6	5.9±3.0	85	57	75	Deficit	Deficit	Deficit
ZINC	7mg/d	10mg/d	8mg/d	3.9±2.6	3.8±1.9	8±19.7	5.1±10.6	57	38	100	Deficit	Deficit	Adequate
VITAMIN C	15mg/d	25mg/d	45mg/d	48±15	55±51.6	46.3±21.4	52.1±42.6	320	220	102	Excess	Excess	Excess
VITAMIN B_6	0.5mg/d	0.6mg/d	1.0mg/d	0.9±0.6	0.8±0.5	7.1±32.9	2.6±17.1	180	133	260	Excess	Excess	Excess
FOLATE	150µg/d	200µg/d	300µg/d	215.7±217.4	177±143.7	172±131	179.6±147.8	143	88.5	59.7	Excess	Deficit	Deficit
VITAMIN B_{12}	0.9µg/d	1.2µg/d	1.8µg/d	1.3±1.8	1.6±6.0	1.1±1.7	1.5±4.8	144	92	83	Excess	Deficit	Deficit
VITAMIN A	300µg/d	400µg/d	600µg/d	135.2±154.8	103.1±109.1	105.4±105.5	107±112.4	45	25.7	17.8	Deficit	Deficit	Deficit
VITAMIN E	6mg/d	11mg/d	11mg/d	5.2±5.7	4.2±2.8	4.1±1.7	4.2±2.9	86.6	38	37.3	Deficit	Deficit	Deficit

Continuous data were presented as means ± SEM and compared with age-specific Recommended Dietary Allowance. Results were interpreted as deficit, excess or adequate. mg/d: miligram per day, µg/d: microgram per day.

4.3. DIETARY PATTERN OF SOME SELECTED FOOD GROUPS

Table **4.3** presents dietary patterns of some selected foods using food, frequency table. Twelve percent (12%) of the participants indicated that they take fruits daily, while 9% indicated green leafy vegetables. From the study, 13% of the children indicated intake of meat daily while intake of fish and offal daily recorded 21% and 9% respectively. Daily intake of eggs recorded the highest prevalence of 61% while dairy products reported 57%. Consumption of cereals and grains daily (51%) were higher, compared with legumes (1%) and nuts (7%).

Table 4.3. Dietary pattern among children with SCD

Food Groups	**Frequency intakes (%)**				
	Daily	**2-3 times Weekly**	**Monthly**	**Occasionally**	**Never**
Fruits	12	47	23	17	1
Vegetables	56	44	0	0	0
Green leafy vegetable	9	77	7	6	1
Cereals and grains	51	45	2	2	0
Legumes	1	56	18	16	9
Nuts	7	60	23	4	6
Meats	13	66	13	5	3
Fish	21	69	8	2	0
Offals	9	23	19	28	21
Eggs	61	30	5	3	1
Dairy products	57	35	4	3	1

Data presented are the frequency (%) of food intake by participants. Green leafy vegetables (Kontonmire, ayoyo, alefu); Cereals (rice, maize, wheat, millet); Fruits (mango, pear, banana, watermelon, orange, pineapple, pawpaw).

4.4. NUTRITIONAL STATUS OF THE CHILDREN WITH SCD USING ANTHROPOMETRY AND BIOCHEMICAL PARAMETERS

Table **4.4** presents the summary of the nutritional status of the study participants, using anthropometric variables. Using BMI-for-Age as an indicator of malnutrition, 40 children were underweight, the proportion females who were underweight were 46.5% and males recorded 35.1%. In terms of weight-for-age, 37 children were underweight, females recorded 46.5% and their males' counterpart recorded (29.8%). Twenty–two children were stunted with females having the higher prevalence (25.6%). Using Mid Upper Arm Circumference-For-Age as an indicator, males had the higher prevalence (73.7%) for underweight. Majority (63%) of the children had low levels of haemoglobin (< 10.0 g/dl) as well as other haematological parameters such as red blood cell count (57%) and Haematocit (79%). Forty children had high Platlet count (40%) above the normal reference range of 450 x 10^3 /µL. Using WHO cut-off point for serum ferritin (< 15 ng/ml), 23% were iron deficient, 45% were normal levels of iron and 32% had above the normal range. Seven percent of the children had low serum protein and 14% also recorded low serum albumin levels.

Table 4.4. Nutritional status among children with sickle cell diseases

Parameter		Total (n=100)	Males (n=57)	Females (n=43)	p-value
BMI-for-Age	Underweight	40	20(35.1)	20 (46.5)	0.16
	Normal	50	33(57.9)	17 (39.5)	
	Overweight	10	4(7.0)	6 (14.0)	
Weight-for-Age	Underweight	37	17(29.8)	20 (46.5)	0.3
	Normal	54	37(64.9)	17 (39.5)	
	Overweight	9	3(5.3)	6 (14.0)	
Height-for-Age	Stunted	22	11(19.3)	11 (25.6)	0.8
	Normal	71	42(73.7)	29 (67.4)	
MUAC-for-Age	Normal	69	42(73.7)	27 (62.8)	0.28
	Underweight	31	15(26.3)	16 (37.2)	
Serum protein	Low levels	7	4(7.0)	3 (7.0)	0.26
	Normal	93	53(93.0)	40 (93.0)	
Albumin	low levels	13	9(15.8)	4 (9.3)	1
	Normal levels	87	48(84.2)	39 (90.7)	
Hb	low levels	63	36(63.2)	27 (62.8)	1
	Normal levels	37	21(36.8)	16 (37.2)	
WBC	Low ranges	1	0(0)	1 (2.3)	0.15
	Normal ranges	57	29(50.9)	28 (65.1)	
	Higher ranges	42	28(49.1)	14 (32.6)	
RBC	Low ranges	57	34(59.6)	23(53.5)	0.5
	Normal ranges	43	23(40.4)	20(46.5)	
HCT	Low ranges	79	46(80.7)	33 (76.7)	0.6
	Normal ranges	21	11(19.3)	10 (23.3)	
MCV	Low ranges	44	24(42.1)	20 (46.5)	0.7
	Normal ranges	49	28(49.1)	21 (48.8)	
	Higher ranges	7	5(8.8)	2 (4.7)	
MCH	Low ranges	48	27(47.4)	21 (48.8)	0.46
	Normal ranges	50	28(49.1)	22 (51.2)	
	Higher ranges	2	2(3.5)	0 (0)	
PLT	Normal ranges	60	36(63.2)	24 (55.8)	0.5
	Higher ranges	40	21(36.8)	19 (44.2)	
Serum Ferritin	Low ranges	23	11(19.3)	12 (27.9)	0.36
	Normal ranges	45	29(50.9)	16 (37.2)	
	Higher ranges	32	17(29.8)	15 (34.9)	

Data presented as number of respondents with the corresponding percentage in brackets. Proportions were compared using chi-square test and Fisher's exact test, where appropriate. BMI: Body Mass Index, MUAC: Mid-Upper Arm Circumference, Hb: heamoglobin, WBC: White Blood Cell, HCT: Haematocrit, MCV: Mean Corposcular Volume, MCH: Mean Corpuscular Haemoglobin and PLT: Platelet

4.5. MEAN VALUES OF ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS OF SCD CHILDREN

The mean ± standard deviation of anthropometric and biochemical parameter of participants are presented in Table 4.5. Total mean for weight, height, MUAC and BMI were as follows: 22.6 ± 10.5 kg, 116.8 ±18 cm, and 18.1 ± 8.9 cm, 117 ± 10 kg/m^2, respectively. Females recorded the higher mean in weight (22.9±8 kg), height (117.7 ± 21.7 cm), MUAC (19.4 ± 13.3 cm) and BMI (17.0 ± 6.8 kg/m^2). The overall mean values of total protein, albumin and serum ferritin were 69.6 ± 8.1 g/L, 38.8 ± 4.3 g/L and 167 ± 173.8 ng/mL, respectively. With regard to the haematological parameters, WBC, RBC, Hb and MCV recorded means of 10.4 ± 5.21 10^3/µL, 3.4 ± 1.2 10^6/µL, 8.6 ± 1.7 g/dL, and 80.56 ± 11.3 fL, respectively. Platelets, haematocrits and MCH also recorded 388.2 ± 155.5 10^3/µL, 27.2 ± 5.9 g/dL and 25.8 ± 3.3 pg, respectively. Females had the higher albumin (39 ± 4.5 g/l), HCT (27% ± 5.9%), serum ferritin (154 ± 164.6 ng/ml), PLT (412 ± 169.3 10^3/µL) and Hb (8.7 ± 1.7 g/dL), while males had the higher WBC (10.8 ± 4.9 10^3/µL) and MCV (81.5 ± 12.4 fL).

Table 4 .5. Mean Values of Anthropometric and biochemical parameter among children with SCD

Parameter	Total mean	Males	Females	P value
Weight (Kg)	22.6 ± 10.5	22.4 ± 12.2	22.9 ± 8.0	0.8
Height(cm)	116.8 ± 18	116.3 ± 15.2	117.7 ±21.7	0.9
MUAC (cm)	18.1 ± 8.9	17.1 ± 2.3	19.4 ± 13.3	0.3
total protein (g/L)	70.2 ± 7.6	70.1 ± 7.2	70.3 ± 8.3	0.9
Albumin(g/L)	39.0 ± 3.4	38.6 ± 4.1	39 ± 4.5	0.9
Serum ferritin(ng/ml)	167.0 ± 173.8	117.9 ±181.3	154.0±164.6	0.5
WBC(10^3/µL)	10.4 ± 5.2	10.8 ± 4.9	9.8 ± 5.5	0.4
RBC(10^6/µL)	3.4 ± 1.2	3.3 ± 0.9	3.5 ± 1.4	0.3
Hb(g/dL)	8.6 ± 1.7	8.4 ± 1.8	8.7 ± 1.7	0.6
HCT (%)	27.2 ± 5.9	26.9 ± 5.8	27.4 ± 5.9	0.7
MCV(fL)	80.6 ± 11.3	81.5 ± 12.4	79.2 ± 9.6	0.3
MCH(pg)	25.8 ± 3.3	25.9 ± 3.5	25.5 ± 3.0	0.6
PLT(10^3/µL)	388.2 ± 155.5	370.1 ± 143.0	412 ± 169.3	0.2

Data represent mean ± SEM of anthropometric and biochemical measures. Mean levels of males and females were compared using independent t-test. BMI: Body Mass Index, MUAC: Mid-Upper Arm Circumference, Hb: haemoglobin, WBC: White Blood Cell, HCT: Hematocrit, MCV: Mean Corposcular Volume, MCH: Mean Corposcular Haemoglobin and PLT: Platelet.

4.6. SUPPLEMENTATION IN CHILDREN WITH SICKLE CELL DISEASES

Majority (42%) of the children were on combination of zinc and folic acid supplement while the rest were on folic acid 34%, multivitamins (9%) and zincovite (1).

Table 4.6. Supplementation in Children with SCD

Supplement Intakes	Frequency
Zincovite	1(1)
Folic Acid	34(34)
Zinc and Folic Acid	42(42)
Multivitamins	9(9)
No supplementation	14(14)

Data represent respondents on dietary supplementation with the corresponding percentage in brackets.

4.7. ASSOCIATION BETWEEN NUTRITIONAL INTAKE AND BIOCHEMICAL STATUS IN CHILDREN WITH SCD

Table **4.7** presents the summary of the association between biochemical parameters and nutritional intakes. A strong positive correlation (r = 0.65, p-value = 0.00) was found between vitamin B_{12} intake and red blood cell count. There was another positive association (r = 0.244, p-value =0.015) between Zinc and HCT. A positive association was found between vitamin B_6 and HCT with a significance of p-value = 0.016, r = 0.244. Additionally, a positive correlation (r = 0.198, p -value = 0.05) was found between zinc and Hb, while vitamin C had a weak positive association (r= correlations 0.197, p = 0.051) with Hb. In the case of vitamin B_6 and HCT, and Zinc and Hb, the correlations were very weak.

Table 4.7. Pearson's Correlation between the Nutritional Intakes and Biochemical Parameters among Children with SCD

Biochemical parameters	Nutritional intakes									
	Calories	Proteins	Iron	Zinc	Vitamin C	Vitamin B_2	Folate	Vitamin B_{12}	Vitamin A	Vitamin E
Protein	-0.071	0.048	0.087	0.111	-0.01	-0.133	0.009	0.021	-0.005	-0.001
Serum albumin	-0.08	0.053	0.083	0.203	-0.022	-0.205	0.093	-0.067	0.011	-0.011
Ferritin	-0.042	0.072	-0.01	0.022	-0.14	-0.038	0.051	-0.016	0.033	-0.12
WBC	-0.119	-0.142	0.143	-0.13	-0.117	-0.11	0.004	-0.179	-0.093	-0.05
RBC	0.161	0.075	0.113	0.013	0.082	0.009	0.046	0.651**	0.086	0.048
HGB	0.106	0.051	0.02	0.198*	-0.007	0.197	0.083	-0.012	0.028	-0.012
HCT	0.194	0.023	0.071	0.244*	0.017	0.244*	0.023	0.017	0.04	0.023
MCV	-0.03	-0.162	0.085	0.078	0.011	-0.051	0.075	0.053	-0.075	-0.12
MCH	-0.173	-0.133	0.123	0.142	-0.05	-0.115	0.045	0.032	0.063	0.011
PLT	-0.018	-0.146	0.054	0.088	0.115	-0.067	0.024	0.141	-0.092	-0.044

Data represent the association between nutritional intakes and biochemical parameters, using Pearson's correlation test. Significant correlation is represented by two stars (**), while weak significant correlation is represented by one star (*).Hb: haemoglobin, WBC: White Blood Cell, HCT: Haematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin and PLT: Platelet

4.8. ASSOCIATION BETWEEN HAEMATOLOGICAL AND ANTHROPOMETRIC PARAMETERS

A summary of the relationship between anthropometric and biochemical parameters are presented in Table **4.8**. There was a positive association (r = 0.28, p-values = 0.005) between Hb and weight-for-age. Another positive association (r = 0.308, p-value = 0.002) was found between HCT and weight-for-age. A positive correlation (r = 0.267, p-value = 0.008) was found between MUAC-for-age and Hb. MUAC-for-age and HCT had a positive correlation coefficient value of 0.225 and p-value = 0.026. In general, the correlations between MUAC-for-age and Hb, HCT and weight-for-age were weak.

Table 4.8. Pearson's Correlation between the Anthropometric measures and Biochemical Parameters among Children with SCD

Parameter	BMI for Age	Weight for Age	Height for Age	MUAC ranges	Total protein	Albumin	Serum Ferritin	WBC	RBC	HGB	HCT	MCV	MCH	PLT
BMI for Age	1	0.703	-0.143	0.45	0.084	0.166	0.065	-0.1	0.001	0.168	0.164	-0.021	0.093	0.002
Weight for Age		1	0.109	0.404	-0.025	0.063	-0.08	-0.137	0.101	0.28*	0.308*	-0.027	0.041	-0.074
Height for Age			1	0.264	-0.09	0.076	-0.085	0.05	0.171	0.141	0.193	0.023	0.155	-0.172
MUAC for Age				1	-0.062	0.031	-0.054	-0.169	0.092	0.267*	0.225*	-0.182	0.085	-0.068

Data represent the association between anthropometric and biochemical parameters, using Pearson's correlation test. Significant correlation is represented by two star (**), while weak significant correlation is represented by one star (*). Hb: haemoglobin, WBC: White Blood Cell, HCT: Haematocrit, MCV: Mean Corpsucular Volume, MCH: Mean Corpscular Haemoglobin and PLT: Platelet.

CHAPTER FIVE

5.0. DISCUSSION

Dietary assessment in children is important for nutritional monitoring, clinical research and interventional studies (McPherson *et al.,* 2000). Accurate assessment of a child's dietary intake is an essential factor in determining the nutritional adequacy of an individual child's diet (Livingstone *et al.,* 2004). Children with sickle cell diseases (SCD) may be malnurioushed, due to inadequate dietary intake, loss of appetite or chronic haemolysis (Hyacinth *et al.,* 2013). Nutritional deficiencies in children with SCD have been associated with adverse disease outcomes (Nelson *et al.,* 2002). Micro and macronutrient deficiency has been associated with immunologic, nutritional and growth defect in SCD patients (Hyacinth *et al.,* 2013).

In this study, a total number of 100 children SCD participated. The mean age of the children was 7.0 ± 2.7 years, (± standard deviation) with age range between 3-12 years. This is similar to a study conducted by Osei-Yeboah *et al.* (2010) in Korle-Bu Teaching Hospital (Ghana), who recorded a mean age of 7.18 ± 3.15 years. A higher percentage of males (57%) in this study indicate a higher prevalence of SCD in males than females. This finding is consistent with a study done by Al-Saqladi et al. (2010), where males constituted 54.9%, compared to females.

From table 4.1, the common genotype of SCD found among the children in this study was SS genotype (65%) followed by SC genotype (25%). This implies that SCD-SS is the most prevalent type of sickle cell disease which affects children (Jacob, 2011, Osei-Yeboah and Rodrigues, 2011), followed by SC sub-type which is the second most prevalent (Steinberg, 2009).

Although majority (62%) of the parents/guardians had education on nutrition, the training was done by nurses instead of dieticians. The lack of professional nutritional counselling may explain why malnutrition still exists in children with SCD (Osei-Yeboah *et al.,* 2010).

Nutrition has the potential for modifying the adverse effect of sickle cell diseases. Dietary intakes of the children were compared with age-specific Recommended Daily Allowance (RDA) for normal children. Children who fell between age groups 3 years and 4-8 years had excess intake of calories and proteins. From table 4.2, calories (852±342.5 kcal) and protein (24.7±10.5 g/d) intakes for children who fell between the age group 9-12 years were inadequate, compared with RDA. Calories and protein intakes among age group 9-12 years were low and some researchers have reported decreased calories and proteins intakes as younger children grow older (Kawchak *et al.,* 2007; Mandese *et al.,* 2016). Adequate caloric intake by the rest of the age groups (3years and 4-8 years) confirm a study done by Kawchak *et al.* (2007) which reported that dietary intake in SCD children may be adequate but high demand of nutrients due to hyper-metabolism that comes along with the disease may render children malnourished.

Although mean intakes of iron for all the various groups were above the 50^{th} percentile of the RDA, they were still inadequate for all the children. This may be a major contributory factor for iron deficiency anaemia in children with SCD (Mandese *et al.,* 2016). Possible causes of insufficient intake of iron in the children may be due to low intake of high biological value proteins, such as meat which is a rich source of iron, compared to plant sources. A study conducted by Cox *et al.* (2011), reported that iron deficiency anaemia in children with SCD in Africa may be due to dietary insufficiency, rather than chronic haemolysis.

Dietary zinc intakes were below the RDA for children who were 3 years (3.8 ± 1.9 mg/d) and those who fell between age group 4-8 years (5.7± 2.6 mg/d). Low dietary intake of zinc has been considered as one of the major causes of zinc deficiency in SCD children and this

may reflect poor growth and development, especially where there is no supplementation (Mohamed, 2015). This finding is consistent with studies that have reported low intake of zinc by children with SCD, despite normal dietary intakes (Hasanato, 2006).

Folate intakes for age groups 4-8 years (177±143.7 µg/d) and 9-12 years (172±131 µg/d), as well as vitamin B_{12} intakes for the same group were inadequate, compared to RDA. Low folate and vitamin B_{12} levels have been associated with low production of red blood cells, as well as high production of homocysteine levels which is associated with stroke in SCD children (Kennedy *et al.*, 2001). A similar observation was made by Hasanato (2006), who reported low dietary intakes of folate. However, the vitamin B_{12} intakes reported were adequate.

Vitamin A is an essential nutrient needed for immune function, growth, development, reproduction and vision. From Table 4.2, the mean intake of vitamin A was insufficient compared to the Recommended Daily Allowance (RDA) for normal children. This may lead to vitamin A deficiency which is prevalent in children with SCD. Vitamin A deficiency has been associated with reduced resistance to infection and increased rate of anaemia in children with SCD. A similar study conducted by Schall *et al*. (2004) reported that suboptimal vitamin A status increases infections and hospital visits in young children with SCD.

Inadequate vitamin E (4.1±1.7 µg/d) intake across all age groups may be a major contributing factor to poor vitamin E status in children with SCD. The low intakes of vitamin E may indicate reduced antioxidant levels, as reported in a study done by Kawchak *et al.* (2007). Vitamin E is an antioxidant that prevents oxidative stress, through the scavenging of free radicals and, therefore, low levels have been associated with vaso-occlussive crisis in children with SCD (Walter *et al.*, 2006).

From Table 4.3, the dietary pattern of the children in this study indicated low intake of fruits (12%) and green leafy vegetable (9%) daily. These food groups are good sources of vitamin

A, C, E and folate, hence irregular intakes of these food groups may account for low intakes of these nutrients which may contribute to their deficiency. Frequent intakes of eggs (61%) and dairy products (57%) daily may account for the adequate protein intakes observed in majority of the children in this study. However, daily low intakes of offals (9%), fish (21%), and meat (13%) may be the major contributing factor for the insufficient dietary intakes of some micronutrients such as iron, vitamin B_{12} and folic acid in the children.

From Table 4.6, majority of the population (86%) taking supplements (zinc, folic acid and multivitamin) indicate that inadequate intake of these nutrients may be compensated for by supplementation as reported in studies conducted by Dekker *et al.* (2012).

The mean haemoglobin levels of all the children in the study was 8.6 g/dl, which is an indicator of anaemia (WHO, 2001). Low serum ferritin levels in the children further confirms that iron deficiency anaemia is prevalent in children with SCD. Low levels of iron stores, may be due to inadequate intake of iron to replenish iron stores and chronic breakdown of red blood cells (Akinbami *et al.*, 2012). The low mean haemoglobin level observed in this study is consistent with studies conducted by Cox *et al.* (2011) who attributed iron deficiency in children with sickle cell diseases in Africa to inadequate dietary intake.

Using serum albumin as a nutritional marker, a total number of 13 children had low serum albumin, and this may be due to increased protein, turn-over or increase in demand of protein, compared to intake. From the study, prevalence of albumin deficiency was low, compared to studies conducted by Drawz *et al.* (2015) who reported 44% of albumin deficiency in children with SCD as a result of albuminuria. Majority of the children were within the normal range of albumin level and this may be due to the adequate intake of proteins as shown in their dietary patterns which meet the high demand of nutrient accompanied by the diseases. However, albumin deficiency reported in this study agrees with studies that have reported

that albumin deficiency is one of the major causes of malnutrition in children with SCD (Hyacinth *et al.,* 2013).

Anthropometric measures reflect the nutritional and growth status of children with SCD. From Table 4.4, malnutrition markers such as body mass index-for-age recorded 40% of underweight, weight-for-age recorded 37% of underweight, mid-upper-arm-circumference-for-age recorded 31% of underweight and height-for-age recorded 22% of stunting. Prevalence of stunting and underweight, using these anthropometric measures suggest that malnutrition is prevalent in children with SCD, despite dietary supplementation. A similar observation was made by Barden *et al*. (2002) who reported low anthropometric measures in children with SCD. Stunting, as a result of chronic malnutrition may hinder normal pubertal growth in children. Equal number of underweight in both males (20) and females (20) are consistent with study conducted by Al-Saqladi *et al*. (2010), who recorded no significant difference in underweight in both males and females.

The strong positive association (r = 0.600, p-value = 0.00) found between vitamin B_{12} intakes and red blood cell count in Table 4.7, implies that increased dietary intakes of vitamin $B_{12,}$ influences the synthesis of red blood cell, thus increasing the red blood cell count. This finding agrees with studies that have revealed that vitamin B_{12} is one of the essential micronutrients needed in the formation of red blood cells (Enwonwu, 1988).

A positive correlation found between (r = 0.28, p= 0.005) haemoglobin and weight-for-age also implies that as haemoglobin increases weight-for-age also increases. This finding agrees with work done by Lowry *et al*. (1977) who reported positive association between height and weight with haemoglobin. However, the correlation between haemoglobin and weight-for-age was very weak. Another positive correlation was found between MUAC-for-age and haematocrit (p= 0.008, r = 0.225) and this indicates that as MUAC-for-age increases, haematocrit also increases and this is consistent with work done by (Adigun and Ajayi,

2001). Correlations between MUAC-for-age and haematocrit and haemoglobin and weight-for-age were, however, was very weak.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The aim of the study was to assess the nutritional status of children with sickle cell diseases using nutritional intakes, anthropometric and biochemical parameters (serum ferritin, albumin, and protein and haemoglobin). From the study, dietary intakes of some micronutrients such as iron, vitamin A, B_{12}, E and folate were inadequate, compared to the Recommended Daily Allowance (RDA) for normal children. Using anthropometric indices as nutritional marker, prevalence of stunting was 22%, underweight using BMI-for-age was 40%, MUAC-for-age was also 31% while weight-for-age was 37%. Prevalence of iron deficiency anaemia in the sickle cell disease children was 23%. It was also observed from the study that only vitamin B_{12} dietary intake had a strong correlation with red blood cell count.

6.2. RECOMMENDATIONS

It is recommended that a longitudinal study be conducted in children with sickle cell diseases to assess the actual nutritional requirements of children with SCD. This could serve as a basis for setting Recommended Dietary Allowance (RDA) for children with Sickle Cell Disease.

6.3. LIMITATIONS OF THE STUDY

Due to financial constraints, the assay of C-reactive protein could not be done to confirm inflammation in children with SCD. Other indicators of iron status like serum transferrin and total iron binding capacity could be assessed in further studies to obtain the actual state of iron in children with sickle cell disease.

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APPENDICES

Appendix A: Questionnaire

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

QUESTIONNAIRE ON THE ASSESSMENT OF NUTRITIONAL STATUS OF CHILDREN WITH SICKLE CELL DISEASE

INFORMATION OBTAIN FROM THIS QUESTIONNAIRE WILL BE PURELY FOR ACCADEMIC PURPOSES AND CONFIDENTIAL.

PLEASE TICK THE ONE APPROPRIATE.

DEMOGRAPHIC DATA

Identification no: Date:……..……

Name (optional):…… Age: ……

Gender Male ☐ Female ☐

Religion of caretaker Christian ☐ Muslim ☐ Traditionalist ☐

Other…….

Educational status of caretaker JHS ☐ SHS ☐ Tertiary ☐ none ☐

Is the child schooling? Yes ☐ No ☐

If yes which class is your child?

Nursery kindergarten ☐ lower primary ☐ upper primary ☐ other……

Is any member of the family suffering from sickle cell diseases? Yes ☐ No ☐

Is any of the siblings suffering from SCD? Yes ☐ No ☐

DIETARY INFORMATION

1. How many times does the child eat? Once ☐ Twice ☐ Three times ☐

Other……..

2. Does the child eat between meals? Yes ☐ NO ☐

If yes state the type of food?……

3. Does the child have any allergy or any food intolerance? Yes ☐ No ☐

If yes state those foods.

……

4. Does the child skip meals? Yes ☐ No ☐

5. Do you buy food from food vendors to feed child? Yes ☐ No ☐

If yes how often? Every day ☐ every other day ☐ weekly ☐ monthly ☐ occasionally

6. How frequent does the child eat fruits and vegetables? Every day ☐ weekly ☐ monthly ☐ others..☐……

7. Is the child on special diet? Yes ☐ No ☐

if yes state the diet

…….

8. Does the child take any food supplement? Yes ☐ No ☐

If yes state the specific supplement

……..

CLINICAL SYMPTOMS

Please take available information from their medical records

11. Is the child suffering from other disease apart from sickle cell disease? Yes ☐ No ☐

12 What type of disease? ……

13. Type of sickle cell disease

SS ☐ SC ☐ S/B^+ ☐ S/B^0 ☐

14. At what age was the child diagnosed of the disease?

At birth ☐ 1year ☐ 2 years ☐ 3 years ☐ other (specify).....

15. At what age of the child did you start attending the sickle cell clinic?

1year ☐ 2 years ☐ 3 years ☐ 4 years ☐ other (specify).....

16. Does the child experience crises? Yes ☐ No ☐

If yes, how often does the child experience the crisis?

Very often ☐ sometimes ☐ other (specify).....

What do you do when child have the crisis?

Take medication ☐ eat/take fluids ☐ nothing, ☐ other (specify)…

17. What medication do you give the child?

Folic acid ☐ aspirin ☐ Paracetamol ☐ other (specify).....

18. Do you consider nutritional care during crisis? Yes ☐ No ☐

What nutritional care?

.....

19. Do you receive nutrition education at the health center? Yes ☐ No ☐

What nutritional advice do you receive?

.....

20. Has the child been on admission in the past 12 months? Yes ☐ No ☐

Reason for admission

Bone pain ☐ abdominal pain ☐ Chest pain ☐ Malaria ☐ anaemia other……..

Number of admissions in the past 12 months? …….....

How long was the child on admission?

Three Days ☐ one week ☐ one month ☐ other (specify).....

21. Have you received blood transfusion in the past 12 months? Yes ☐ No ☐

How many times have you been transfused? ……

Amount of blood transfused in the past 12 months ……

21. How often do you visit the sickle cell clinic?

Weekly ☐ two weeks ☐ monthly ☐ when I have crisis ☐

Other (specify).....

Name	
Identification number	
DIRECTIONS: The questionnaire is to assess the number of times of fruits and vegetables, plant oils, fish oils food the child has consumed over the **past 6 months.** Where possible provide **one answer to a question**	

Meal consumed	Code	Daily	Weekly (1-3 times)	Monthly	Occasionally	Never	Portion size (g)
FRUITS							
Watermelon	A1						
Banana	A2						
Citrus (Orange,	A3						

tangerine)							
Grape fruit	A4						
Mango	A5						
Pineapple	A6						
Pawpaw	A7						
Apple	A8						
Avocado Pear	A9						
Guava	A10						
Others	A11						
VEGETABLES							
Tomatoes	B1						
Garden eggs	B2						
Kwansosaa(abedru)	B3						
Lettuce	B4						
Kontomire	B5						
Okra	B6						
Carrot	B7						
Cabbage	B8						
Other leafy dark vegetables	B9						
Ayoyo leaves/dandelion	B10						
Cucumber	B11						
LEGUMES AND NUTS							
Cowpeas	C1						
Soya beans	C2						
Lentils	C3						
Cashew nut	C4						
Groundnut/walnut	C5						
Other							
NUTS AND OILS							
plant oils (Corn, soy, canola, coconut, sunflower oils)	D1						
Groundnut							
FISH OIL							
Salmon	E1						
Herrings	E2						
Anchovies	E3						
Tuna	E4						
Oysters	E5						

MEAT, POULTRY AND ANIMAL PRODUCT							
Pork	F1						
Meat (cow, goat, lamb)	F2						
Chicken poultry	F3						
Offal (kidney, liver,heart…)							
Milk							
Whole egg							
Egg yolk							
cheese							
Others							
CEREAL AND GRAINS							
Whole meal oats							
Whole meal wheat							
Brown rice							
Polished rice							
Whole grain (maize)							

NB: I kindly request you list any food the child has consumed which does not appear on the food

table……

……

……

THANK YOU

Appendix B: Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research:

Assessing the nutritional status of children aged 3-12 years and below with sickle cell disease in the Ashanti Region

Name(s) and affiliation(s) of researcher(s):

The study is being conducted by Osei Bonsu Tracy, KNUST, Kumasi and Dr FC Mills-Robertson of Department of Biochemistry, KNUST, and Kumasi.

Background (Please explain simply and briefly what the study is about):

In Ghana sickle cell disease is a major cause of childhood morbidity and mortality. It affect 2% of all babies in and 95% of the children with die before are five years old. Research shows that children with sickle cell anaemia may have increases risk of developing malnutrition due poor appetite levels. Much attention has been given to micronutrient such as iron, zinc, vitamin C, vitamin D and vitamin A and iron over the year. However growth retardation in sickle cell children has also been attributed to protein and energy deficiency. Malnutrition in children can affect their growth and cognitive development as well as increase mortality and susceptibility to infections. Therefore, for children with sickle cell disease to benefit from extensive treatment, it is important that treatment goes in conjunction with good nutrition

Purpose(s) of research:

The purpose of this research is to form part of the assessment leading to the award of MPhil Human Nutrition and Dietetics.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

The research will randomly select children aged 5 and below with sickle cell diseases from sickle cell Clinic at Komfo Anokye Teaching Hospital. Each participant will undergo questionnaire interview after their caretakers give consent to partake in the study. The participants (children) will have their weight and height checked at biochemistry laboratory at Komfo Anokye Teaching Hospital using weighing scale and stadiometer respectively. Venous blood sample will be taken and analysed at clinical Analysis Laboratory at Biochemistry department, KNUST.

Risk(s):

The child may experience pain when blood is being drawn.

Benefit(s):

Participants will receive nutritional education and counselling.

Results obtained will be used by healthcare professionals to improve on nutritional intervention strategies that will benefit children with sickle cell diseases.

Confidentiality:

All information collected in this study will be given code numbers and no name will be recorded.

Voluntariness:

Taking part in this study should be out of the free will of participants.

Alternatives to participation:

If you choose not to participate, this will not affect your treatment in this hospital.

Withdrawal from the research:

Participant (s) can withdraw out of their own will.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to participant(s) if they choose to withdraw from the study.

Costs/Compensation:

For the time and inconvenience, participants will receive snacks as appreciation.

Contacts

If you have any question concerning this study, please do not hesitate to contact Miss. Osei Bonsu Tracy and Dr FC Mills on 0208633759and 0208970091 respectively.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____ _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____(Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

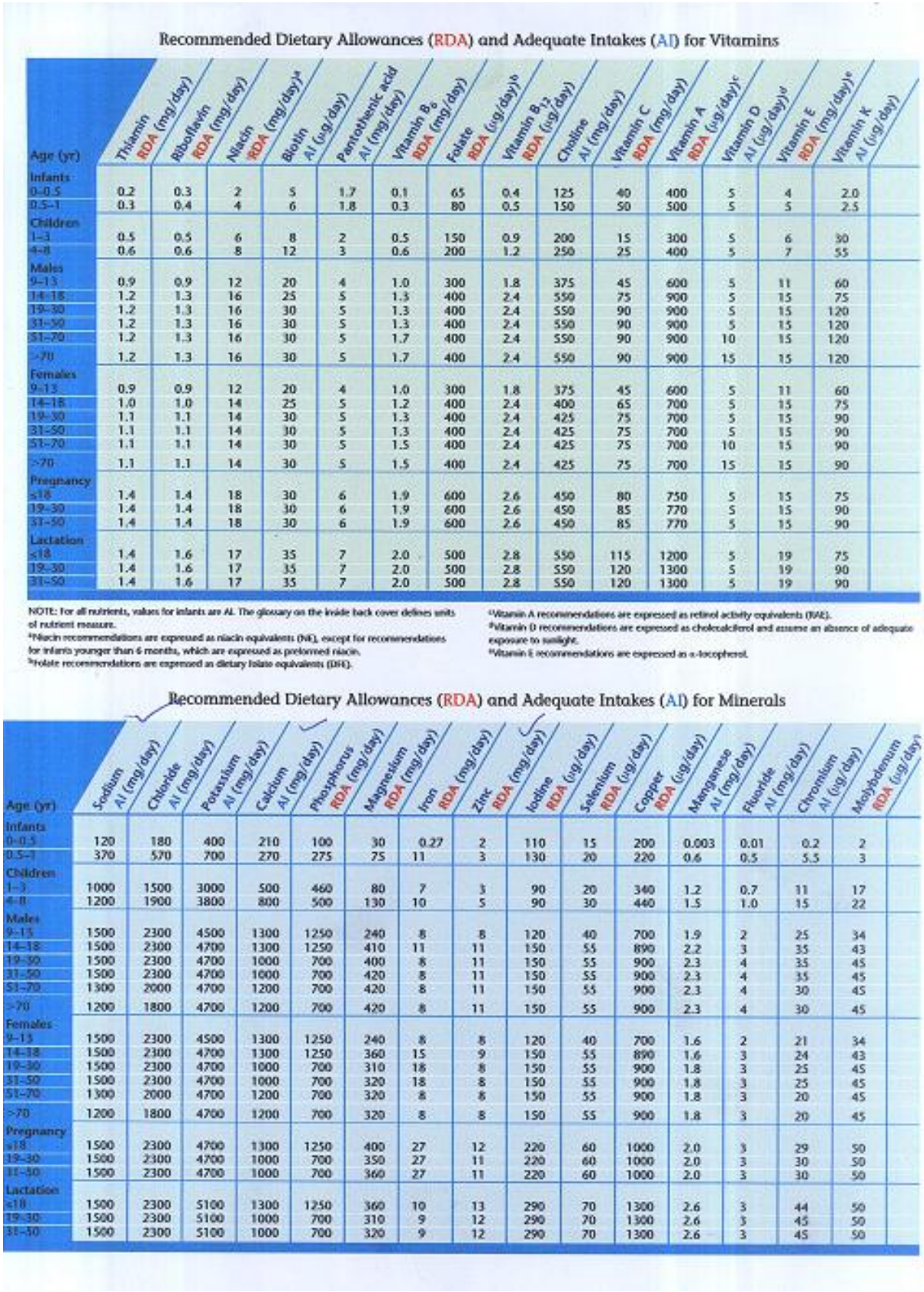
MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'S NAME: _____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'S NAME: _____

Appendix C: Recommended daily allowance for children



Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Vitamins

Age (yr)	Thiamin RDA (mg/day)	Riboflavin RDA (mg/day)	Niacin RDA (mg/day)[a]	Biotin AI (µg/day)	Pantothenic acid AI (mg/day)	Vitamin B_6 RDA (mg/day)	Folate RDA (µg/day)[b]	Vitamin B_{12} RDA (µg/day)	Choline AI (mg/day)	Vitamin C RDA (mg/day)	Vitamin A RDA (µg/day)[c]	Vitamin D AI (µg/day)[d]	Vitamin E RDA (mg/day)[e]	Vitamin K AI (µg/day)
Infants														
0–0.5	0.2	0.3	2	5	1.7	0.1	65	0.4	125	40	400	5	4	2.0
0.5–1	0.3	0.4	4	6	1.8	0.3	80	0.5	150	50	500	5	5	2.5
Children														
1–3	0.5	0.5	6	8	2	0.5	150	0.9	200	15	300	5	6	30
4–8	0.6	0.6	8	12	3	0.6	200	1.2	250	25	400	5	7	55
Males														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	5	11	60
14–18	1.2	1.3	16	25	5	1.3	400	2.4	550	75	900	5	15	75
19–30	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	5	15	120
31–50	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	5	15	120
51–70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	10	15	120
>70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	15	15	120
Females														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	5	11	60
14–18	1.0	1.0	14	25	5	1.2	400	2.4	400	65	700	5	15	75
19–30	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	5	15	90
31–50	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	5	15	90
51–70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	10	15	90
>70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	15	15	90
Pregnancy														
≤18	1.4	1.4	18	30	6	1.9	600	2.6	450	80	750	5	15	75
19–30	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	5	15	90
31–50	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	5	15	90
Lactation														
≤18	1.4	1.6	17	35	7	2.0	500	2.8	550	115	1200	5	19	75
19–30	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	5	19	90
31–50	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	5	19	90

NOTE: For all nutrients, values for infants are AI. The glossary on the inside back cover defines units of nutrient measure.

[a]Niacin recommendations are expressed as niacin equivalents (NE), except for recommendations for infants younger than 6 months, which are expressed as preformed niacin.

[b]Folate recommendations are expressed as dietary folate equivalents (DFE).

[c]Vitamin A recommendations are expressed as retinol activity equivalents (RAE).

[d]Vitamin D recommendations are expressed as cholecalciferol and assume an absence of adequate exposure to sunlight.

[e]Vitamin E recommendations are expressed as α-tocopherol.

Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Minerals

Age (yr)	Sodium AI (mg/day)	Chloride AI (mg/day)	Potassium AI (mg/day)	Calcium AI (mg/day)	Phosphorus RDA (mg/day)	Magnesium RDA (mg/day)	Iron RDA (mg/day)	Zinc RDA (mg/day)	Iodine RDA (µg/day)	Selenium RDA (µg/day)	Copper RDA (µg/day)	Manganese AI (mg/day)	Fluoride AI (mg/day)	Chromium AI (µg/day)	Molybdenum RDA (µg/day)
Infants															
0–0.5	120	180	400	210	100	30	0.27	2	110	15	200	0.003	0.01	0.2	2
0.5–1	370	570	700	270	275	75	11	3	130	20	220	0.6	0.5	5.5	3
Children															
1–3	1000	1500	3000	500	460	80	7	3	90	20	340	1.2	0.7	11	17
4–8	1200	1900	3800	800	500	130	10	5	90	30	440	1.5	1.0	15	22
Males															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.9	2	25	34
14–18	1500	2300	4700	1300	1250	410	11	11	150	55	890	2.2	3	35	43
19–30	1500	2300	4700	1000	700	400	8	11	150	55	900	2.3	4	35	45
31–50	1500	2300	4700	1000	700	420	8	11	150	55	900	2.3	4	35	45
51–70	1300	2000	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
>70	1200	1800	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
Females															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.6	2	21	34
14–18	1500	2300	4700	1300	1250	360	15	9	150	55	890	1.6	3	24	43
19–30	1500	2300	4700	1000	700	310	18	8	150	55	900	1.8	3	25	45
31–50	1500	2300	4700	1000	700	320	18	8	150	55	900	1.8	3	25	45
51–70	1300	2000	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
>70	1200	1800	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
Pregnancy															
≤18	1500	2300	4700	1300	1250	400	27	12	220	60	1000	2.0	3	29	50
19–30	1500	2300	4700	1000	700	350	27	11	220	60	1000	2.0	3	30	50
31–50	1500	2300	4700	1000	700	360	27	11	220	60	1000	2.0	3	30	50
Lactation															
≤18	1500	2300	5100	1300	1250	360	10	13	290	70	1300	2.6	3	44	50
19–30	1500	2300	5100	1000	700	310	9	12	290	70	1300	2.6	3	45	50
31–50	1500	2300	5100	1000	700	320	9	12	290	70	1300	2.6	3	45	50

Tolerable Upper Intake Levels (UL) for Vitamins

Age (yr)	Niacin (mg/day)[a]	Vitamin B_6 (mg/day)	Folate (µg/day)[a]	Choline (mg/day)	Vitamin C (mg/day)	Vitamin A (µg/day)[b]	Vitamin D (µg/day)	Vitamin E (mg/day)[c]	
Infants									
0–0.5	—	—	—	—	—	600	25	—	
0.5–1	—	—	—	—	—	600	25	—	
Children									
1–3	10	30	300	1000	400	600	50	200	
4–8	15	40	400	1000	650	900	50	300	
9–13	20	60	600	2000	1200	1700	50	600	
Adolescents									
14–18	30	80	800	3000	1800	2800	50	800	
Adults									
19–70	35	100	1000	3500	2000	3000	50	1000	
>70	35	100	1000	3500	2000	3000	50	1000	
Pregnancy									
≤18	30	80	800	3000	1800	2800	50	800	
19–50	35	100	1000	3500	2000	3000	50	1000	
Lactation									
≤18	30	80	800	3000	1800	2800	50	800	
19–50	35	100	1000	3500	2000	3000	50	1000	

[a]The UL for niacin and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

[b]The UL for vitamin A applies to the preformed vitamin only.
[c]The UL for vitamin E applies to any form of supplemental α-tocopherol, fortified foods, or a combination of the two.

Tolerable Upper Intake Levels (UL) for Minerals

Age (yr)	Sodium (mg/day)	Chloride (mg/day)	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day)[a]	Iron (mg/day)	Zinc (mg/day)	Iodine (µg/day)	Selenium (µg/day)	Copper (µg/day)	Manganese (mg/day)	Fluoride (mg/day)	Molybdenum (µg/day)	Boron (mg/day)	Nickel (mg/day)	Vanadium (mg/day)	
Infants																	
0–0.5	—[b]	—[b]	—	—	—	40	4	—	45	—	—	0.7	—	—	—	—	
0.5–1	—[b]	—[b]	—	—	—	40	5	—	60	—	—	0.9	—	—	—	—	
Children																	
1–3	1500	2300	2500	3000	65	40	7	200	90	1000	2	1.3	300	3	0.2	—	
4–8	1900	2900	2500	3000	110	40	12	300	150	3000	3	2.2	600	6	0.3	—	
9–13	2200	3400	2500	4000	350	40	23	600	280	5000	6	10	1100	11	0.6	—	
Adolescents																	
14–18	2300	3600	2500	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
Adults																	
19–70	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8	
>70	2300	3600	2500	3000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8	
Pregnancy																	
≤18	2300	3600	2500	3500	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
19–50	2300	3600	2500	3500	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—	
Lactation																	
≤18	2300	3600	2500	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—	

[a]The UL for magnesium applies to synthetic forms obtained from supplements or drugs only.
[b]Source of intake should be from human milk (or formula) and food only.

NOTE: An Upper Limit was not established for vitamins and minerals not listed and for those age groups listed with a dash (—) because of a lack of data, not because these nutrients are safe to consume at any level of intake. All nutrients can have adverse effects when intakes are excessive.

Dietary Reference Intakes (DRI)

The Dietary Reference Intakes (DRI) include two sets of values that serve as goals for nutrient intake—Recommended Dietary Allowances (RDA) and Adequate Intakes (AI). The RDA reflect the average daily amount of a nutrient considered adequate to meet the needs of most healthy people. If there is insufficient evidence to determine an RDA, an AI is set. AI are more tentative than RDA, but both may be used as goals for nutrient intakes. (Chapter 1 provides more details.)

In addition to the values that serve as goals for nutrient intakes (presented in the tables on these two pages), the DRI include a set of values called Tolerable Upper Intake Levels (UL). The UL represent the maximum amount of a nutrient that appears safe for most healthy people to consume on a regular basis. Turn the page for a listing of the UL for selected vitamins and minerals.

Estimated Energy Requirements (EER), Recommended Dietary Allowances (RDA), and Adequate Intakes (AI) for Water, Energy, and the Energy Nutrients

Age(yr)	Reference BMI (kg/m²)	Reference height, cm (in)	Reference weight, kg (lb)	Water[a] AI (L/day)	Energy EER[b] (kcal/day)	Carbohydrate RDA (g/day)	Total fiber AI (g/day)	Total fat AI (g/day)	Linoleic acid AI (g/day)	Linolenic acid[c] AI (g/day)	Protein RDA (g/day)[d]	Protein RDA (g/kg/day)
Males												
0–0.5	—	62 (24)	6 (13)	0.7[e]	570	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8[f]	743	95	—	30	4.6	0.5	11	1.2
1–3	—	86 (34)	12 (27)	1.3	1046	130	19	—	7	0.7	13	1.05
4–8	15.3	115 (45)	20 (44)	1.7	1742	130	25	—	10	0.9	19	0.95
9–13	17.2	144 (57)	36 (79)	2.4	2279	130	31	—	12	1.2	34	0.95
14–18	20.5	174 (68)	61 (134)	3.3	3152[h]	130	38	—	16	1.6	52	0.85
19–30	22.5	177 (70)	70 (154)	3.7	3067[h]	130	38	—	17	1.6	56	0.8
31–50				3.7	3067[h]	130	38	—	17	1.6	56	0.8
>50				3.7	3067[h]	130	30	—	14	1.6	56	0.8
Females												
0–0.5	—	62 (24)	6 (13)	0.7[e]	520	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8[f]	676	95	—	30	4.6	0.5	11	1.2
1–3	—	86 (34)	12 (27)	1.3	992	130	19	—	7	0.7	13	1.05
4–8	15.3	115 (45)	20 (44)	1.7	1642	130	25	—	10	0.9	19	0.95
9–13	17.4	144 (57)	37 (81)	2.1	2071	130	26	—	10	1.0	34	0.95
14–18	20.4	163 (64)	54 (119)	2.3	2368	130	26	—	11	1.1	46	0.85
19–30	21.5	163 (64)	57 (126)	2.7	2403[i]	130	25	—	12	1.1	46	0.8
31–50				2.7	2403[i]	130	25	—	12	1.1	46	0.8
>50				2.7	2403[i]	130	21	—	11	1.1	46	0.8
Pregnancy												
1st trimester				3.0	+0	175	28	—	13	1.4	+25	1.1
2nd trimester				3.0	+340	175	28	—	13	1.4	+25	1.1
3rd trimester				3.0	+452	175	28	—	13	1.4	+25	1.1
Lactation												
1st 6 months				3.8	+330	210	29	—	13	1.3	+25	1.3
2nd 6 months				3.8	+400	210	29	—	13	1.3	+25	1.3

NOTE: For all nutrients, values for infants are AI. Dashes indicate that values have not been determined.

[a]The water AI includes drinking water, water in beverages, and water in foods; in general, drinking water and other beverages contribute about 70 to 80 percent, and foods, the remainder. Conversion factors: 1 L = 33.8 fluid oz; 1 L = 1.06 qt; 1 cup = 8 fluid oz.

[b]The Estimated Energy Requirement (EER) represents the average dietary energy intake that will maintain energy balance in a healthy person of a given gender, age, weight, height, and physical activity level. The values listed are based on an "active" person at the reference height and weight and at the midpoint ages for each group until age 19. Chapter 8 and Appendix F provide equations and tables to determine estimated energy requirements.

[c]The linolenic acid referred to in this table and text is the omega-3 fatty acid known as alpha-linolenic acid.

[d]The values listed are based on reference body weights.

[e]Assumed to be from human milk.

[f]Assumed to be from human milk and complementary foods and beverages. This includes approximately 0.6 L (~3 cups) as total fluid including formula, juices, and drinking water.

[g]For energy, the age groups for young children are 1–2 years and 3–8 years.

[h]For males, subtract 10 kcalories per day for each year of age above 19.

[i]For females, subtract 7 kcalories per day for each year of age above 19.