BASELINE SERUM AND URINE OSMOLALITY AND FACTORS THAT AFFECT IT IN STEADY STATE SICKLE CELL DISEASE PATIENTS

By

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DECLARATION

I, YAW ADUBOFOUR KUSI-MENSAH, author of this thesis do hereby declare that, with the exception of references to other people's work which has been duly cited, this work has entirely resulted from my personal original research under supervision of Rev. Dr. C. Antwi-Boasiako and Dr Ebenezer Owusu-Darkwah and has not been presented for another degree elsewhere.

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I dedicate this work to my late father Mr John Oboadum Kusi-Mensah, who constantly reminded me of the value of a good education. Though departed you shall never be forgotten.

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LIST OF ABBREVIATIONS

ADH	Antidiuretic hormone
KBTH	Korle-Bu Teaching Hospital
HB	Haemoglobin
HBS	Haemoglobin S
HB SC	SC-Genotype
HB AA	AA-Genotype
Hb F	Foetal Haemoglobin
HBC	Haemoglobin C
HBA	Haemoglobin A
HB SS	SS-Genotype
IV	Intravenous fluid
PLT	Platelet
RBC	Reb blood cell
SCD	Sickle cell disease
WBC	White blood cell

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ABSTRACT

It is over a century since the discovery of Sickle Cell Disease (SCD) and since then, a lot more is still being discovered about the pathophysiology of the disease. Notable amongst the various factors that affect the disease's pathophysiology and management is red cell dehydration. Factors that affect cellular dehydration and serum osmolality in SCD patients are not very clearly understood. An objective method among of assessing SCD patient's hydration status is by measuring the serum and urine osmolality. Limited data exist on factors that affect the hydration status of sickle-cell disease patients especially in Ghana and sub-Saharan Africa. There is therefore the need to collate data about these factors to help in the management of SCD.

Aim: The aim of this study was to determine factors that affect serum and urine osmolality in steady-state HbSS and HbSC SCD patients.

Methodology: This was a Case-Control study that was conducted at the Department of Haematology (Sickle Cell Clinic), Korle-Bu Teaching Hospital (KBTH) in Accra. 120 cases of SCD (80 HbSS and 40 HbSC genotype) in their steady-state were matched for age to 60 controls. A structured questionnaire was used to obtain the demographic characteristics of recruited subjects, medical history, physical examination findings, a 24-hour dietary recall and frequency of consumption of sodium-rich food. Five millilitres (mls) of venous blood and 5mls of mid-stream urine samples were obtained from each subject. Serum, urine osmolality, full-blood count, blood urea, electrolytes and creatinine (BUE & Cr) were determined. Urine routine examination (Urine R/E) was also done to assess urine pH and specific gravity. Bivariate analysis was used to compare the variables measured, with a p-value < 0.05 deemed as significant.

Results: Compared to the mean serum osmolality of the control group (354.50±82.37)mmol/kg, SCD patients (HbSS and HbSC) had a higher mean serum osmolality (440.87±113.14 and

422.90±88.59)mmol/kg respectively (*p-value* <0.001). The mean urine osmolality of HbSS and HbSC patients (452.28 ± 109.41 and 498.28±87.26)mmol/kg respectively was significantly lower compared to HbAA individuals (681.23±276.82) mmol/kg (*p-value* <0.001). Comparing HbSS and HbSC patients, there was no significant difference in the mean serum osmolality (p-value =0.382), but a significant increase in the mean urine osmolality of HbSC patients compared to HbSS patients (p-value =0.020).

There was a weak negative correlation between serum osmolality and dietary Sodium (*r= -0.192*), between serum osmolality and average water intake (*r= -0.025*), between serum osmolality and dietary Protein (*r= -0.098*) and between serum osmolality and dietary Potassium (*r= -0.052*) in SCD patients.

Analysing data from the Food Frequency Table, majority (> 50%) of study participants (HbSS, HbSC AND HbAA) did not consume most sodium-rich foods (salted meals) very often. The only salt-rich food assessed that was consumed often was stock cubes. Majority (>60%) of study participants (HbSS, HbSC AND HbAA) obtained their food from Food Vendors making it difficult to accurately quantify their food consumption.

Conclusion: Sickle Cell Disease patients have a high serum osmolality and a low urine osmolality. The HbSC patients have a higher urine osmolality compared with HbSS patients. In general, study participants did not frequently consume sodium-rich foods. Serum osmolality decreases with increasing consumption of sodium-rich diets, thus the high serum osmolality and low urine osmolality observed in SCD patients is mainly due to the disease pathophysiology.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Sickle Cell Disease (SCD) is caused by a structural abnormality in Haemoglobin as a result of a point mutation in the β-globin gene, a substitution of thymine for adenine, at the sixth codon of the β globin gene found on Chromosome 11. The result is the formation of a defective Haemoglobin called the Sickle Haemoglobin (HbS). Clinically, SCD is defined as an individual who has inherited two HbS or an individual who inherited one HbS in association with another abnormal Haemoglobin example; HbS/beta-thalassemia, HbSC, HbSD and HbSO-Arab (Asare et al., 2018)(Provan, 2009). In Ghana HbSS and HbSC are the commonest phenotypes (Asare et al., 2018). The condition has an autosomal recessive inheritance pattern and so preventive measures are aimed at couples knowing their Hb electrophoresis status before having children (Kaur, Dangi, & Singh, 2013).

Sickle Cell disease affects millions of people globally with serious complications associated with the condition. It affects all organs in the body with serious socio-economic implications(Kaur et al., 2013). Sickle Cell disease is a major haematological health problem in Malaria-prone sub-tropical regions such as obtains in West Africa (Piel, Steinberg, & Rees, 2017)(Rees, Williams, & Gladwin, 2010). In Ghana, the disease prevalence is 1.9% of all live births per year (C Antwi-Boasiako et al., 2015).

Under low oxygen tension, HbS undergoes polymerization within the Red Blood Cell (RBC) changing the shape of the RBC into the characteristic Sickle-shaped Red Blood Cell of SCD, a deviation from the usual Biconcave-Circular shape (Galkin & Vekilov, 2004). This causes damage to the membrane of the RBC, causing it to lyse or be easily destroyed in circulation. Further, HbS polymerization increases the propensity of the defective RBC to adhere to vascular endothelium significantly leading to occlusion of the vessels especially in the microcirculation (Galkin & Vekilov, 2004)(Provan, 2009).

Red-Cell dehydration is a distinguishing characteristic of sickle cell disease and an important contributor to the disease pathophysiology (C. Brugnara, 1995). The Red Blood Cell (RBC) in SCD is more prone to dehydration compared to RBCs in controls HbAA (Bookchin & Lew, 2002). This makes dehydration common in SCD patients even when they are not in overt crisis (Bookchin & Lew, 2002)(Provan, 2009). Cellular dehydration observed in the disease results from the loss of intracellular (K^+) to the extracellular domain which is partially compensated by an increase in cellular (*Na*). The loss of *K* and the resultant cell dehydration is as a result of four (4) major mechanisms; Calcium (Ca^{2+}) -activated Potassium (K^+) channel (Gardos Pathway), Potassium-Chloride (Cl) cotransport, Deoxygenation induced by the (*Na*) and (*K*) fluxes (Na-K pump) and Oxidative damage of the red cell membrane and net (*K*) loss (C. Brugnara, 1995)(De Franceschi L, D Bachir, Galacteros F, 1997)(C., 2003). Due to the unique dependence of Hb S polymerization on cellular Hb S concentration, cell dehydration promotes polymerization and sickling (C., 2003). Based on this knowledge, SCD patients are encouraged to drink extra fluids to prevent dehydration. Being adequately hydrated is key in maintaining homeostasis in the body (Ruth Williams-Hooker, 2013) and thus clinicians need to have an objective way of assessing patients' hydration status to guide in management. Serum osmolality is clinically useful in

determining the hydration status of the SCD patient and the urine osmolality helps to determine the tubular function of the patient's kidneys (Sherwood, n.d.). Thus, knowledge of osmolality in SCD patients, particularly in their steady-state, is a good clinical indicator of the hydration state of the SCD patient influencing SCD patient education on fluid intake and the overall management of SCD patients.

Sickle Cell Disease is primarily a disease of haemoglobin found in the Red Blood Cells (Haemoglobinopathy) however, the White Blood Cells and the Platelets are also affected by the β-globin gene mutation (Akinbami et al., 2012). Several clinical manifestations arise from the disease process in SCD patients. The common clinical manifestations of the disease are haemolysis, chronic anaemia, recurrent vaso-occlusive phenomenon and infections (Provan, 2009)(Rees et al., 2010). Sickle Cell Disease patients are usually in a steady-state (a state in which they are not experiencing any acute painful episodes or any changes due to therapy) but periodically get crisis which more often than not requires some form of intervention (Ballas, 2012). Although there is a recent cure for SCD through haemopoietic stem-cell transplantation, this treatment is not readily available in the sub-region and very expensive. Management of the disease in this part of the world is aimed at keeping patients in their steady-state, alleviating the symptoms and preventing complications (Edition, 2010). As a chronic condition in this part of the world, it is associated with frequent acute exacerbations (L. W. Diggs, n.d.), poor dietary habits, underweight, frequent hospital admissions, frequent blood transfusions, frequent Intravenous (IV) fluid and drug administration (antibiotics, antimalarials, analgesics) and an overall sub-optimal state of health (Provan, 2009). Management of the disease complications poses a nightmare to clinicians (Provan, 2009) and even more so when these patients are coming for surgeries. Several

factors affect Osmolality mainly through changes in the ECF concentration of Na. In Sickle Cell Disease, these factors may be due to the pathophysiology of the disease itself, from the management of the disease or lifestyle patterns.

High dietary sodium has been associated with a raised blood pressure but not much is known on its effect in SCD patients (Brown, Tzoulaki, Candeias, & Elliott, 2009). Excessive dietary salt intake is known to increase serum Osmolality which triggers protective physiological mechanisms to offset this increase (Sherwood, 2010)(Kjeldsen et al., 1985). The first mechanism the body employs is the release of Vasopressin/Anti Diuretic hormone (ADH) from the posterior pituitary. Vasopressin acts on the collecting ducts of the kidneys causing an increase in its permeability to water and to a lesser extent sodium (Sherwood, 2010)(Kjeldsen et al., 1985). The second mechanism is the polyol mediated aldose reductase enzyme activation in renal tubules (Koc University, Istanbul, n.d.). The overall effect of these mechanisms is to increase the amount of water reabsorbed by the kidneys and thus produce urine which is low in volume and high in concentration. It has been established that Sickle Cell Disease patients have an impaired ability to concentrate urine (ITANO, KEITEL, & THOMPSON, 1956)(Pediatric & Clinic, 1968). Children with Sickle Cell Anaemia (SCA) consume higher than recommended sodium-rich foods (chips, snack foods and processed foods) and lower than recommended fluid intake in a study carried out in the United States (Ruth Williams-Hooker, 2013) worsening their hydration status which can be assessed by their osmolality. Preservatives used in storing blood together with the changes in electrolytes and pH that occurs when blood and blood products are stored can affect serum osmolality when patients are given in large quantities or frequently. Apart from isotonic IV fluids, all other IV fluids affect the osmolality of serum (James & Schneider, 1993)(Boulard, Marguinaud,

& Sesay, 2003) with a majority of these IV fluids containing *Na*. Intravenous fluids are given usually in large quantities during the management of crisis (E. L. Williams, Hildebrand, McCormick, & Bedel, 1999). Sodium (Na) is the most abundant electrolyte in the ECF and also the most osmotically active particle in the ECF due to its position on the electrochemical series (Provan, 2009)(Hoyle, 2009). Movement of Na across most cell membranes is accompanied by a movement of water in the same direction (Provan, 2009)(Hoyle, 2009). Sodium is, therefore, a key determinant of osmolality in the human body.

1.2 Problem statement

Even though cellular dehydration and invariably chronic dehydration is one of the distinguishing features of SCD, it's difficult objectively to assess the hydration status of SCD patients. Osmolality which is one of the physiological parameters used to objectively assess one's hydration status is poorly investigated in SCD. Very little data has been published on the serum osmolality levels of SCD patients especially within the Ghanaian context to the best of the author's knowledge. Most of the data published focus on urine osmolality looking specifically at Hyposthenuria (inability to concentrate urine) and the study population are mainly children.

It is very important to study serum Osmolality in SCD patients because firstly, the repeated microvasculature occlusion can lead to end-organ damage with the Kidneys being commonly affected. This can develop in childhood or adulthood (K.A. & R.P., 2015). Hyposthenuria (inability to concentrate urine) is one of the renal complications that develop commonly in childhood (ITANO et al., 1956). The loss of urine concentrating ability means that Sickle Cell patients produce urine with low Osmolality. This affects the body's ability to maintain a normal serum osmolality in times of reduced fluid intake (Sherwood, n.d.).

Secondly, osmolality is affected by several factors. In Sickle Cell Disease patients these factors may be from the pathophysiology of the disease itself or management. No clear association has been demonstrated previously between these factors and osmolality. The factors being investigated in this research are; diet and sodium-rich food consumption. Modifiable factors such as diet and adequate hydration can be modified to help improve the general well-being of SCD patients in the sub-region. Clinicians and caregivers would have a sound scientific basis for these modifications.

This project would, therefore, seek to throw much-needed light on this important parameter and factors that may affect it.

1.3 Justification

Sickle cell disease (SCD) is one of the most common genetic diseases in the world. Africans are disproportionately affected by this disease and have a more severe phenotype (Pandey et al., 2012)(Makani, Williams, & Marsh, 2007). An estimated 15 to 30% of individuals in Ghana have sickle cell trait (C Antwi-Boasiako et al., 2015). There are geographical differences associated with mortality in SCD patients and disease-related complications and mortality in Sickle Cell are ethnic driven (Davis, Gergen, & Moore, 1997). Serum osmolality is clinically useful in determining the hydration status of individuals and urine osmolality looks more at the tubular function of the kidneys. This can be an early monitoring tool to pick up disease complications of SCD and in measuring disease severity. There is the paucity of data within the Sub-region on serum and urine osmolality amongst individuals with Sickle Cell Disease despite the clinical relevance of osmolality. Research carried out in the sub-region would provide context-appropriate knowledge that would be more relevant in management in the sub-region.

This study will help to bridge the knowledge gap and create more awareness among clinicians.

This study seeks to contribute to the wider scholarly discussion on Sickle Cell Disease and add to the body of knowledge on contemporary theories on the subject.

1.4 Aim

To determine the serum and urine osmolality and factors that affect it in steady-state SCD patients (HbSS and HbSC).

1.5 Specific Objectives

1. To determine the baseline steady-state serum and urine osmolality in patients with and without sickle cell disease
2. To identify risk factors for / protective factors against the development of deranged urine and serum osmolality in patients with and without sickle cell disease.

1.6 Research Questions

1. What is the baseline mean serum and urine osmolality in Sickle Cell patients in their steady-state attending the Sickle Cell Clinic?
2. What is the baseline mean serum electrolytes (Na, Cl, K, Mg, Ca), urea & creatinine, urine pH and urine specific gravity in HbSS and HbSC patients?
3. What is the dietary sodium consumption of Sickle Cell patients attending the Sickle Cell Clinic?
4. Is there an association between sodium consumption and osmolality?
5. What is the frequency of consumption of sodium-rich (salted meals) foods by Sickle Cell patients?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sickle Cell Disease

2.1.1 Definition of Sickle Cell Disease

Red Blood Cells (RBCs) contain Haemoglobin which is responsible for the transport of oxygen in the body. Haemoglobin is made up of two parts; the globin (polypeptide) portion and iron-containing heme-group. The globin portion is a heterotetramer that consists of two pairs of identical highly folded polypeptide chains; a pair of alpha (α) globin and a pair of beta (β) globin chains (Thom, Dickson, Gell, & Weiss, 2013)(Sherwood, 2010). Sickle Cell Disease (SCD) is a common genetic disorder (single-gene) which affects the beta (β) globin of Haemoglobin found in Red Blood Cells (RBC) (Creary, Williamson, & Kulkarni, 2007)(Konotey-Ahulu, 1991). It is inherited as an Autosomal Recessive Mendelian trait and results from a single base-pair change, thymine for adenine, at the sixth codon of the β globin gene (Chromosome 11) (Asare et al., 2018). The defective Haemoglobin formed from the point mutation is called the Sickle Haemoglobin (HbS). Sickle cell disease (SCD) generally refers to a group inherited Haemoglobinopathies in which Hb S is paired with another abnormal Haemoglobin (Asare et al., 2018)(Provan, 2009). The condition derived its name from the characteristic sickle shape the Red Blood Cells adopt under conditions of low oxygen tension, a deviation from the normal Biconcave-Circular shape of RBCs (Dorn-Beineke A, n.d.). Sickle Cell Disease is the first human disease to be described at the molecular level, three-quarters of a century after its first documentation by Africanus.

2.1.2 History of Sickle Cell Disease

It has been over a century since the description of an abnormally elongated red blood cell in an anaemic black student in Chicago, U.S.A who also had a history of leg ulcers and jaundice was documented (Konotey-Ahulu & Ringelhann, 1969). However, SCD is believed to have existed long before any documentation in literature. Folktales of African origin have described a chronic disease with recurrent episodes of bone pain associated with cold weather (Konotey-Ahulu, 1968). Africanus in 1874 described an inherited disease characterized by a persistent blood abnormality with painful episodes more frequent in the cold season and associated with a fever. The aetiology of the disease, a point mutation in the β globin gene (Chromosome 11) was discovered by Linus Pauling et al in 1949 and in that same year, the Autosomal Recessive inheritance of the disease was established (Galkin & Vekilov, 2004). Subsequent studies have characterized the structural and physical properties of Hb S (Ferrone & Rotter, 2004)(Ferrone, 2004), molecular basis underpinning the sickling phenomenon (Galkin & Vekilov, 2004)(Ferrone, 2004), development of molecular diagnostic tools (Pandey et al., 2012) and the establishment of prenatal diagnosis (Kwaku Ohene-Frempong, Oduro, Tetteh, & Nkrumah, 2008). A lot more is yet to be discovered about the disease as research improves the survival rate of the condition.

2.1.3 Classification of Sickle Cell Disease

Sickle Cell Disease (SCD) patients are classified based on whether they are homozygous (Hb SS) or heterozygous (with co-inheritance with other abnormal Haemoglobin) (Mehta & Hoffbrand, 2014). In both types, the genetic defect results in the defective expression of both β globin in Haemoglobin. In the strict sense; Sickle Cell Anaemia (SCA), the most common type of this condition (60-70% of SCD in the US(M, Bender, 2003)), refers to an individual who is

homozygous for the HbS mutation whereas Sickle cell disease (SCD) is defined as Hb S plus another abnormal Haemoglobin example; HbSC, HbSD, HbSO-Arab, and HbS/beta-thalassemia (Asare et al., 2018)(Provan, 2009). In Ghana HbSS and HbSC are the commonest types encountered (Asare et al., 2018). Haemoglobin C (which results from a substitution of the Glutamic acid residue for a Lysine residue at the 6^{th} position of the β globin chain) is found almost exclusively amongst people of West African descent with a high prevalence in northern Ghana and Burkina Faso (M, Bender, 2003). Heterozygotes have a milder form of the disease with fewer symptoms compared to the homozygotes (Asare et al., 2018).

Haemoglobin S/beta-thalassemia (Hb Sβ-Thalassemia) which is a compound heterozygous form of the disease is believed to be rare in most parts of Sub-Saharan Africa. Two types of Hb Sβ-Thalassemia exits; Hb $S\beta^{+}$-Thalassemia (where the expression of the β^{+}-Thalassemia gene results in a reduction in the production of normal Haemoglobin and this is coupled with the expression of Hb S) and Hb $S\beta^{\circ}$-Thalassemia (where the expression of the β°-Thalassemia gene results in the production of no β globin coupled with the expression of Hb S). Hb $S\beta^{\circ}$-Thalassemia is more severe compared to Hb $S\beta^{+}$-Thalassemia (Provan, 2009).

Sickle Cell Trait (Hb AS) refers individuals with the sickle Haemoglobin (Hb S) and a normal Haemoglobin (Hb A). They are not considered as SCD patients because they are carriers with no clinical manifestation of the disease. The prevalence of Hb AS is 1 in 10 Afro-Caribbean, 1 in 5 Ghanaian and 1 in 4 Nigerian (Powars, 1991). Haemoglobin C trait is when an individual has a normal Haemoglobin and Haemoglobin C (Hb AC) this is different from the Sickle Cell Trait and such individuals are not at risk of developing sickle cell disease. In this write-up, however, SCD refers to both SS-genotype and SC-genotype patients.

2.1.4 The Burden of Sickle Cell Disease Globally, in Africa and Ghana

There is overwhelming scientific evidence demonstrating the abundance of genetic disorders globally. According to the World Health Organization (WHO) Bulletin in 2008, 7 million babies are born each year with a genetic abnormality (Modell & Darlison, 2008). Sickle Cell Disease (*SCD*) is one of the more common severe monogenic disorders in the world (Rees et al., 2010) and the most well studied genetic disorder known to man. It is the commonest Haemoglobinopathy (Modell & Darlison, 2008). The global burden of *SCD* is now becoming increasingly realized. Sickle Cell Disease is of public health significance in sub-Saharan Africa, the Middle East, some regions of India, the Caribbean and Brazil (Aygun & Odame, 2012). The United Nations has recognized SCD as a global public health concern, and the World Health Organization (WHO) recommends that 50% of member states have established *SCD* control programs by 2020 (Mulumba & Wilson, 2015).

Africa is the main birthplace of sickle mutations; the number of new-borns affected by sickle cell disease is estimated at 200,000 per year (Diallo & Tchernia, 2002)(T. N. Williams, 2016). In the past, in the absence of new-born screening and early detection, most of these children died undiagnosed (T. N. Williams, 2016). The prevalence of the disease is high in our part of the world because of the remarkable protection the sickle cell trait (heterozygosity for the sickle cell mutation) provides against malaria (Piel et al., 2017).

There is the abundance of abnormal Haemoglobin in Ghana, with 1 in 5 carrying an abnormal Haemoglobin (either the sickle-cell trait Hb AS or Haemoglobin C Hb C (Hb AC)) (Konotey-Ahulu & Ringelhann, 1969). In Ghana, the prevalence of sickle cell disease is 1.9% of

all live births per year and it contributes significantly to morbidity and mortality in the country (C Antwi-Boasiako et al., 2015). In 2003, SCD was ranked 36th in the out-patient morbidity records in a survey carried out by the Ghana Health Service.

2.2 Pathophysiology of Sickle Cell Disease

2.2.1 Red Blood Cells and Haemoglobin

Red Blood Cells (RBC) are flat (2µm thick), biconcave disc-shaped (8µm in diameter) cells found in blood (Sherwood, 2010). The main function of RBCs is the efficient transport of oxygen in the body (Sherwood, 2010). This function is achieved by RBCs because of two main features; i) their unique structure (biconcave shape provides a large surface area for diffusion of O_2, thinness enables O_2 to diffuse rapidly and flexibility of the cell membrane allows easily reversible deformity of the cell as they squeeze single file through capillaries (as narrow as 3µm in diameter) and ii) Haemoglobin a molecule found in only RBCs (Sherwood, 2010).

Globin found in Haemoglobin are polypeptides, synthesized from α-like globin genes (ζ, α1, and α2) located on chromosome 16 and β-like globin genes (ε, Gγ, Aγ, δ, and β) located on chromosome 11(Okpala, 2004). In the normal RBC, three (3) types of haemoglobin exists; i) Haemoglobin F (Hb F) which is predominant during foetal life and decreases gradually after birth to 6 months ii) Haemoglobin A (which accounts for 95% of haemoglobin in adults and iii) Haemoglobin A2 (accounts for less than 2.5% of circulating haemoglobin in adults (Thom et al., 2013)(Bank, 2006). Any genetic damage that can alter any of the steps in the globin production, the folding of the subunit, the attachment of the heme group to the globin subunits, dimerization or tetramerization can destroy the stability of the Haemoglobin and impair its function.

2.2.2 Haemoglobinopathy

Haemoglobinopathy is defined as any abnormality in the structure, function and/or production of Haemoglobin (Longo Dan, Fauci Anthony, Kasper Dennis, 2012)(Provan, 2009). Haemoglobinopathy can be classified into five (5) main categories; i) Structural Haemoglobinopathies- Haemoglobin with altered amino acid sequences that result in deranged function or altered physical and chemical properties example Sickle Haemoglobin. ii) Thalassaemic Haemoglobin- defective biosynthesis of globin chains example β-Thalassemia.

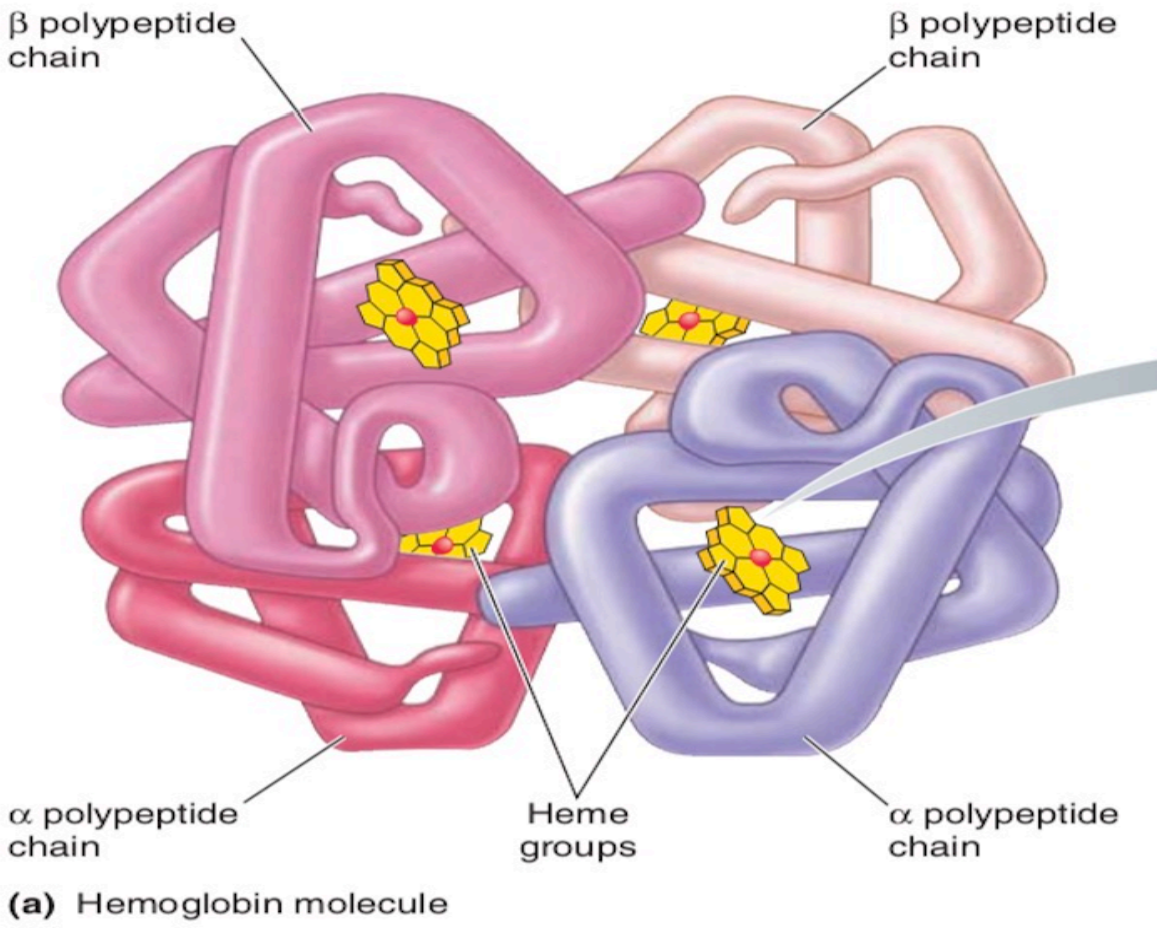

Figure 2. 1: Showing a normal Haemoglobin Molecule

iii) Thalassaemic Haemoglobin variants- structurally abnormal haemoglobin associated with co-inherited Thalassaemic phenotype. iv) Hereditary persistence of foetal Haemoglobin- persistence of high levels of Hb F even in adulthood. v) Acquired Haemoglobinopathies- example Carboxyhaemoglobin (Longo Dan, Fauci Anthony, Kasper Dennis, 2012). There are over 800 Haemoglobin (*Hb*) variants arising from substitutions, insertions or point mutations (*16*, n.d.). Sickle Cell Disease (*SCD*) is the commonest haemoglobinopathy known to mankind (Asare et al., 2018).

2.2.3 The Sickle Haemoglobin-Haemoglobin S

As already established, the Sickle Haemoglobin (*Hb S*) is a structural variant of the normal Haemoglobin (*Hb A*) which comes about as a result of a point mutation in the β-globin gene, a substitution of thymine for adenine, at the sixth codon of the β globin gene (Chromosome 11) (Asare et al., 2018). Due to this mutation, the sixth amino acid expressed in the β globin chain is valine instead of glutamic acid. This alteration in the amino acid sequence is the underlying pathology in SCD (Steinberg, 1998).

Over time, scientists have demonstrated DNA polymorphism or different haplotypes associated with the Sickle Haemoglobin. Four major sickle haplotypes of *Hb S* exist in Africa: "Senegal" (West Africa), "Benin" (Central West Africa), "Bantu" or "CAR" (Central African Republic) and "Cameroon" (Mehta & Hoffbrand, 2014)(Dorn-Beineke A, n.d.). The prevalence of SCD is high in Africa with seventy per cent (70%) of sufferers worldwide living in Africa (Makani et al., 2007). The sickle cell gene is also prevalent in the Middle East, some regions of India, the Caribbean and Brazil (Aygun & Odame, 2012). In Central and Western Africa, the homozygous state (*HbSS*) occurs in 3% of the population and the heterozygous form "sickle cell trait" (*HbAS*)

occurs in 25% of the population. Among black Americans in North, Middle and South America, the incidence of *HbSS* is 0.15% and 8% of *HbAS*. In Jamaica, 10% of the population have sickle cell trait. In recent data, 30,000 patients with sickle cell trait and 800 to 900 homozygous sickle cell patients are reported to be living in Germany(Dorn-Beineke A, n.d.).

Due to the Hb S mutation and its pathogenesis, heterozygotes are nearly resistant to malaria since infected red blood cells are more susceptible to sickling and intracellular parasites are lysed by sickling(Dorn-Beineke A, n.d.).

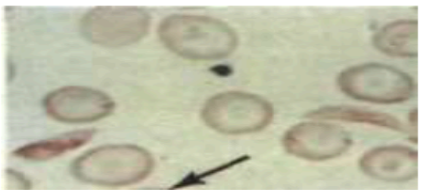

Figure 2. 3: Microscope slide showing Sickle Cell &Target Cell

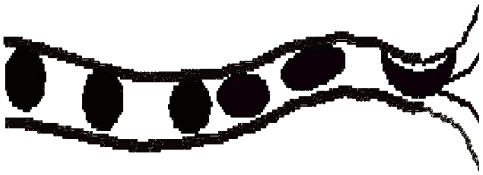

Figure 2. 2: Schematic Diagram of Sickle Cell in a small vessel

2.3.5 Haemoglobin S Polymerization and Sickling

The Sickle Haemoglobin (*HbS*) has abnormal physiochemical properties which makes it prone to polymerization with other Haemoglobin molecules under low oxygen tension. Ordinarily, Haemoglobin molecules exist as single, isolated units in RBCs whether oxygen is present or otherwise. Valine is hydrophobic and it replaces Glutamic acid which is hydrophilic in *Hb S.* During periods of low oxygen tension, the Valine residues form hydrophobic interactions with the Valine residues of other *Hb S* molecules also experiencing low oxygen tension (Odièvre, Verger, Silva-Pinto, & Elion, 2011)(Ferrone, 2004). Polymerization of *Hb S* forms the basis for most of the complications that arise from the disease (Vekilov, 2007). This abnormality leads to a defect

in the red blood cell (Erythrocyte) membrane. This causes the RBC to be more rigid and to be deformed especially under low oxygen tension, giving rise to the characteristic sickle-shaped RBC (Rees et al., 2010). The deformed RBCs have a life span of 10-20 days as compared to 120 days for normal RBCs and are easily destroyed (lysed) by the spleen. The deformed RBCs also occlude blood vessels especially small vessels (vaso-occlusion) impairing oxygen supply to end organs (Provan, 2009)(Hoyle, 2009).

The polymerization process of *Hb S* is in two steps, which requires time to be primed (delay time) before it occurs (Galkin & Vekilov, 2004). The first step is homogeneous nucleation, in which single fibres are randomly generated in the bulk of a supersaturated solution followed by their growth. The growing fibres serve as substrates for the nucleation of newer fibres. The second step is the branching of new fibres on top of the pre-existing one called heterogeneous nucleation. Together the process is called a double-nucleation mechanism (Galkin & Vekilov, 2004). The delay time is also an important feature of the polymerization process. Studies of the kinetics of HbS polymerization have shown a latent period (delay time) preceding the formation of the polymer strands and their subsequent explosive growth inside the RBC.

In the steady-state (basal conditions), the delay time is much longer than the time taken for the RBC to transit through the microcirculation (capillary transit time <1sec (Sherwood, 2010)), a region which has low oxygen tension. This phenomenon is vital because before the *Hb S* is primed to start polymerizing, it would have left the microcirculation, sickling in the venules instead of the capillaries (Galkin & Vekilov, 2004). If the delay time is shorter than the capillary transit time, polymerization and thus sickling would occur in the capillaries with likely vaso-occlusion. On the

other hand, if the delay time is more than the capillary transit time, sickling occurs in the venules which have a larger diameter compared to the capillaries, thus, there would be no vaso-occlusion (Galkin & Vekilov, 2004)(Carlo Brugnara, 2018)(C. Brugnara, 1995).

Seeing how crucial the delay time is in the pathophysiology of the disease, it is important to study factors and elements that affect or modify the delay time. Studies have shown that the delay time is inversely proportional to the 30^{th} to the 50^{th} power of *Hb S* concentration (Cao & Ferrone, 1996). Red Blood Cells with high Haemoglobin S concentrations will be most susceptible to accelerated *HbS* polymerization (Cao & Ferrone, 1996)(Ferrone & Rotter, 2004). Small changes in HbS concentration markedly affect Haemoglobin polymerization and cell sickling (C., 2003)(Mueller BU, n.d.). Dehydration of red blood cells containing sickle haemoglobin will, therefore, contribute to the pathophysiology of sickling because of the high dependence of polymerization on the concentration of HbS (C. Brugnara, 1995).

2.4 Cellular Dehydration in Sickle Cell Disease

Cell dehydration is a distinguishing characteristic of sickle cell disease and an important contributor to disease pathophysiology (C. Brugnara, 1995). The Red Blood Cell (RBC) in Sickle Cell Disease is more prone to dehydration compared to RBCs in controls *HbAA* (Bookchin & Lew, 2002). This makes dehydration common in SCD patients even when they are not in Crisis (Bookchin & Lew, 2002)(Provan, 2009). Cellular dehydration in the disease is due to **loss of intracellular potassium (K^+)** to the extracellular domain which is partially offset by an increase in cellular *Na*. The loss of *K* and the resultant cell dehydration is as a result of four (4) major mechanisms; Calcium (Ca^{2+}) -activated Potassium (K^+) channel (Gardos Pathway), Potassium-

Chloride (Cl) cotransport, Deoxygenation induced *Na* and *K* fluxes (Na-K pump) and Oxidative damage of the cell membrane and *K* loss(C. Brugnara, 1995)(De Franceschi L, D Bachir, Galacteros F, 1997)(C., 2003).

2.4.1 Calcium-activated Potassium Channel (Gardos Pathway)

Calcium ions (Ca^{2+}) play a very vital role in the body's physiology. They are needed in signal transduction pathways as secondary messengers, in neurotransmitter release from neurons, in muscle contraction and fertilization (Sherwood, 2010). Calcium ions are more abundant outside the cell (extracellular domain) compared to inside the cell. When intracellular free Ca^{2+} increases in RBCs, there is a large loss/movement of Potassium (K^{+}) extracellularly with an accompanying loss of Chloride (*Cl*) and water. This is due to the activation of Ca^{2+}-activated K^{+} channel (Gardos channel, named after György Gárdos who first discovered it in 1958).

All RBCs contain Gardos Channels and their function is in preventing colloid-osmotic lysis of the cell during transient complement activation on the Red Cell surface via the loss of K^{+} (Carlo Brugnara, 2018)(C. Brugnara, 1995). The Gardos channel of RBCs with the sickle haemoglobin, either alone or in conjunction with K-Cl cotransport, play a major role in RBC dehydration(C. Brugnara, 1995). Studies done in vitro have shown that dehydration of sickle erythrocytes depends on external Ca^{2+} and can be prevented by inhibitors of the Gardos channel such as charybdotoxin, nifedipine or clotrimazole(Mueller BU, n.d.)(C. Brugnara, 1995).

2.4.2 Potassium (K^+)-Chloride(Cl^-) Co-Transport (*KCC*)

The Potassium-Chloride Cotransporter (*KCC*) was first identified in RBCs of individuals with Haemoglobin C (Carlo Brugnara, 2018). Further studies of the cotransporter revealed an abundance of *KCCs* in the least dense, reticulocyte-rich fraction of normal RBCs (AA genotype individuals) and RBCs of sickle cells (Bookchin & Lew, 2002). The Potassium-Chloride Cotransporter (*KCC*) is not found only in RBCs but in a variety of cells in the human body (I. Bize & Dunham, 2017). It acts by mediating the electroneutral symport of K^+ and Cl^- ions across the plasma membrane outwardly (Rust et al., 2007). The outward movement of the ions is accompanied by water (Rust et al., 2007). Thus the *KCC* functions mainly in regulating cell volume and it's the main determinant of cell volume and density (I. Bize & Dunham, 2017).

The activity of KCCs in RBCs has been found to be dependent on key features of the cell; the age of the cell (hence its increased activity in reticulocytes and not normal matured RBCs),the presence of positively charged haemoglobin (Haemoglobin S and C) (Carlo Brugnara, 2018) and oxidative damage to the cell membrane. It is indeed noteworthy that the activation of the KCC is not a feature of all relatively positively charged haemoglobin but a feature of those with the positive charge at position six (6) and seven (7) of the beta globin chain (Carlo Brugnara, 2018)(Mueller BU, n.d.). This explains why KCC activity is increased in SCD (Mueller BU, n.d.).

The Potassium-Chloride Cotransporter is also influenced by several activators and inhibitors extrinsic to the cell. The Potassium-Chloride Cotransporter is also activated by cellular swelling, thiol oxidation, cellular magnesium depletion, slight cellular acidification (not less than pH of 7), protein kinase inhibitors, low Hb F levels and free radicals (William, 2015)(Carlo

Brugnara, 2018). On the other hand, KCC is inhibited by cell shrinkage, most bivalent cations, marked cellular acidification (pH < 6.5), cellular alkalinisation (pH>7.4), polyamines and protein phosphatase inhibitors (Carlo Brugnara, 2018)(William, 2015). Clinically, specific pharmacological inhibitors and magnesium modulation have been thought to be useful in mitigating the activity of KCCs however not much evidence supports their continuous use in recent times (Than, Soe, Palaniappan, Abas, & De Franceschi, 2014).

2.4.3 Deoxygenation induced Sodium (*Na*) and Potassium(*K*) fluxes and Sodium-Potassium Pump (*Na-K* Pump)

The contribution to the loss of *K* and accompanying water via this mechanism is small (C. Brugnara, 1995). There is an increased membrane permeability to *Na* and *K* during sickling, however, deoxygenation induced *Na* and *K* fluxes can be distinguished as a significant mechanism for *K* loss because of certain reasons (C. Brugnara, 1995). First of all, this mechanism is insensitive to inhibitors of other red cell transport system, secondly, it's independent of *Cl*- and membrane potential and lastly they have an inhibitory effect on di-isothiocyano-disulfonyl stilbene (DIDS) (a potent anion transporter inhibitor)(C. Brugnara, 1995). The increased Na^+ and K^+ permeability of sickling are associated with an increased entry of Ca^{2+}, which is also sensitive to DIDS and inhibitors of Ca^{2+} channels such as Nifedipine, which may be useful in clinical practice (C. Brugnara, 1995)(Bookchin & Lew, 2002). Activation of the Na-K pump, which has a 3 Na^+ out/ 2 K^+ in stoichiometry, may also lead to cell dehydration (C. Brugnara, 1995).

2.4.4 Oxidative damage of the Cell Membrane and Potassium Loss.

Oxidative damage to the sickle cell membrane is the result of:

1. Accelerated (auto)oxidation of *Hb S* (C. Brugnara, 1995)
2. Decompartmentalization of iron, with membrane deposition of denatured haemoglobin, ferritin, free haem and iron (C. Brugnara, 1995)
3. Abnormal membrane deposition of iron induces oxidative damage of protein thiols and lipids (C. Brugnara, 1995)

Oxidative damage is responsible for activation of K-Cl cotransport in an *in vitro* model of human thalassemia and a portion of the abnormal activity of K-Cl cotransport in sickle erythrocytes (based on the inhibitory effect of dithiothreitol, DTT) (C. Brugnara, 1995)(Carlo Brugnara, 2018). A possible therapeutic approach based on reducing membrane-associated iron with the use of the orally -absorbable iron chelator L1 (1,2-dimethyl-3- hydroxypyrid-4-one) is currently being considered for thalassemia and sickle cell disease(C. Brugnara, 1995).

2.5 Hyposthenuria in Sickle Cell Disease

The inability to concentrate urine (Hyposthenuria) is a known feature in SCD (Miller et al., 2010)(Pediatric & Clinic, 1968). This defect is seen in childhood and progressively gets worse with age (Pediatric & Clinic, 1968)(ITANO et al., 1956). Studies have shown some improvement in urine concentrating ability in some patients after blood transfusion (Pediatric & Clinic, 1968) and a genetic association with hyposthenuria in SCD (ITANO et al., 1956)(Pediatric & Clinic, 1968). Hyposthenuria is commoner in homozygous individuals and has been shown to exist in individuals with sickle cell trait (ITANO et al., 1956). McCrory et al in 1928 established that hyposthenuria in SCD was not due to a defect in Vasopressin (ITANO et al., 1956) however recent evidence postulates that the renal damage from intravascular sickling and the presence of sickle haemoglobin in the renal tubule cells may be the cause(ITANO et al., 1956).

2.6. Sickle Disease: Diagnosis, Management and Complications

Sickle Cell disease can be diagnosed in utero by DNA analysis of amniotic fluid cells (Kan & Dozy, 1978) but this is not routinely done. Diagnosis is commonly made after 6 months of life when the majority of Hb F (foetal Haemoglobin) needed in foetal life has been replaced by the infants Haemoglobin based on the infant's genetic library (Provan, 2009). The gold standard of diagnosis is by conducting an Hb Electrophoresis to identify the specific genotype of the patient (Hoyle, 2009)(Provan, 2009). There are several types of electrophoresis but the commonest used is the cellulose acetate electrophoresis. There have been significant improvements in the morbidity and mortality rates for children with SCD in high resource countries such as the United States due to factors such as early diagnosis through new-born screening programs, prophylactic therapy, comprehensive care programs including hydroxyurea therapy, and bone marrow transplant (Mulumba & Wilson, 2015).

SCD accompanied with various clinical manifestations due to three pathological processes: sickling of red blood cells, vaso-occlusion and susceptibility to infections. The disease is associated with acute exacerbations called crisis which can manifest as Vaso-occlusive crisis (VOC), Haemolytic crisis, Aplastic anaemia, Sequestrative crisis and Hyposplenism/Infection (L. W. Diggs, n.d.). The two most common acute exacerbations are Vaso-occlusive pain crisis, caused by physical and adhesive entrapment of red cells containing Haemoglobin S in the microcirculation, and acute chest syndrome, a lung injury syndrome (Gladwin & Vichinsky, 2008). Currently, the only accepted cure for the disease is haemopoietic-stem-cell transplantation (Psatha N, Papayanni PG, n.d.) which is not readily available in Sub-Saharan Africa. In Africa, management, therefore, is aimed at alleviating the symptoms, reducing the frequency of crisis and preventing complications of the disease making it a chronic disease. Common precipitants of crisis

are dehydration, infections, stress, hypoxia and hypothermia or hyperthermia (Edition, 2010). Patients are put on folic acid to replace folate stores, which are depleted due to the high RBC turn over (Provan, 2009) and hydroxyurea which raise the level of Hb F and reduces the incidence of painful episodes (Platt, 2008). Although management aims to prevent a crisis, crises are inevitable in most patients. Management protocol for crisis include IV fluid therapy to ensure adequate hydration, Pain relief usually with Opioids and NSAIDS, blood transfusion to improve the proportion of normal Haemoglobin in the blood compared to Hb S and Antibiotics & Antimalarial to treat any precipitating infection (Edition, 2010)(Provan, 2009).

Although *HbS* polymerization, Vaso-occlusion, and Haemolytic Anaemia are central to the pathophysiology of sickle cell disease, they precipitate a cascade of pathologic events. These, in turn. lead to a wide range of complications. These processes include vascular–endothelial dysfunction, functional nitric oxide deficiency, inflammation, oxidative stress and reperfusion injury, hypercoagulability, increased neutrophil adhesiveness, and platelet activation (Piel et al., 2017)(Piel et al., 2010).

SCD thus affects all organs of the body and complications can be seen in all organs, starting from the head right down to the toe. A common organ affected by the disease is the Kidneys, affecting 30-50% of adults with *SCA* (Sundaram et al., 2011). A broad spectrum of renal changes is observed in patients with sickle cell anaemia, and ideal therapeutic measures for the management of these alterations are still being studied (Feltran, n.d.)(Ataga & Orringer, 2000a). In the past, sickle cell nephropathy was believed to be confined to renal medullary changes due to the medullary vasculopathy associated with the disease. In recent times, studies have shown changes seen in the cortical function of *SCD* patients; an elevated renal blood flow, increased Glomerular Filtration Rate (GFR) and an increased Proximal Tubular activity have all been

observed (Jong, n.d.). By and large, affected patients have deficient urinary concentration and potassium excretion but an intact urinary diluting ability (Feltran, n.d.)(Ataga & Orringer, 2000a). Some, howbeit rare, may develop end-stage renal failure and would require Renal Replacement Therapy (RRT) (Feltran, n.d.). Despite this defect, the occurrence of an overt metabolic acidosis and hyperkalaemia is infrequent in *SCD* patients (Ataga & Orringer, 2000a). Hyposthenuria (inability to concentrate urine) can develop in *SCD* regardless of other evidence of renal impairment (ITANO et al., 1956). Hyposthenuria develops as a result of damage to the renal tubules, from impaired blood supply to the kidneys from vaso-occlusion and proteinuria due to an increased RBC breakdown. The loss of urine concentrating ability means that Sickle Cell patients produce urine with low Osmolality (ITANO et al., 1956)(Sherwood, n.d.)(Miller et al., 2010).

2.7 Osmolality and Osmolarity: Definitions, Clinical Relevance and Measurements

There are two types of osmotically active particles in the body; organic (amino acids, polyols, trimethylamines) and non-organic (electrolytes) (Boulard et al., 2003). Osmolality is the number of solute particles per 1Kg of water (independent of temperature) and Osmolarity is the number of solute particles per 1L of water (solution), temperature-dependent (Sherwood, 2010). These two measures are technically different but functionally the same. The clinical relevance of Osmolality is that the cell membrane is permeable to water, thus the Extracellular fluid (ECF) osmolality is approximately equal to that of the Intracellular fluid. Changes in the osmolality of the ECF would affect that of the ICF and vice versa to ensure homeostasis (Sherwood, 2010) (Hoyle, 2009). If the ECF is hypotonic (low Osmolality), more water would move inside the cell, causing it to swell and lead to lysing of the cell (the reverse occurring in hypertonicity (high osmolality)). Serum Osmolality is clinically useful in determining the hydration status of the individual, whereas urine osmolality looks more at the tubular function of the kidneys.

Physiologically, an increase in serum Osmolality triggers some protective mechanisms to balance out the increase. The first mechanism the body employs is the release of Vasopressin/Anti Diuretic hormone (ADH) from the posterior pituitary. Vasopressin acts on the collecting ducts of the kidneys causing an increase in its permeability to water and to a lesser extent sodium (Sherwood, 2010). The second mechanism is the polyol mediated aldose reductase enzyme activation in renal tubules (Koc University, Istanbul, n.d.). The overall effect of these mechanisms is to increase the amount of water reabsorbed by the kidneys and thus produce urine which is low in volume and high in concentration. This leads to more water being reabsorbed in the kidneys, producing more concentrated urine. Osmolality is measured using a device called an Osnometer, the physiological range is between 275-300 milli-osmoles per Kilogram water. Sodium (Na) is the most abundant electrolyte in the ECF and also the most osmotically active particle in the ECF because it has a high size to charge ratio on the electrochemical series compared to the other electrolytes found in the ECF (Sherwood, 2010). Movement of *Na* across most cell membranes is accompanied by a movement of water in the same direction (Eries, n.d.). Potassium (*K*) is more abundant intracellularly but has a lesser osmotic activity compared to *Na* (Sherwood, 2010). Thus, any factor that affects the plasma electrolytes especially Na would affect the Osmolality. Although it's known that *SCD* patients have renal tubular damage, not much is known about their plasma osmolality or how the management protocols affect their Plasma Osmolality.

2.8 Diet

Over the years due to urbanization, the dietary pattern of Ghanaians vary (Galbete et al., 2017). The contribution of macronutrients to daily energy intake varies between rural and urban dwellers in Ghana (Galbete et al., 2017). The Recommended Daily Allowance (RDA) of macronutrients for *SCD* patients differ from that of the general population although there is a

paucity of data concerning the need for macronutrients supplementation in *SCD* patients (Hyacinth, Gee, & Hibbert, 2011). Over the past 30years, research has shown that the (RDA) of macronutrients in the general population is inadequate for SCD patients (Hyacinth et al., 2011).

High dietary sodium has been associated with a raised blood pressure but not much is known on its effect in *SCD* patients (Brown et al., 2009). In a study carried out in St Jude's Hospital, Memphis U.S.A, children with *SCA* were shown to consume higher than recommended sodium-rich foods (such as chips, snack foods and processed foods) and a lower than recommended fluid intake (Ruth Williams-Hooker, 2013). Excessive dietary salt intake is known to increase serum Osmolality which triggers protective physiological mechanisms to offset this increase (Sherwood, 2010)(Spinelli et al., 1987). High dietary levels of Na affect Osmolality and invariably the individual's hydration status. In an adequately hydrated person, overall total body water (TBW) accounts for 55-65% of overall body weight (Sherwood, n.d.). Poor hydration increases the viscosity of blood which is much more exaggerated in SCD (Hoyle, 2009)(Provan, 2009). Hyposthenuria commonly found in SCD worsens the risk of dehydration in Sickle Cell patients (Ruth Williams-Hooker, 2013). When patients with SCD take in too little fluid and too much sodium, their RBC which is already prone to cellular dehydration (as a result of the disease pathophysiology) lose more water and become dehydrated and sickle.

Not much is known about the diet of SCD patient in the sub-region and whether the same picture seen in the United States is true for our geographical location. The research was however limited with regards to the renal function of the patients studied. In the Sub-region however, there is limited data about the dietary pattern of Sickle Cell patients. If the picture in Memphis is similar to our part of the world, dietary modifications must be inculcated in the management of SCD patients.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study site

The study was conducted at The Ghana Institute of Clinical Genetics (Sickle Cell Clinic), Korle-Bu Teaching Hospital (KBTH), Accra. KBTH is currently the third-largest Hospital in Africa and the leading national referral centre in Ghana. Established on 9th of October 1923, KBTH is the premier health facility in the country. It gained teaching hospital status in 1962 when the University of Ghana Medical School was established. It has a bed capacity of 2000 with 17 clinical and diagnostic units. It has an average daily attendance of 1,500 patients and about 250 patient admissions. Clinical and diagnostic departments of the hospital include Medicine, Child Health, Obstetrics and Gynaecology, Pathology, Laboratories, Radiology, Anaesthesia, Surgery, Polyclinic, Accident Centre and the Surgical/Medical Emergency as well as Pharmacy. It is currently the training institution for the College of Health Sciences of the University of Ghana. The Sickle Cell Clinic is under the supervision of the Ghana Institute of Clinical Genetics, KBTH/MOH. It has over 25,000 registered SCD patients. It is made up of an Outpatient unit and an Urgent-care unit. It has an average daily Out-Patient attendance of almost 50 patients and admits over 10,000 SCD patients every year. It's equipped with its Laboratory and has counsellors and support groups available for patients. The commonest sickle cell genotypes seen are SS and SC in a ratio of 2:1. The Clinic is run Monday to Friday 8 am to 5 pm, with a number Consultant Haematologists on hand every working day.

Healthy Controls for this study were obtained from voluntary blood donors coming to donate blood at the Southern Area Blood Centre (Korle-Bu Blood Bank). The Southern Area Blood

Centre, supervisors the provision of safe blood and related services in Eastern, Central, Volta, Greater Accra regions and parts of the Western region. Several remote sites are found in the Greater Accra region, one of which is the Korle-Bu Blood Bank. Situated in the Korle-Bu Teaching Hospital (Next to the Central Cafeteria, Opposite the National Radiotherapy Unit), the Blood Bank attends to 25-30 healthy blood donors majority of whom are voluntary donors.

3.2 Study design

This was a Case-Control, carried out between May 2018 to August 2018 to assess osmolality and factors that affect it in individuals with Sickle Cell Disease.

3.3 Study Population

Haemoglobin electrophoresis diagnosed Sickle Cell Patients of HbSS and HbSC genotypes were recruited between May 2018 to July 2018 at The Ghana Institute of Clinical Genetics (Sickle Cell Clinic), KBTH. Apparently healthy blood donors with HbAA genotype were also recruited between May 2018 to July 2018 from the Southern Area Blood Centre (Korle-Bu Blood Bank).

3.4 Inclusion Criteria

Subjects included in the study fulfilled the following criteria:

1. Steady-state HbSS and HbSC patients (SCD patient who has not had any sickle cell crisis in the past 4 weeks or blood transfusion in the past 4 months or antibiotic therapy in the past 3 weeks)
2. Age 18 and above attending The Sickle Cell Clinic
3. HbSS, HbSC and apparently Healthy Control-group (HbAA) with no comorbidities.

3.5 Exclusion Criteria

- Patients with a history of other comorbidities namely; chronic hypertension, diabetes mellitus, urinary tract infection and any renal disease.
- Smokers
- Patients who are unable to give informed consent or unwilling to comply with the requirements of the study
- Sickle Cell patient in Crisis
- Pregnant Women (Sickle-Cell and Non-Sickle Cell)

3.6 Sampling technique

Systematic Sampling technique was used in recruiting participants. A sample frame was obtained from The Sickle Cell Clinic Registry and people scheduled for blood donation for the day at The Blood Bank. Six (6) patients were recruited per day. Using a sampling interval k = total clinic or blood donor booking / 6, a total of 6 folders or scheduled blood donor were chosen each weekday by selecting every k^{th} folder or blood donor that met the eligibility criteria. The 1st patient was chosen by simple balloting from the clinic registry and every kth patient after him/her in a steady state or coming to donate blood was recruited into the study until the sample size was obtained. Cases were recruited based on a 2:1 ratio, with SS-genotype being more than SC.

3.7 Sample Size Calculation

A study by Miller et al. reported mean serum osmolality to be 286 ±6 mOsm/KgH_2O. Thus, it was expected that in this study the mean serum osmolality in the sickle cell patients (cases) would

be higher than the controls (assumed to be 283 mOsm/KgH_2O). Using a power of 80% and 95% confidence level, the minimum sample size was calculated using the formula:

Where

Z_β = value on the standard normal distribution at the power of 80% = 0.84

Z_α = value on the standard normal distribution at the 95% confidence level = 1.96

d = the mean difference between the two study groups = 3

σ = standard deviation=6

$$n = \frac{2\sigma^2(Z_\beta + Z_\alpha)^2}{d^2}$$

$$n = \frac{2*3^2(0.84+1.96)^2}{(286-283)^2}$$

$$n = \frac{2*3^2(0.84+1.96)^2}{(3)^2}$$

n=62.7=63

A total sample size of 180, comprising of 80 Sickle Cell (SS) clinic attendants, 40 Sickle Cell (SC) clinic attendants (ratio of SS to SC at the clinic being 2:1) and 60 Non-Sickle Cell individuals donating blood at The Southern Area Blood Centre were recruited for the study.

3.7 Methods and Materials

3.7.1 Interview

The study population were interviewed by means of a well-structured questionnaire (See Appendix 2) to find out about their:

- Demographics
- 24hr Dietary Recall of 3 separate days (one day being a weekend) with emphasis on High Sodium diet
- Sodium-rich Food Frequency Consumption
- Number of Blood Transfusions in the past 4 months
- Frequency of Crisis in the past 1 year

All information was corroborated with the patient's medical history where applicable. Using an Omron digital weighing scale and a standard metre rule, patient's weight and height were obtained and the BMI calculated using the BMI formula- Weight (in Kg) divided by Height (in Metres) squared. Patient's blood pressure and heart rate were measured with an Omron digital sphygmomanometer/ heart rate monitor and temperature determined with a VitalTemp infrared thermometer.

3.7.2 Blood Sampling and Analysis

Five millilitres (5mls) of venous blood was collected using the method outlined in the Standard Operating Procedure for performing venepuncture in the Southern Area Blood Bank (KBTH, 2010) for the purpose of the study. A rubber tourniquet was tied to the arm about 8cm above the elbow joint for less than a minute and the skin over the antecubital fossa (Brachial vein) disinfected with 70% alcohol swab. Venous blood sample was taken into two vacutainer tubes, labelled with a permanent marker for analysis, using a 19G hypodermic needle fixed on a 5ml syringe. One bottle contained Sodium (Na)EDTA and the other without. The blood in the EDTA tube was mixed in the bottle to prevent clotting by gently inverting the tube four times manually. Samples taken into the EDTA bottle were analysed using lab system Multiskan MS (manufactured

by Amisham Bioscience LTD, UK) to obtain a Full Blood Count. Samples taken in the tubes without EDTA were spun in a centrifuge at 2500rpm for 10 minutes immediately they were taken. Each Sera obtained was divided into two parts and put into two separate Eppendorf tubes. One part of the sera was kept at -20 ^{o}C before analysing for electrolytes, electrolyte levels (Na, Cl, K, Mg and Ca) were quantified using a Flame Atomic Absorption Spectrometer (Variant 240FS manufactured by VARIAN Australia Pty Ltd) and the Sherwood Flame Photometer (Model 420 by Sherwood Scientific Ltd). On the other part of the sera, Osmolality was determined using VAPRO Pressure Osmometer (Model 5600 manufactured by ELI Tech Group, Utah USA).

3.7.3 Measurement of Haematological profile

Full blood count analysis of samples in the Na EDTA tubes was done on the same day of collection using lab system Multiskan MS (manufactured by Amisham Bioscience LTD, UK), a three-part auto analyser able to run 19 parameters per sample including haemoglobin concentration, packed cell volume, red blood cell concentration, mean corpuscular haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, white blood cells and platelet parameters.

3.7.4 Procedures for estimation of serum Magnesium, Calcium, Potassium, Sodium, Urea and Creatinine levels

Determination of serum magnesium, calcium, urea and creatinine were done using a Flame Atomic Absorption Spectrometer (Variant 240FS manufactured by VARIAN Australia Pty Ltd). Serum sodium and potassium were analysed using Sherwood Flame Photometer (Model 420 by

Sherwood Scientific Ltd). All electrolyte measurements were done at the Chemical Pathology Lab of the Department of Chemical Pathology, University of Ghana.

Two (2) grams of serum sample was weighed into a 100ml class A beaker. Twelve (12ml) of concentrated HNO_3 was added to the weighed sample. This was done in a fume chamber. The beaker was then covered with a clean film and placed on a hot plate for a hot plate acid digestion. The sample was digested for 3 hours at a temperature of 45°C. After acid digestion, the sample was transferred into a 50ml measuring cylinder. The sample was then topped up to the 30ml mark with distilled water. The whole content was transferred into a test tube for the electrolyte analysis. Reference standards and blanks were also digested under the same conditions as the samples. These served as internal positive controls. Reference standards that were used are from Fluka Analytical, Sigma-Aldrich Chemie GmbH, a product of Switzerland.

The digested sample was then assayed for the presence of Mg, Ca, K, Na, Urea and Creatinine. The digestate was used directly for magnesium and calcium assessment using the Atomic Absorption Spectrometer in an acetylene-air flame. For potassium analysis, 5ml of the digestate was transferred into a 10ml measuring cylinder. Two (2ml) of lithium solution was added to the 5ml digestate and measurement taken. For sodium analysis, a 1:5 dilution of the digested sample was transferred in a 10ml measuring cylinder. Two (2ml) of lithium solution was added to the 1:5 diluted digestate in the measuring cylinder and measurement taken. Sodium and potassium were measured using the Sherwood Flame Photometer (Model 420). All electrolyte determinations were done in duplicates.

3.7.5 Procedure for estimation of serum osmolality

Before running the samples, the Vapro Pressure Osmometer was calibrated using 10µL samples at the 290, 1000 and 100 mmol/kg calibration set points. The Whatman #1 filter paper (Paper disc) was also cut (punched) to 1/8-inch diameter as very small sample volumes (2µL) would be used. The sample hold was changed from the default 10µL to a 2µL sample holder. The 2µL sample holder was aligned with the sample slide and then pushed down so it positions inside the sample slide. The instrument was allowed about a minute to reach temperature equilibration as ambient temperature can affect readings. A single disc paper was loaded into the center of the special sample holder, and using the 2µL Micro-pipettor serum sample was placed onto the centre of the paper disc ensuring that the pipette tip touched the disc. The sample chamber was closed and the measurement run. When the measurement was completed, the sample was retracted from the sample holder and the sample holder cleaned with lint-free tissue and a cotton-tipped applicator. The process was then repeated for all the samples taken

3.7.6 Mechanism of Action for the Instruments

The Flame Atomic Absorption Spectrometer and the Sherwood Flame Photometer, work based on a similar principle. This principle is that each ionic atom (electrolyte) when excited absorbs and emits a specific wavelength of light and the amount of light absorbed at that wave light can be quantified and its directly proportional to the amount of the element present in the mixture. The serum contains (in aqueous solution) the electrolytes to be determined. When put in the machine, the serum is vaporized into charged atoms by a flame (energy) producing a gaseous mixture made up of atoms of the various elements. Light of specific wavelengths (i.e. specific wavelengths of light of which the elements being determined are known to emit and absorb

(specific to each element)) is passed through the gaseous mixture. The light then passes through a monochromator (a device which picks up each wavelength of light) and the various wavelengths are separated and focused on a photodetector (a device that is able to quantify the amount of light at a particular wavelength focused on it). The amount of light and the different wavelengths detected by the photodetector is able to compute two things; the electrolytes in the mixture based on the wave lengths absorbed and the amount of the electrolyte in the mixture based on the amount of the light at the specific wavelength absorbed.

Osmolality is an expression of the total concentration of dissolved particles in a solution without regard for the particle size, density, configuration or electrical charge. The presence of the particles (solute) in the solution alters the cardinal properties (vapour pressure, freezing point and boiling point) of the solvent (substance with the ability to dissolve). Compared with the pure solvent (solvent without any dissolved particle), the vapour pressure and freezing point of a solution (solvent + solute) are lowered while its boiling point is increased, provided a single solvent is present in the solution. In single-solvent solutions, the relative changes in solution properties are linearly related to the number of particles added to the solvent. The measurement of Osmolality can thus be determined indirectly by comparing the properties in solution with the cardinal properties of the pure solvent. The first practical laboratory instruments developed for osmolality measurements were based solely upon the depression of the freezing point. The VAPRO Pressure Osmometer however uses a newer technology. This technology is based upon the measurement of Vapour Pressure depression using a thermocouple hygrometer. The vapour pressure method has significant advantage over the previous methods of measuring the depression of the freezing point or measuring the elevation of the boiling point in that it can be performed

without the need to change the physical state of the specimen. This makes this method devoid of measurement artefacts that can often occur when the specimen to be tested must be altered physically(GROUP, 2015).

3.7.3 Urine Sampling and Analysis

About 5mls of mid-stream urine was collected into a small plastic container. Urine was analysed using the urine r/e dipstick (MEDI-TEST Combi 10 SGL, Manufactured by Macherey-Nagel GmbH & Co, Germany) and urine Osmolality determined with the VAPRO Pressure Osmometer (Model 5600 manufactured by ELI Tech Group, Utah USA). Procedure and mechanism is the same as described for blood samples.

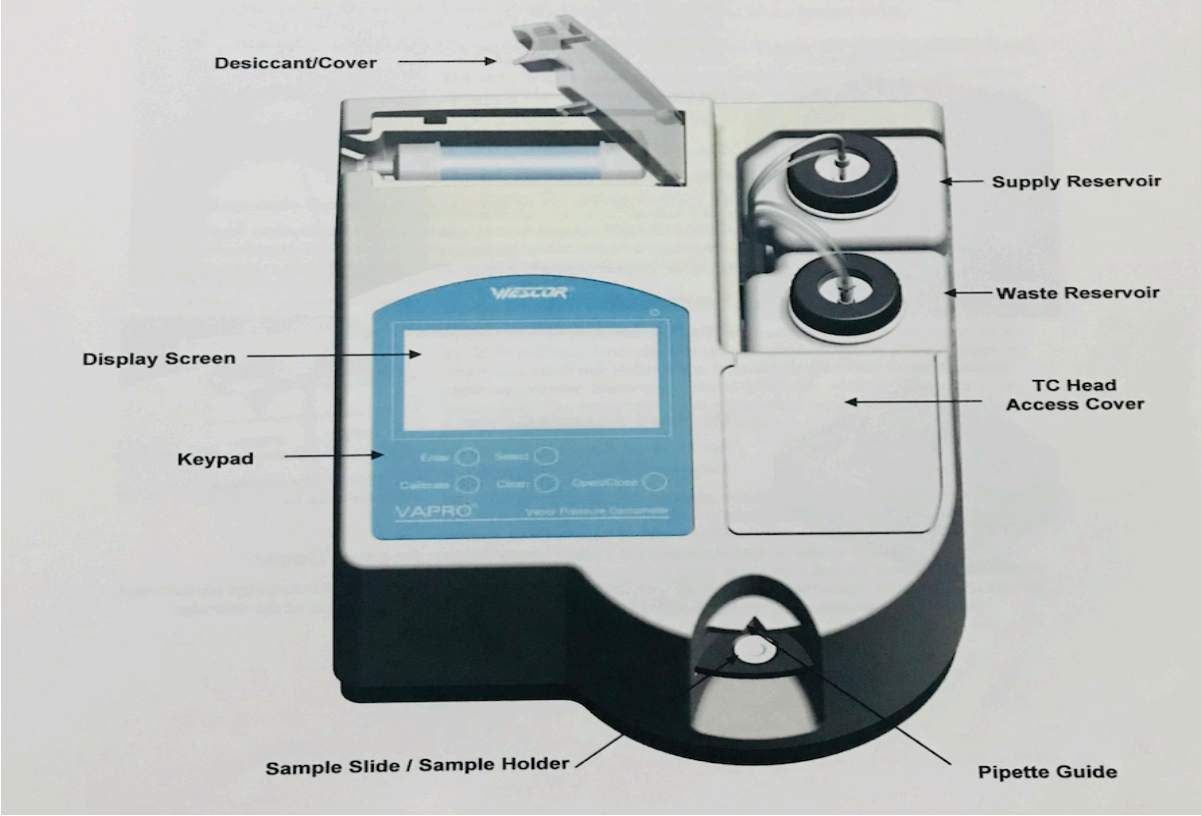

Figure 3. 1: Showing a labelled image of the VAPRO Pressure Osmometer (Source: VAPRO Pressure Osmometer user's manual)

Table 3. 1: Laboratory Reference Ranges

Investigation	Units	Reference Range
Sodium	mmol/l	135.0 - 150.0
Potassium	mmol/l	3.5 – 5.5
Chloride	mmol/l	96.0 – 115.0
Magnesium	mmol/l	0.73 – 1.06
Calcium	mmol/l	2.20 – 2.65
Urea	mmol/l	2.5 – 7.5
Creatinine	μmol/l	53.0 – 123.0
Osmolality	mmol/kg	279-300

3.8 Data Management and Analysis

To minimize missing data and ensure confidentiality, the following strategies were employed:

1. The data gathered was entered into the Department of Physiology Research Computer system with back-ups in the department.
2. Data was stored anonymously with each patient having a unique identifier.
3. No information was released, nor patient participation in the research acknowledged, to any party.
4. In order to maintain confidentiality, the principal investigators kept records in locked cabinets and results of tests coded to prevent association with patient names. Patient records were made available to the study staff only.

All data were exclusively handled by the Investigators with no access granted to any unauthorized person for any purposes. Data was password protected and securely kept on the department computer. Data was entered into an access spread sheet (Microsoft Company, USA) and analysed using SPSS 10.0 software.

The Shapiro-Wilk test was used to test for normality for data with continuous variables. Data with normal distribution was presented as mean ± standard deviation whereas those not normally distributed was presented as median (interquartile range). Categorical data were presented as frequencies (percentage). The differences between the three groups of subjects were analysed statistically using Analyses of variance (ANOVA) and differences between two groups analysed using Student T-test. Chi square goodness of fit was used to verify the agreement of the observed genotype frequencies with those of the expected. Correlation was used to determine two relationships; i) the association between the frequency of blood transfusion and Osmolality and ii) the association between the frequency of crisis and osmolality. Odds ratio [95%confidence interval (CI)] was calculated as an index of association between high sodium diet and osmolality. Statistical significance was defined as p-value <0.05.

3.9 Dissemination of Results

The outcome of the study was presented at a seminar in the Department of Physiology of the University of Ghana School of Biomedical and Allied Health Sciences as part of the Master of Philosophy programme in Physiology. The finished paper would be presented as thesis in partial fulfilment for the award of M.Phil. Degree in Physiology. Copies of the completed work would be made available at the Physiology Department of the University of Ghana School of Biomedical and Allied Health Sciences, The Balme Library and the School of Graduate Studies of the University of Ghana to be accessed by other interested researchers.

The outcome of the study was also presented at a seminar to the Haematology Unit and Diet Therapy Unit of the Korle-Bu Teaching Hospital and a copy of the report given to the Southern Area

Blood Centre. Some of the findings have been submitted for publication in peer-reviewed journals, and it's still being reviewed for possible publication.

3.10 Ethical Consideration

Ethical approval was sought from The Ethical and Protocol Review Committee of the College of Health Science, University of Ghana and the Korle-Bu Teaching Hospital (KBTH) (Number: CHS-Et/M.8-P2.13/201-2018), see Appendix 4. Informed consent and assent was sought and documented for all participants selected for the study, see Appendix 1. Token gifts such as pens and notebooks were given to all participants as appreciation after they had successfully cooperated with the field workers.

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

A total of 180 participants (120 SCD patients and 60 controls) were recruited for this study. The 120 SCD patients were made up of 80 HbSS and 40 HbSC.

This chapter is divided into **four (4) sections**. The first section describes the **socio-demographic** characteristics of the participants. Section II gives results of the **physical examination** findings of participants, whiles Section III presents data on the **electrolytes, urea & creatinine, full blood count and osmolality** of participants. The last section, Section IV describes the **dietary pattern** of participants looking particularly at their Sodium (*Na*) consumption.

Section I: Socio Demographic Characteristics

4.2 Comparative socio-demographic characteristics

In comparing the Case group (HbSS and HbSC) to the Control group (HbAA), it is noteworthy that there were no statistical differences *(p-value = 0.321)* in the average age HbSS-(27.14 ± 9.86)years, HbSC(28.41 ± 11.92)years and HbAA(31.04 ± 8.92)years . The sex distribution (male to female ratio) (HbSS 42:38, HbSC17:23, HbAA 38:22) revealed more males in the control group.

Section II: Physical Examination (Blood Pressure and Weight)

4.3 Physical Examination

Comparing the mean body weights of study participants using one way ANOVA, the results revealed a significant difference among the 3 groups (HbSS, HbSC and HbAA). Post-Hoc analysis showed that, sickle cell disease patients HbSS and HbSC had a significantly lower body weight (56.59±9.72 and 59.43±8.33)kg respectively compared to the Controls HbAA (74.70±16.58)kg (*p-value* <0.001) and the HbSS patients had a significantly lower body weight compared to HbSC patients (*p-value* <0.001). The SCD patients had a mean Body Mass Index (BMI) of (21.17 ± 2.98) kg/m^2. Comparing the systolic blood pressure of HbSS (109.85 ± 13.23)mmHg, HbSC (114.10 ± 13.25)mmHg and HbAA (124.57±13.24)mmHg using one way ANOVA, the results showed a significant difference among the 3 groups (*p-value* <0.001). Post-hoc analysis showed that HbSS and HbSC patients had a significantly lower systolic blood pressure compared to HbAA controls, there was however no difference between the systolic blood pressure of HbSS and HbSC patients. Comparing the diastolic blood pressure of HbSS (65.20 ± 10.58)mmHg, HbSC (72.68 ± 10.85)mmHg and HbAA (76.75±8.58)mmHg using one way ANOVA, the study revealed a significant difference among the 3 groups (*p-value* <0.001). Post-hoc analysis showed that HbSS patients had a lower diastolic pressure compared to HbSC patients and HbAA controls. HbSC patients also had a lower diastolic blood pressure compared to HbAA controls. These findings are highlighted in **Table 4.1**.

Table 4. 1: Physical Examination (Blood Pressure and Weight)

Variable	HbAA (n=60) Mean ± SD	HbSS (n=80) Mean ± SD	HbSC (n=40) Mean ± SD	p-value
Systolic Blood Pressure(mmHg)	124.57±13.24	109.85±13.23	114.10±13.25	<0.001*
Diastolic Blood Pressure(mmHg)	76.75±8.58	65.20±10.58	72.68±10.84	<0.001*
Weight (Kg)	74.70±16.58	56.59± 9.72	59.43±8.33	<0.001*

***Significant at p<0.05 n= number of participants, SD=standard deviation, HbSS= Haemoglobin SS patients, HbSC=Haemoglobin SC patients, HbAA=Healthy controls**

Section III Electrolytes, Urea & Creatinine, Full Blood Count, Serum & Urine Osmolality

4.4 A comparison of haematological parameters of study participants

Comparing the haemoglobin of HbSS(8.02± 1.50)g/dl, HbSC(10.45±1.80)g/dl and HbAA controls (14.38±1.92)g/dl using one way ANOVA, the results revealed there was a significant difference among the 3 groups (*p-value* <0.001). Post-Hoc analysis showed the HbSS had a lower haemoglobin compared to HbSC patients and HbAA controls and the HbSC also had a lower haemoglobin compared to HbAA controls. Comparing the platelet count, using one way ANOVA of HbSS(506.04±138.90)X10^9/L, HbSC(329.59±125.70)X10^9/L and HbAA controls (240.61±51.85)X10^9/L revealed a significant difference among the 3 groups (*p-value <0.001*). Post-hoc analysis showed that HbSS patients had a higher platelet count compared to HbSC and HbAA controls and the HbSC patients had a higher platelet count compared HbAA controls. Using ANOVA, the white cell count of HbSS (12.04±5.44)X10^9/L, HbSC(8.43±3.11) X10^9/L and HbAA controls(4.75±1.36)X10^9/L were observed to be significant among the 3 groups(*p-value <0.001*). Post-hoc analysis showed that HbSS patients had a higher white cell count compared to HbSC

and HbAA controls and the HbSC patients had a higher white cell count compared HbAA controls. These findings are highlighted in Table **4.2**.

Table 4.2: Haematological Parameters of The Study Participants

Variable	HbAA (n=60) Mean ± SD	HbSS (n=80) Mean ± SD	HbSC (n=40) Mean ± SD	p-value
Haemoglobin level (g/dL)	14.38±1.92	8.02±1.50	10.45±1.80	<0.001*
Platelet count (per L)	240.61±51.85	506.04±138.90	329.59±125.70	<0.001*
Number of WBC per mm^3	4.75±1.36	12.04±5.44	8.43±3.11	<0.001*

***Significant at p<0.05 n=number of participants, SD=Standard Deviation, HbSS=Haemoglobin SS patients, HbSC=Haemoglobin SC patients, HbAA=Healthy controls**

4.5 Comparison of serum osmolality of study participants

The mean serum osmolality, by one way ANOVA, was significantly different amongst HbSS (440.87±113.14)mmol/kg, HbSC (422.90±88.50)mmol/kg and HbAA (354.50±82.37) (p-value <0.001). The HbSS and HbSC patients had a higher mean serum osmolality compared to the HbAA controls. Post-hoc analysis however showed no significant difference between HbSS and HbSC patients (p =0.382). These findings can be seen in **Fig 4.1**.

4.6 Comparison of urine osmolality of study participants

Comparing the urine osmolality of the study participants, HbSS (452.28±109.41)mmol/kg, HbSC (498.28±87.26)mmol/kg and HbAA (681.23±276.82)mmol/kg, using one way ANOVA, the result revealed a significant difference between the 3 groups (*p-value* <0.001). However, post-hoc analysis showed no difference in the urine osmolality of HbSS and HbSC participants. These findings can be seen in **Fig 4.2**.

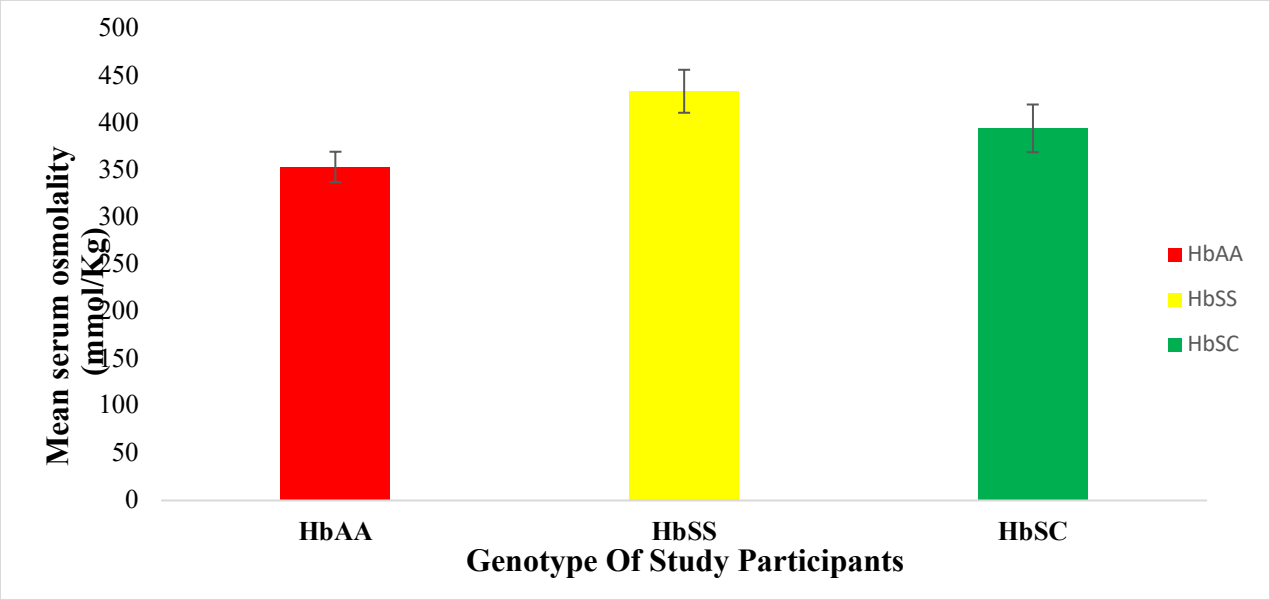

Figure 4. 1: Showing the Mean Serum Osmolality of Study Participants

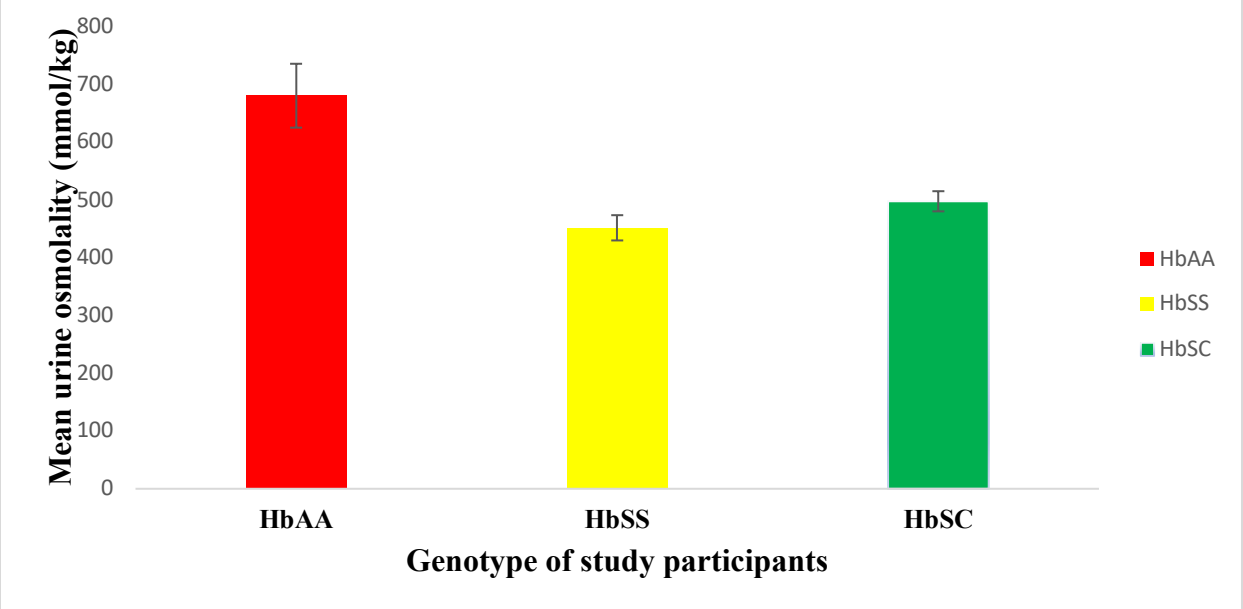

Figure 4. 2: Showing the Mean Urine Osmolality of Study Participant

4.7 A comparison of serum sodium, chloride, potassium, calcium, magnesium and calcium: magnesium ratio for HbAA HbSS and HbSC

Comparing the serum sodium for the HbSS (135.01±4.71)mmol/l, HbSC(136.10±3.91)mmol/l and HbAA(140.72±4.06)mmol/l of study participants by one way ANOVA, the result revealed there was a significant difference among the 3 groups (*p-value* <0.001). The HbSS and HbSC patients had a significantly lower serum sodium compared to HbAA controls. However, the post-hoc analysis showed no significant difference between the HbSS and HbSC study participants (*p-value =0.21*). Comparing the serum potassium for the HbSS (5.15±0.63)mmol/l, HbSC(4.81±0.60)mmol/l and HbAA(4.57±0.39)mmol/l by one way ANOVA, there was a significant difference among the 3 groups (*p-value* <0.001). The Post-hoc analysis showed that HbSS patients had a significantly higher serum potassium compared to HbSC (*p-value* =0.015) and HbAA (*p-value* =0.006) study participants and HbSC patients had a significantly higher serum potassium compared to HbAA controls (*p-value* <0.001). Analysing by one way ANOVA, the mean serum Magnesium (Mg) in HbSS (0.79±0.25) mmol/l, HbSC (0.82±0.21) mmol/l and HbAA (0.90 ± 0.11)mmol/l of study participants were significantly different (*p-value* < 0.001). However, the post-hoc analysis showed that there was no significant difference between the HbSS and HbSC study participants. Comparing the mean serum calcium (Ca) in HbSS (2.07±0.39)mmol/l, HbSC (2.17±0.36)mmol/l and HbAA (2.28±0.53) mmol/l using one way ANOVA there was a significant difference among the 3 groups (*p-value* =0.01). However, the post-hoc analysis showed no significant difference between the HbSS and HbSC study participants. Serum Chloride *(Cl)* levels evaluated using one way ANOVA between HbSS (102.57±12.11)mmol/l, HbSC (103.56±4.16)mmol/l, HbAA (100.58±3.60)mmol/l showed no significant difference between the 3 groups (*p-value =0.62*).When calcium to magnesium ratio

was computed and compared using one way ANOVA with post-hoc analysis, it was observed that HbSS (2.79±0.71) and HbSC (2.82±0.76) patients had significantly higher Calcium to Magnesium ratio compared to the Control-group (2.54 ± 0.89) (*p-value* 0.031). There was no significant difference between the calcium to magnesium ratio of HbSS and HbSC patients (*p- value* =0.10). These findings are highlighted in **Table 4.3**.

Table 4. 3: Serum sodium, potassium, magnesium, calcium, chloride and calcium: magnesium ratio of the study participants

Variable (mmol/l)	HbAA (n=60) Mean ± SD	HbSS (n=80) Mean ± SD	HbSC (n=40) Mean ± SD	p-value
Sodium	140.72±4.06	135.01±4.71	136.10±3.91	<0.001*
Potassium	4.57±0.39	5.15±0.63	4.81±0.60	<0.001*
Magnesium	0.90±0.11	0.79±0.39	0.82±0.21	<0.001*
Calcium	2.28±0.53	2.07±0.39	2.17±0.36	<0.001*
Chloride	100.58±3.60	102.57±12.11	103.56±4.16	0.214
Calcium : Magnesium	2.54±0.89	2.79 ± 0.71	2.82 ± 0.76	0.031

***Significant at p<0.05 n=number of participants, SD=Standard Deviation, HbSS=Haemoglobin SS patients, HbSC=Haemoglobin SC patients, HbAA=Healthy controls**

4.8 A comparison of serum urea, creatinine, urine pH and specific gravity

Comparing the mean serum urea for the HbSS (2.61±0.95)mmol/l, HbSC (3.27±1.22) and HbAA (3.75±1.04) of study participants by one way ANOVA, revealed there was a significant difference among the 3 groups *(p-value* <0.001*)*. The post-hoc analysis showed that HbSS patients had a significantly lower urea compared to HbSC (*p-value* =0.012) and HbAA *(p-value* <0.001*)* study participants. However, there was no significant difference between HbSC and HbAA study

participants (*p-value* =0.115). Comparing the mean serum creatinine for the HbSS (47.78±18.11)µmol/l, HbSC (69.33±21.83)µmol/l and HbAA (100.26±15.26)µmol/l by one way ANOVA, the result showed that there was a significant difference between the 3 groups (*p-value* <0.001). The post-hoc analysis showed that HbSS patients had a significantly lower serum creatinine compared to HbSC (*p-value* <0.001) and HbAA (*p-value* <0.001) study participants. Similarly, HbSC patients had a significantly lower serum creatinine compared to HbAA controls (*p-value* <0.001). Analysing by one way ANOVA, urine pH of HbSS patients (6.05±0.42), HbSC patients (6.17±0.53) and HbAA controls (6.46±1.09) were found to be significant *(p-value* =0.006*)*. However the post-hoc analysis showed that the significance was only between HbSS and HbAA study participants. Using one way ANOVA, there was no significant difference in the Specific Gravity between HbSS (1.02±0.01), HbSC (1.02±0.02) and HbAA (1.02±0.01). These findings are highlighted in **Table 4.4**.

Table 4. 4: Biological Data of Tubular Function in the Study Participants

Variable	HbAA (n=60) Mean ± SD	HbSS (n=80) Mean ± SD	HbSC (n=40) Mean ± SD	p-value
Urine specific gravity	1.02±0.01	1.02±0.01	1.02±0.02	0.106
Urine pH	6.46±1.09	6.05±0.42	6.17±0.53	0.006*
Creatinine(µmol/l)	100.26±15.26	47.78±18.11	69.33±21.83	<0.001*
Urea(mmol/l)	3.75±1.04	2.61±0.95	3.27±1.22	<0.001*

***Significant at p<0.05. n=number of participants, SD=Standard Deviation, HbSS=Haemoglobin SS patients, HbSC=Haemoglobin SC patients, HbAA=Healthy controls**

Section IV Comparing the dietary pattern of study participants

4.9 Comparing the 24hr dietary recall and sodium-rich dietary consumption of study participants

The average daily intake of dietary calcium amongst study participants was significantly different using ANOVA (*p-value =0.007*). Post-Hoc analysis showed that the dietary calcium of HbSS patients (189.57±71.41)mg and HbSC patients (198.10±69.03)mg was significantly lower compared to HbAA controls (132.88±71.31)mg. However, there was no difference in the dietary calcium of HbSS and HbSC patients (*p-value =0.694*). Comparing by one way ANOVA, there was no statistical difference amongst the 3 groups in the average daily dietary Sodium, Potassium, Magnesium, Zinc and Phosphorous (*p-values* shown on **Table 4.5**). Comparing by one way ANOVA, the average daily intake of Macronutrients (Carbohydrate, Protein and Fat) between the 3 groups was not significantly different (*p-values* shown on **Table 4.5**).

Analysing data from the Food Frequency Table, majority (> 50%) of study participants (HbSS, HbSC AND HbAA) did not consume most sodium-rich foods (salted meals) very often (**Fig 4.3**). The only salt-rich food assessed that was consumed often was stock cubes (**Fig 4.3**). Majority (>60%) of study participants (HbSS, HbSC AND HbAA) obtained their food from Food Vendors (**Fig 4.4**).

Table 4. 5: Daily Dietary Intake of Macro and Micro Elements

Variable	HbAA (n=60) Mean ± SD	HbSS (n=80) Mean ± SD	HbSC (n=40) Mean ± SD	p-value
Dietary Sodium (mg)	1917.40±504.44	1990.86±538.95	1882.69±521.81	0.812
Dietary Potassium (mg)	1321.69±306.95	1343.58±333.85	1348.68±395.30	0.981
Dietary Calcium (mg)	132.88±71.31	189.57±71.41	198.10±69.03	0.007*
Dietary Magnesium (mg)	184.53±86.50	180.09±74.58	185.36±87.51	0.942
Dietary Zinc (mg)	4.47±2.16	7.54±2.51	5.13±3.14	0.562
Dietary Phosphorous (mg)	100.26±11.26	47.78±10.11	69.33±11.83	0.627
Dietary Carbohydrate (g)	161.64±73.02	172.77±71.55	176.25±72.28	0.672
Dietary Protein (g)	26.09±12.11	31.42±11.20	31.84±12.90	0.126
Dietary Fat (g)	27.66±8.84	33.67±9.22	33.07±9.78	0.311
Daily Water (g)	552.82±134.66	612.17±128.84	603.80±111.96	0.420

***Significant at p<0.05 n=number of participants, SD=Standard Deviation, HbSS=Haemoglobin SS patients, HbSC=Haemoglobin SC patients, HbAA=Healthy controls**

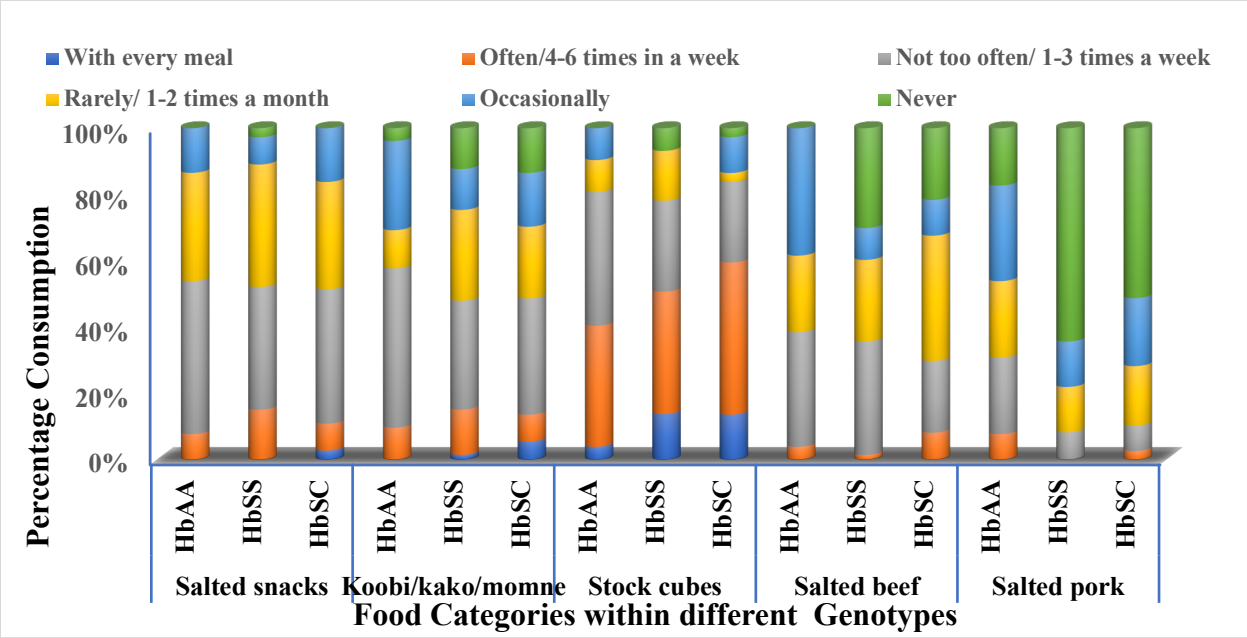

Figure 4. 3: Frequency of Consumption of Sodium-rich Diet

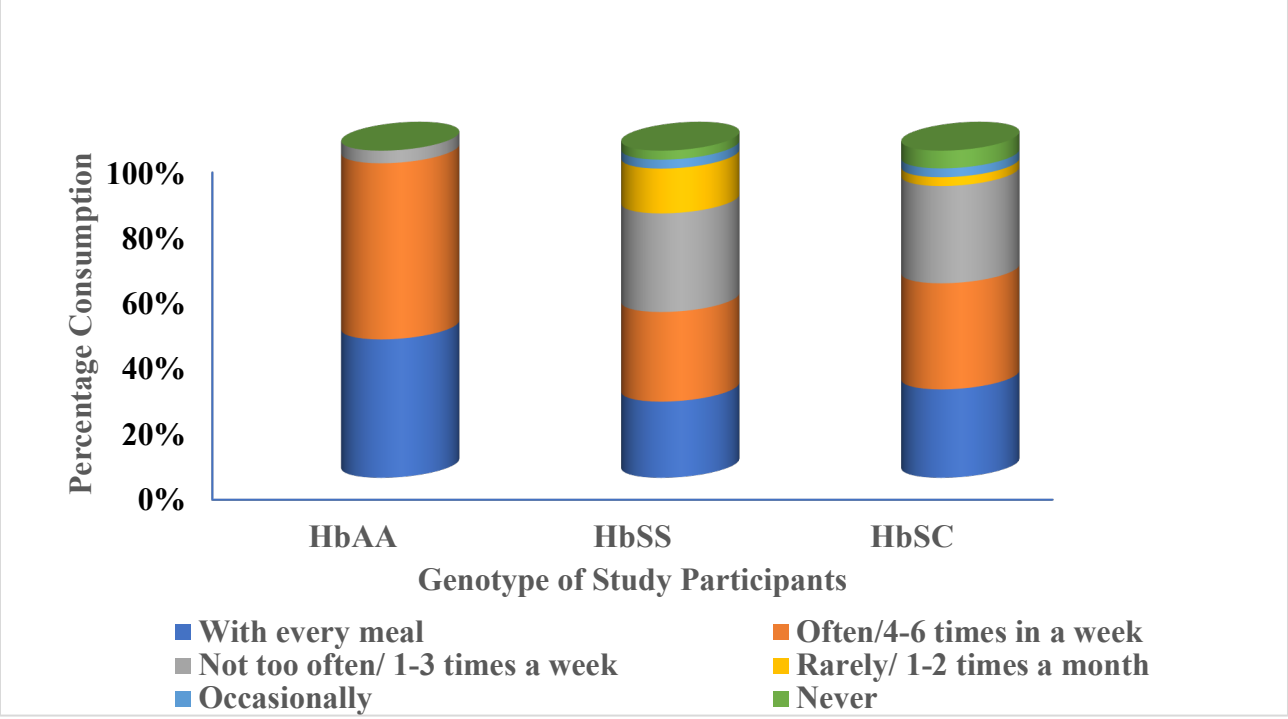

Figure 4. 4: Frequency of Patronizing Food Vendors

4.10 Correlations between Serum Osmolality and Dietary Sodium, Potassium, Water and Protein of sickle cell patients

There was a weak negative correlation between serum Osmolality and dietary Sodium (*r= -0.192*), between serum Osmolality and dietary Potassium (*r= -0.052*), between serum Osmolality and daily water intake (*r= -0.025*) and between serum Osmolality and dietary protein (*r= -0.098*) in SCD patients. These findings are graphically represented on **Fig 4.5**, **Fig 4.6**, **Fig 4.7** and **Fig 4.8** respectively.

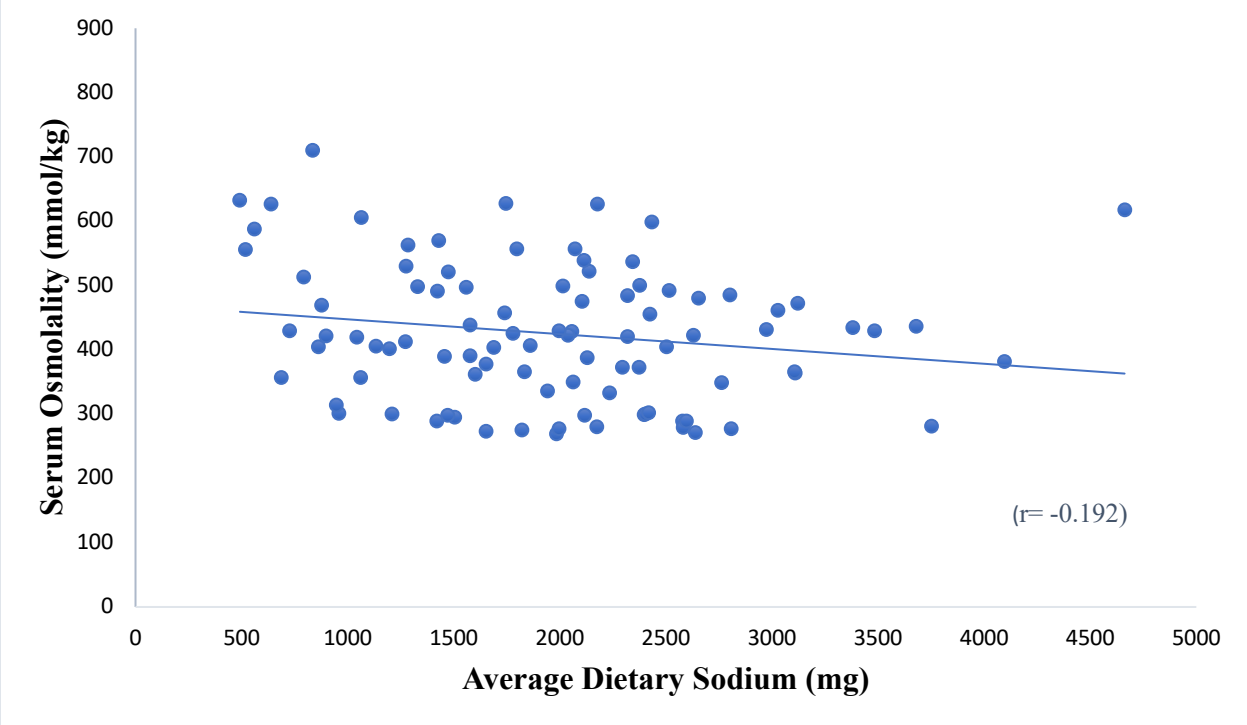

Figure 4. 5: Correlation Between Serum Osmolality and Dietary Sodium of Sickle Cell Patients

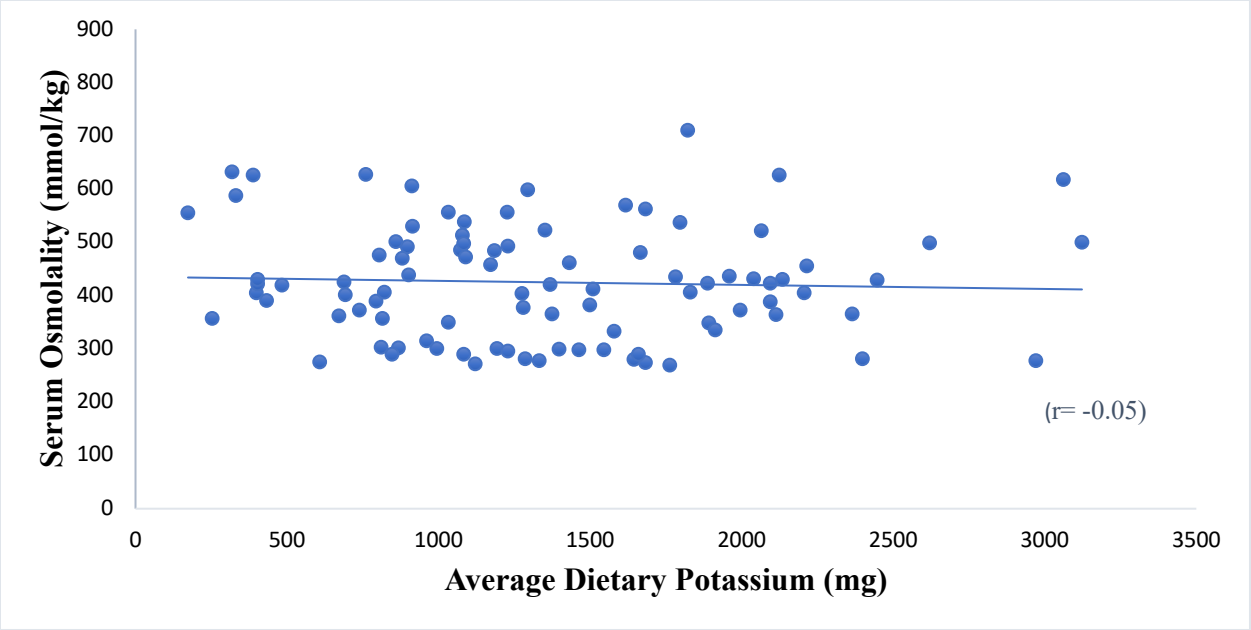

Figure 4. 6: Correlation Between Serum Osmolality and Dietary Potassium of Sickle Cell Patients

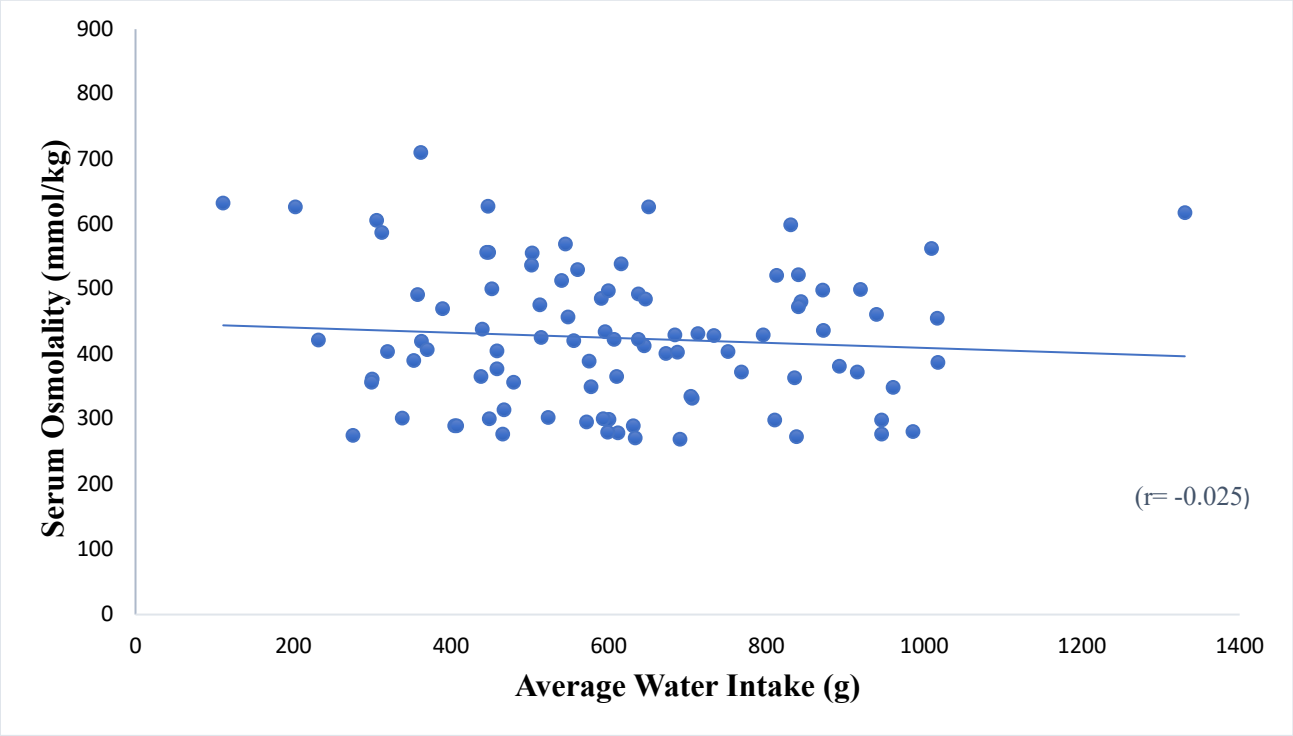

Figure 4. 7: Correlation between Serum Osmolality and Average Water Intake of Sickle Cell Patients

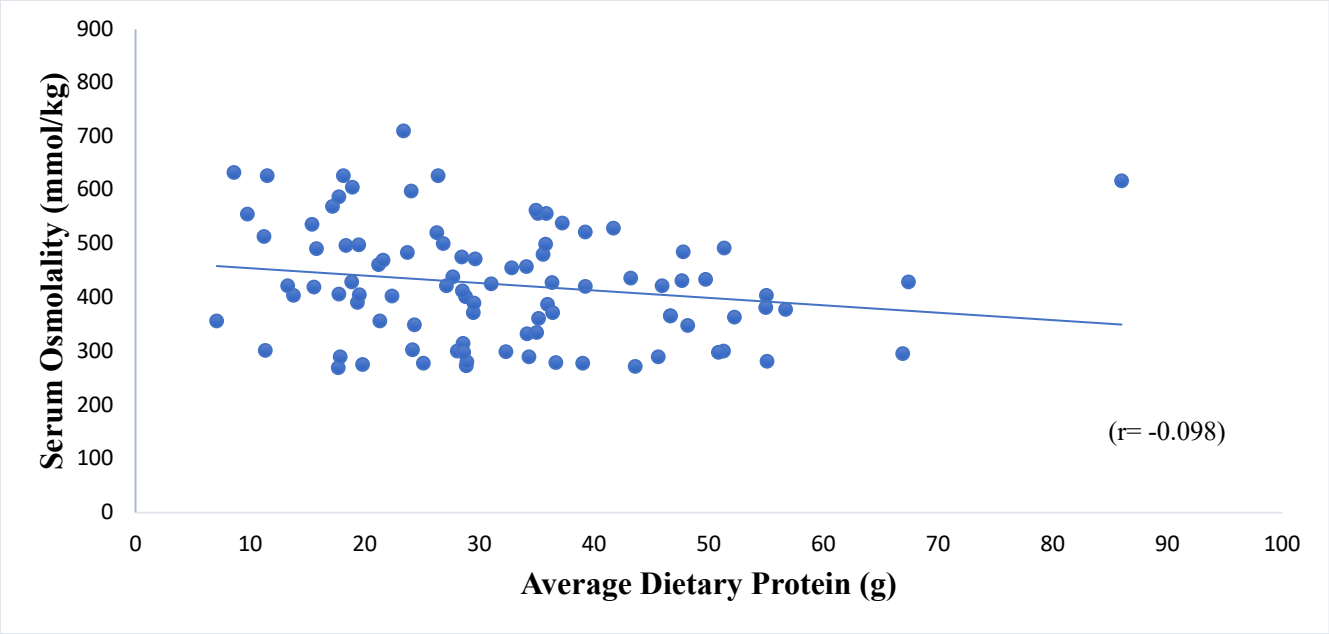

Figure 4. 8: Correlation Between Serum Osmolality and Dietary Protein of Sickle Cell Patients

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

Sickle cell disease (SCD) is an autosomal recessive inherited haemoglobinopathy with well-known complications found in various populations worldwide (Kwaku Ohene-Frempong et al., 2008). The disease mainly affects black populations worldwide with a prevalence of 1.9% of all births in Ghana (Asare et al., 2018). Red cell dehydration is an established feature of the disease (C. Brugnara, 1995). This study was a case-control survey carried out to determine the baseline serum & urine osmolality, serum electrolytes, blood urea &creatinine of steady-state sickle cell clients at the Sickle Cell Clinic and the effect of high sodium diet on osmolality. This chapter scientifically considers five (5) key things;

1. The demographic characteristics of the sample population,
2. The physical examination of the sample population,
3. The differences in the full blood count and serum & urine osmolality amongst study participants, the role of serum electrolytes in osmolality and renal tubular function amongst the study participants
4. The dietary pattern (Sodium consumption) of Study participants
5. Correlation and Associations between Serum Osmolality and Factors investigated.

5.2 Socio-Demographic Characteristics of the Study Population.

In the study, the average age of HbSS, HbSC and HbAA were comparable with no significant difference among the 3 groups. The sex distribution (male to female ratio) of all the

study participants was 1:1 in the study. This is expected given the age range of the study (18-70yrs) and the fact that the cases and controls were age-sex matched.

5.3 Physical examination of the study participants

In this study, Sickle Cell patients on the average had a lower body weight compared with the Control-group. Sickle Cell Disease patients although having a lower body weight, have a normal BMI. Historically, children with sickle cell disease are known to be underweight and this is due to the high basal metabolic rate observed in SCD patients and the study corroborated this known fact (Chawla et al., 2013). However, recent studies carried out in New England states of the United States of America have shown a rather increased prevalence of obesity in SCD children (Chawla et al., 2013).The prevalence of overweight and obesity in the general population has increased exponentially over time with increasing urbanization and wealth. Limited studies done in adults with SCD have also shown an increasing incidence of obesity amongst SCD patients in line with the trend amongst the general population (Farooqui Marwah W, Hussain N, 2014). Zivot et al in 2017, tried but failed to demonstrate an association between the extremes of BMI and frequency of hospitalization for VOC (Zivot, Apollonsky, Gracely, & Raybagkar, 2017).

In this study also, the HbSS patients had a significantly lower body weight compared to HbSC patients and this finding is in line with two other studies (Chawla et al., 2013)(Zivot et al., 2017). The HbSS patients have a severe form of the disease and thus have worse complications from the disease and this fact can explain the weight differences observed.

In this study, SCD patients had a significantly lower systolic and diastolic blood pressure compared to the Control-group. There was no significant difference in the blood pressure between HbSS and HbSC patients. It has been shown in previous studies that HbSS and HbSC patients have lower blood pressures compared to the general population (Pegelow et al., 1997). Sickle Cell

Disease patients with hypertension have an increased risk of developing a stroke and other complications (Pegelow et al., 1997).

5.4 Full blood count, serum & urine osmolality, role of serum electrolytes on serum osmolality and renal tubular function.

5.4.1 Haemoglobin, Platelet Count and White Cell Count

In this study, HbSS patients had a lower haemoglobin level compared to HbSC and HbAA individuals as expected. The HbSC patients also had a lower haemoglobin level compared to HbAA controls. The study also showed that HbSS patients and HbSC patients had a higher platelet count and a higher white cell count compared to HbAA controls. The HbSS patient who have the severest form of the disease also had a higher platelet count and white cell count compared to HbSC patients These findings are in line with other studies carried out on the haematological parameters of SCD patients (Charles Antwi-Boasiako et al., 2018)(Omoti, 2005).

There are significant differences in the haematological parameters of Sickle Cell Disease patients and that of normal individuals (Hb AA). Sickle Cell patients however adapt to their baseline haematological values and remain apparently healthy (Steady-State). Some of the "Steady-State" haematological values such as the Haemoglobin (HB) concentration, the White Blood Cell count (WBC) and the Platelet (PLT) count have been used as a predictive tool in the disease severity as well as in the management of SCD patients (Okpala, 2006)(Emmanuelchide, Charle, & Uchenna, 2011).

One of the characteristic features in the pathophysiology of SCD is a chronic haemolytic anaemia, painful crises, acute chest syndrome, stroke and susceptibility to bacterial infection (Kaur

et al., 2013). Chronic haemolysis, shortened red cell survival (10-20days), as well as low erythropoietin response associated with SCD (Charles Antwi-Boasiako et al., 2018) can explain the reduced Hb levels seen in SCD patients. Lower steady state Hb and a high white cell count is associated with higher risk of stroke (silent infarcts) and the recurrence of stroke(K Ohene-Frempong et al., 1998). Sickle cell patients with higher Hb values are reported to have higher rates of severe pain (Platt et al., 1991) this was not thoroughly investigated in this study. Red cell transfusions beyond the steady state Hb may increase blood viscosity (Kaul, Fabry, Windisch, Baez, & Nagel, 1983) with attendant consequences such as worsening of Vaso-occlusion and osteonecrosis.

Although SCD is essentially a disease affecting haemoglobin in the Red Blood Cells (erythrocytes), SCD patients have been shown to have high White Blood cell (leucocyte) and platelet (thrombocyte) counts (Charles Antwi-Boasiako et al., 2018). A study carried out in 2009 by Okpala et al. demonstrated that vascular lumen obstruction seen in SCD is not only as a result of the polymerization of Hb S but as a result of a more complex interaction between Red Blood Cells, White Blood Cells, Platelets, Plasma proteins and the vessel wall (Okpala, 2004). The severity of SCD increases with Leukocyte count and this explains the differences between HbSS and HbSC white cell counts observed in the study. Leukocytes contribute to SCD by adhering to blood vessel walls and obstructing the lumen, aggregating with other blood cells with more effective blockage of the lumen, stimulating the vascular endothelium to increase its expression of ligands for adhesion molecules on blood cells, and causing tissue damage and inflammatory reaction which predispose to Vaso-occlusion (Okpala, 2004). Although SCD patients have an increase in the White cell count, the function of the white cells are impaired. With an impaired ability of leukocytes to kill microbes. SCD patients are therefore more prone to infections, which

precipitate sickle cell crisis (Omoti, 2005). Similarly, targeted blockade or reduced synthesis of specific leukocyte adhesion molecules and their ligands might confer clinical benefit in SCD (Okpala, 2004)(Okpala, 2006).

Previous studies have shown an increase in prothrombin fragment 1.2, thrombin-antithrombin complexes, plasma fibrinogen products, D-dimer and decreased factor V (De Franceschi, Cappellini, & Olivieri, 2011). This culminates into an enhanced generation of thrombin and a chronic thrombophilic state in SCD patients, thus SCD patients are known to have a hypercoagulable state (De Franceschi et al., 2011). Also observed from studies in SCD patients is an increase in the production of Thromboxane-A2 and Prostaglandin metabolites which are associated with a decrease in Platelet Thrombospondin-1 levels, suggesting a chronic activation of platelets (De Franceschi et al., 2011)(Provan, 2009).

5.4.2 Serum and Urine Osmolality

The serum osmolality was observed to be significantly high in HbSS patients compared to HbSC and HbAA individuals in this study. The study also showed that HbSC patients had a higher serum osmolality compared to HbAA controls. However not much is known in literature about the serum osmolality of SCD patients. Osmolality simple measures the amount or number of solute particles per 1Kg of water (independent of temperature) (Sherwood, 2010). In serum, the solute particles (osmotically active particles) are mainly the electrolytes and proteins (Boulard et al., 2003). The key determinants of osmolality in serum are the electrolytes (Sherwood, 2010). An increase in the serum electrolytes or decrease in water in the serum would cause an increase in serum osmolality. Sodium and potassium are the most abundant electrolytes in the body and play

a key role in the determination of serum osmolality (Sherwood, 2010) although all other electrolytes in the serum do contribute to osmolality. The Vapro osmometer, determines serum osmolality taking into account all osmotically active particles in the serum. Cell dehydration is a distinguishing characteristic of sickle cell disease and an important contributor to disease pathophysiology (C. Brugnara, 1995). Cell dehydration occurs as a result of the abnormal activity of the Sodium-Potassium ATPase pump, the Gardos Channels and the Potassium-Chloride Cotransporter(KCC) in SCD patients (Rust et al., 2007)(C. Brugnara, 1995)(Carlo Brugnara, 2018). Sickle Cell patients are known to have a significantly high serum potassium and chloride (Nduka, Kazem, & Saleh, 1995)(Silva Junior et al., 2014) and a significantly low serum sodium (JR, n.d.). Osmolality is measured relative to the amount of water in a solution. Although serum sodium is low, the extent of cellular dehydration is more and this explains the high serum osmolality observed in SCD.

In this study, a lower urine osmolality was observed in HbSS and HbSC patients compared to the HbAA controls, which is in line with a similar study (Silva Junior et al., 2014). SCD patients are known to have Hyposthenuria (urine with low Specific Gravity (SG) from impaired urine concentrating ability) albeit reversal which is seen in childhood (ITANO et al., 1956) and this can explain the low urine osmolality observed in SCD patients. Renal manifestations of SCD vary from renal ischaemia to microinfarcts, to renal papillary necrosis to renal tubular abnormalities (Alhwiesh, 2015). Sickle Cell nephropathy, affects the disease prognosis and the overall quality of life of the patients (Pham & Pham, 2018). Generally, a dysfunction of the proximal tubules would manifest as an impaired ability to concentrate urine (hyposthenuria) where as a distal tubule dysfunction would manifest in an impaired potassium excretion (Alhwiesh, 2015). Low urine

osmolality is a feature of proximal tubular dysfunction and thus an assessment of SCD patients tubular function is imperative in studies of urine osmolality (ITANO et al., 1956).

5.4.3 The role of serum sodium, potassium and chloride in serum osmolality

In this study, HbSS and HbSC patients had lower serum sodium compared to HbAA controls, however there was no difference in the serum sodium of HbSS and HbSC patients. The study also showed that HbSS patients had a higher serum potassium compared to HbSC and HbAA controls. The HbSC patients also had a higher serum potassium compared to HbAA controls. In the study, however, there was no difference in the serum chloride levels among the 3 groups. These findings are in line with previous studies (Damanhouri et al., 2015)(Ibe et al., 2009)(Pandey et al., 2012).

Studies have shown several variations in biochemical makers in SCD patients, which can be used as a tool in monitoring the disease severity (Nduka et al., 1995)(Silva Junior et al., 2014). Serum electrolyte imbalances, including sodium, potassium and chloride have been described in relation with sickling and increased dehydration in sickle cell patients in eastern Saudi Arabia, India and some few African countries (Nduka et al., 1995)(Sundaram et al., 2011)(Silva Junior et al., 2014)(Ibe et al., 2009). Cellular dehydration and hypoxia is a prominent feature of SCD which results from the loss of potassium from the cell into the extracellular fluid and thus an increase in serum potassium (*K*) (C. Brugnara, 1995). Implicated in cellular dehydration is the abnormal activity of the Sodium-Potassium ATPase pump, the Gardos Channels and the Potassium-Chloride Cotransporter(KCC) in SCD patients (Rust et al., 2007)(C. Brugnara, 1995)(Carlo Brugnara, 2018). Cellular dehydration is a precursor for sustained and rapid haemoglobin S polymerization which leads to vaso-occlusion and associated painful episodes. In addition, SCD patients have

impaired renal excretion of potassium (*K*) although overt hyperkalaemia is not seen in them (Feltran, n.d.)(Ataga & Orringer, 2000a) contributing to the high levels of serum potassium. The Gardos channel implicated in the efflux of potassium has been shown to be very active in HbSS compared to HbSC (C. Brugnara, 1995)(Vikas G, Ajay KS, 2012) and this explains the differences seen between the two genotypes.

The low sodium levels can partly be explained by loss of some body fluids and electrolytes as part of the disease process, the inflammation from the recurrent hypoxia, as well as the underlying chronic nature of the condition (Steinberg, 1998)(Barrett & Boitano, 2012).

In sickle cell, Chloride and water movement from the intracellular fluid is observed when calcium is increased in the red cells (Carlo Brugnara, Bunn, & Tosteson, 1986)(Carlo Brugnara & Tosteson, 1987). A rise in the erythrocyte concentration of Calcium levels, (although not demonstrated in the current study) in the sickle cell patients has been shown to cause a higher efflux of Potassium accompanied by Chloride (Isabel Bize, Taher, & Brugnara, 2013)(Engelmann, 1991). This is as a result of the abnormal activation of the Gardos channel and the K-Cl cotransport, and thus, may lead to cell dehydration (C. Brugnara, 1995). Thus, erythrocyte Calcium levels may play a role in Chloride homeostasis (De Franceschi, 2009). Significant changes in Chloride levels in sickle erythrocytes may influence cell dehydration and provoke certain clinical presentations of sickle cell disease. The main limitation of the study was the small sample size as well as the inability to assess severity of the sickle cell disease.

All together these mechanisms described above in SCD patients, lead to a high serum potassium and chloride, a low serum sodium and reduced water, leading to a high serum osmolality.

5.4.4 The role of serum calcium, magnesium and calcium-to-magnesium ratio in serum osmolality

In this study, the mean serum magnesium of HbSS and HbSC patients where seen to be significantly lower compared to HbAA controls. However, there was no significant difference between the serum magnesium of HbSS and HbSC patients. Studies on serum Mg^{2+}levels in SCD have yielded varying results, Akenami in 1999 and Oladipo in 2005 showed normal circulating levels of Mg^{2+}(Akenami, Aken'Ova, & Osifo, 1999)(Oladipo, Temiye, Ezeaka, & Obomanu, 2005). Olukoga in 1990, Zehtabchi in 2004 and Agbozo in 2015 all demonstrated low serum Mg^{2+} levels in sickle cell patients (William, 2015)(Zehtabchi S, Sinert R, Rinnert S, Chang B, Heinis C, Altura RA, Altura BT, 2004)(Olukoga, Adewoye, Erasmus, & Adedoyin, 1990). Previous studies have also demonstrated low levels of Mg^{2+} in Red Blood Cells due to an abnormality in the red cell membrane permeability to double charged ions such as Calcium and Magnesium during periods of low oxygen tension (Than et al., 2014). Increase in haemolysis in SCD is due to HbS polymerization, this phenomenon coupled with the associated complications seen in SCD rapidly depletes Mg^{2} (De Franceschi L, D Bachir, Galacteros F, 1997; Zehtabchi S, Sinert R, Rinnert S, Chang B, Heinis C, Altura RA, Altura BT, 2004).

In SCD, the abnormally high red cell permeability and loss of Potassium (K) favours higher propensity for red cell dehydration. The Potassium-Chloride (K-Cl) co-transport is one of the pathways through which K is lost; which is abnormally activated by low intracellular Mg^{2+}(De Franceschi L, D Bachir, Galacteros F, 1997). In principle, K^{+} and Cl^{-} ions are rapidly and irreversibly lost with a very significant amount of water following, as a result of osmosis (Hyacinth et al., 2011). It could be postulated therefore that higher levels of intracellular Mg^{2+} could block this pathway, and reduce dehydration as well as sickling in SCD patients (C. Brugnara, 1995).

Mg^{2+} supplementation in these patients could help regulate the channels through which K^+ are lost; thereby reducing painful episodes frequently encountered by patients with sickle cell (Badaki-Makun et al., 2014)(Brousseau, Paul Scott, Hillery, & Panepinto, 2004). However, a Cochrane review in 2017 concluded with moderate to low quality of evidence that neither intravenous magnesium and oral magnesium therapy had an effect on reducing painful crisis, length of hospital stay and changing quality of life in treating sickle cell disease. Therefore, no definitive conclusions can be made regarding its clinical benefit.

It was also observed that, patients with the HbSS genotype had lower levels of Mg^{2+} compared with the HbSC individuals, although not significant. In line with the current study, a previous study observed that, homozygous sickle cell patients (SS) had an increased frequency of painful vaso-occlusive crises, intense haemolysis and a lower study state haemoglobin (Hb) and these patients generally had low levels of Magnesium (Platt OS, Thorington BD, Brambilla DJ, Milner PF, Rosse WF, Vichinsky E, 1991)(Serjeant et al., 1994). Findings from this current study suggest in part that, there may be similar underlying conditions in the two SCD genotypes studied (SS and SC), which resulted in the comparable Mg^{2+} levels. Therefore, it is possible that, the clinical complications that arise as a result of the relatively low Magnesium levels seen in the *SS* genotype are as common as those seen in the *SC* genotypes (Nagel, Fabry, & Steinberg, 2003). Magnesium is an electrolyte with osmotic activity and thus low levels of magnesium should result in low serum osmolality. However as described about, low magnesium levels, activate the potassium-chloride co-transporter. This results in rapid and irreversible loss of K^+ and Cl^- ions to the extracellular domain accompanied with a significant amount of water, although not in an equal ratio. The overall effect is an increase in serum osmolality. Thus low levels of magnesium indirectly contribute to a high serum osmolality.

In the study, serum calcium was observed to be low in HbSS and HbSC patients compared to HbAA controls with no significant difference between HbSS and HbSC serum calcium levels. These findings were consistent with other studies (JR, n.d.)(Vikas G, Ajay KS, 2012). In human red cells, Ca is mainly bound to the inner side of the plasma membrane. A smaller part may be present within intracellular Ca storing vesicles, while only a small percentage would be found in the ionized form in red cells (Engelmann, 1991).In SCD, there is an increase in the number of endocytotic Ca vesicles and Ionized Ca in red cells. This is due to an increase in the Ca inward transport rates and/or a decrease in Ca efflux through the Ca pump (Engelmann, 1991). Serum Ca is lower in SCD patients because of the movement of Ca into the red cells. Ionized serum Calcium (Ca^{2+}) was found to be significantly low in SCD patients compared with healthy controls in the study.

Although both Ca^{2+} and Mg^{2+} levels were low in the study, serum Mg^{2+} was much lower in SCD patients resulting in a high Ca^{2+}/Mg^{2+} ratio. High serum Ca^{2+}/Mg^{2+} ratio observed in the SCD patients suggests that, the Gardos channel could be activated, leading to K^{+} loss and dehydration (Zehtabchi S, Sinert R, Rinnert S, Chang B, Heinis C, Altura RA, Altura BT, 2004). Thus, Ca^{2+}/Mg^{2+} ratio could help predict the extent of dehydration in SCD. Low calcium levels alone seen in SCD patients is expected to lead to low serum osmolality, but as described above, the activation of the Gardos channel by the high calcium to magnesium ratio leads to the loss of potassium into the extracellular domain resulting in an increase in serum osmolality. Low calcium levels indirectly cause an increase in serum osmolality.

5.4.5 Tubular Function

In line with other studies (Silva Junior et al., 2014)(Rees & Gibson, 2012)(Damanhouri et al., 2015), this study showed that HbSS and HbSC patients had significantly low serum urea, creatinine and urine pH compared to the HbAA controls. Many renal structural and functional abnormalities have been associated with Sickle Cell Disease. Sickle Cell Nephropathy affects 30-50% of adults with Sickle Cell Anaemia (Sundaram et al., 2011). Proximal tubular function (PCT) is exaggerated in SCD patients and this is manifested by an increased reabsorption of sodium and phosphorous with and an increased secretion of Creatinine and Uric acid (Ataga & Orringer, 2000a). The exaggerated PCT function in SCD patients gives a paradoxically higher glomerular filtration rate (GFR) in SCD patients as creatinine clearance is increased in SCD (Jong, n.d.)(Ataga & Orringer, 2000b). The presence of uric acid in the urine decreases the pH of urine making SCD patients produce a more acidic urine. Sickle Cell patients thus have defects in both urinary acidification and potassium (*K)* excretion (Silva Junior et al., 2014). These findings demonstrate both a proximal and distal tubule dysfunction in SCD patients.

5.5 Dietary Pattern (Sodium Consumption)

In this study, based on the quantification of the averages obtained from the 24-hour dietary recall, there was no difference in the dietary Sodium, Potassium, Magnesium, Zinc and Phosphorous consumption among the 3 groups (SCD patients and the control group). The dietary consumption of macronutrients and water was no different between all study participants. These findings differ from what is known in other literature (Kawchak, Schall, Zemel, Ohene-Frempong, & Stallings, 2007) and this can be explained by the fact that majority of participants purchased their food from food vendors, making it hard to efficiently quantify the amount they consumed

and hence obtain the different components of the food. Sickle Cell Anaemia is associated with low Calcium intake, Vitamin D deficiency and an overall poor appetite (Moore, n.d.). However, in this study, SCD patients had a significantly higher dietary calcium intake compared to the control-group. This can be explained by the fact that diet in urban Ghana is dominated by animal-based products which are rich in Calcium (Galbete et al., 2017). Participants in the study did not frequently consume sodium-rich diets. This can be explained by the fact that in recent times a lot of emphasis has been placed on a massive reduction in sodium consumption as a preventive measure against hypertension and cardiovascular diseases (Pegelow et al., 1997)(Brown et al., 2009). Participants from the study bought their food from food vendors, this can be explained by the age group and the fact that majority of participants single.

In general, there are differences in the dietary pattern of Ghanaians, based on their geographical location and the effect of urbanization (Galbete et al., 2017). Children in Memphis, United States of America with Sickle Cell Anaemia were shown to consume higher than recommended sodium-rich foods (such as chips, snack foods and processed foods) and a lower than recommended fluid intake (Ruth Williams-Hooker, 2013). High levels of dietary sodium consumed in the form of common salt has been associated with hypertension and other cardiovascular disease (Brown et al., 2009). Excessive dietary salt intake is known to increase serum Osmolality which triggers protective physiological mechanisms to offset this increase (Koc University, Istanbul, n.d.). This was however not corroborated in the study.

5.6 Correlation and Associations between Serum Osmolality and Sodium-rich Diet

This study showed a negative although weak correlation between serum osmolality and average water intake. This is in line with other studies (Kjeldsen et al., 1985). Osmolality is a

measure of the number of solutes in one (1) kg of water (Sherwood, 2010). If the amount of water increases, the amount of solute relative to the water decreases (Barrett & Boitano, 2012)(Sherwood, 2010). Osmolality therefore decreases with increasing water intake.

As already stated, excessive dietary sodium stimulates Vasopressin activity via the stimulation of osmoreceptors in the hypothalamus, which eventually counteracts the transient increase in serum Osmolality (Sherwood, 2010)(Barrett & Boitano, 2012)(Koc University, Istanbul, n.d.). Serum Osmolality therefore reduces with dietary sodium as a result of the compensatory mechanism of Vasopressin (Koc University, Istanbul, n.d.). This correlation was weakly established in the study. Dietary potassium and dietary protein all had weak negative correlation with serum osmolality probably on account of vasopressin activity.

CONCLUSIONS

This study provides the first baseline data for serum and urine osmolality for SCD patients in Ghana. This study reveals that SCD patients in general have a high serum osmolality and a low urine osmolality as compared to controls. This study demonstrated that SCD patients have an impaired renal tubular function. Renal tubular function of SCD patients must be assessed periodically to pick up worsening function in SCD patients. Serum urea alone is not a good determinant of the hydration status of SCD patients at it may deceive the clinician into thinking the patient is well hydrated. In general, study participants did not frequently consume sodium-rich foods and bought food frequently from food vendors. In SCD patients, serum osmolality decreases with increasing consumption of sodium-rich diets. The high serum osmolality and low urine osmolality observed in SCD patients is mainly due to the disease pathophysiology.

Clinical Relevance of this study:

Routine treatment of SCD includes the use of drugs to relieve pain and the maintenance of adequate fluid levels in patients. It is clear from the conclusion of this study that the serum osmolality of SCD patients are high even in their steady state. Therefore, it is recommended that extra fluids be given routinely as adjunct treatment clinically, regardless of the individual's state of hydration. This will aid in slowing or stopping the sickling process and thereby keeping them in their steady state or alleviating pain. The choice of fluids based on the high serum osmolality should be hypotonic or isotonic, however, since this study was done in the steady state it would be hard to draw a conclusion on the type of fluid (Intravenous Fluid (IVF)) that would be beneficial to give SCD patients during crisis. Serum urea alone is not a good determinant of the hydration status of SCD patients at it may deceive the clinician into thinking the patient is well hydrated.

Osmolality maybe more accurate in assessing the hydration status of SCD patients. Furthermore, knowing that the serum osmolality of SCD patients is high, SCD patients should be educated to take adequate fluid especially the HbSS patients, as their serum osmolality would further increase if their fluid intake does not match their fluid lost through the kidneys because SCD patients do not concentrate urine properly.

Limitation of the study

- Study participants were not able to accurately recall their meals in the past 24hrs with majority eating from food vendors, making accurate quantification a challenge
- Study was done with steady-state SCD patients and thus may not be reflective of conditions prevailing when patients are in crisis.

Recommendations

This study was done in steady-state SCD patients, it would be useful to note the osmolality of SCD patients in crisis and the effect interventions such as blood transfusion and intravenous fluids have on osmolality. It is therefore recommended that this work is repeated in SCD patients in crisis. This may guide clinicians in their management of SCD patients when they are in crisis. Further studies should be carried out in SCD patients in crisis.

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APPENDIX

Appendix 1: Consent Form

Inform Consent Form For Study Participants

STUDY ID								
	Group			***Patient No.***	***Unique No.***			

Study Title

BASELINE SERUM AND URINE OSMOLALITY AND FACTORS THAT AFFECT IT IN STEADY STATE SICKLE CELL PATIENTS AT THE KORLE-BU TEACHING HOSPITAL

What the study is about:

Sickle Cell Disease is a condition associated with several complications one of which is inability to concentrate urine. Sickle Cell patients are prone to dehydration. Osmolality gives an idea of one's hydration status and it is affected by some factors. The main purpose of this study is to determine factors that affect the steady state serum and urine osmolality in *SCD* patients. You are being asked to take part in this research study because you meet the eligibility requirement for the study.

Procedures for the study

- Your weight, height, BP and Temperature would be taken.
- You will be asked to provide certain information about yourself which include your contact, socioeconomic status, marital status, level of education, age/DOB and your medical history. You are not obliged to answer all the questions.
- We will take blood samples from your veins for blood tests.
    - This amount of blood we will take is not very different from what you will normally be asked to provide when you visit the laboratory for a blood test.
    - Only sterile techniques and disposable, single-use equipment will be used at all times.
    - Rarely, you might experience minor bruising at the site of taking of blood sample as with any blood test. All study participants will receive appropriate treatment as necessary.
    - The blood we take will help in performing special tests such as Full blood count, Blood Urea & Creatinine, Serum Ca^{2+}, $Mg^{2+}$and Plasma Osmolality. Some of the blood samples may be stored for analysis later.
    - **Any future analyses of the blood samples will be done with prior approval from the Ethics Committee of the College of Health Sciences, University of Ghana.**

- You will also be given a bottle for the collection of mid-stream urine sample for a Urine R/E and Urine Osmolality.
- All the tests we conduct will be at no cost to you.

Confidentiality

- All information collected from this research will be kept confidential. The findings of this study may be reported at meetings or in medical journal.
- If this study is reported you will not be identified by

My right to refuse or withdraw:

- Taking part in the research is entirely voluntary.
- You may refuse to take part or withdraw from the study at any time without anyone objecting and without penalty or loss of any benefits to which you are otherwise entitled

Who to contact

The following people will be available to answer any questions you may later have in relation to this study:

- Yaw Kusi-Mensah, Department of Physiology, School of Biomedical and Allied Health Sciences, University of Ghana College of Health Sciences (Telephone: 0244444329 ykusimensah@yahoo.com)
- Rev Charles Antwi-Bosiako, School of Biomedical and Allied Health Sciences, University of Ghana College of Health Sciences (antwibosiako@gmail.com)

I have fully explained to ……
the nature and purpose of the above described study and risks that are involved in its performance. I have answered and will answer all questions to the best of my ability.

_____ _____

(Signature) Full Name of Staff Member Date

Your signature or thumbprint below indicates that you are willing to participate in this study. You do not lose any of your legal rights by signing this consent document.

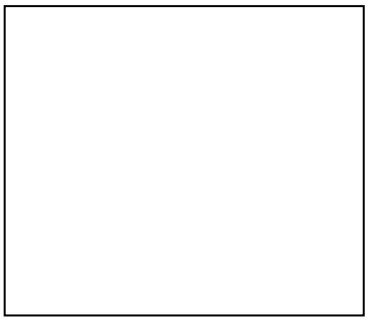
_____ _____

(Signature **study participant**) Date

If illiterate, signed by the investigator……..…

In the presence of an independent literate witness……..
(where possible this person should be selected by the participant).

Thumbprint (for the illiterate participant):



Appendix-2: Questionnaire

Baseline serum and urine osmolality and factors that affect it in steady-state Sickle Cell Disease Patients At The Korle-Bu Teaching Hospital

Questionnaire

1.	SUBJECT'S NUMBER							
		Group	***Unique No.***				***Patient Number***	

PART 1: DATE, TIME AND INTERVIEWER'S ID		
2.	Date of completion of the instrument	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *(DD MM YYYY)*
3.	Time of interview (24-hour clock)	⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ HRS MINS
	Interviewer's ID (Name)	

PART 2: CONSENT, INTERVIEW LANGUAGE NAME AND CONTACTS						
4.	Interview Language *(Insert Language)*	1. English	2. Akan	3. Ga		
		4. Ewe	5. Hausa	6. Other (specify)		
5.	Consent has been read and obtained	1. Yes		2. No	**If NO, END**	
	Contact phone number of participant (1)					
	Contact phone number of participant (2)					
	Contact e-mail of participant					
	Family Name					
	First Name					

PART 3: SOCIODEMOGRAPHIC INFORMATION OF PARTICIPANT				
6.	Gender *(Tick Male / Female as observed)*	1. *Male*	*2. Female*	
7.	Date of Birth	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Don't Know 77 77 7777* *DD MM YEAR*		
8.	Age (in years)			
	[Check against the DOB; if there is a discrepancy, confirm of both DOB and AGE and correct accordingly]			
9.	Tribe *(please tick)*	*1. Akan*	*2. Ewe*	*3. Ga/Dangbe*
		4. Mole/Dagomba	*5. Mamprusi*	*6. Gruma*
		7. Guan	*8. Other (please specify)*	

10.	Marital status *(please tick)*	*1.Never Married*	*2.Currently married*	*3. Separated*
		4. Divorced	*5. Widowed*	*6. Cohabitating*
11.	Highest level of education you have completed *(please tick)*	*1. No formal schooling*		
		2. Basic (Primary to JHS)		
		3. Secondary school		
		4. Tertiary		
12.	Work Status (*please tick) (Which of the following best describes your main work status over the past 12 months?)*	*1. Government employee*	*2. Non-government employee*	
		3. Self-employed	*4. Student*	
		5. Homemaker / housewife	*6. Retired*	
		7.Unemployed (able to work)	*8.Unemployed (unable to work)*	
		9. Other (please specify)		
13.	What is your occupation?			

PART 4: MEDICAL HISTORY				
14.	Do you have any known chronic medical illness? **[Aside Sickle Cell Disease]**	*1.Yes*	*2.NO*	
15.	If Yes *(please tick)*	*1. Hypertension*	*2. Diabetes*	*3. Asthma*
		4. Peptic Ulcer	*5.Bleeding Disorder*	*6.Other*
16.	Have you had any Surgeries in the past?	*1.Yes*	*2.NO*	
17.	If Yes *(please tick)*	*1.Hip Replacement*	*2. Cholecystectomy*	*3.Bowel Surgery/Laparatomy*
		4.Kidneystones Surgery	*5.Caesarian Section*	*6.Other (Specify)*
18.	Are you on any medication aside Hydroxyurea and Folic Acid?	*1.Yes* *2. No*		
19.	If Yes *(please tick)*	*1.Antihypertensives*	*2.Oral Hypoglycaemics Agents*	*3. Food supplements*
		4.Inhaler(For Asthma)	*5. Anticoagulant*	*6.Analgesic*
		7. Herbal Medications	*8. Other (please specify)*	

PART 5: STEADY STATE HISTORY (FOR SICKLE CELL PATIENTS ONLY)				
20.	At what age were you first diagnosed with Sickle Cell Disease? *(please tick)*	*1.0-1yr*	*2. >1-5yrs*	*3.>5-12yrs*
		4. >12-19yrs	*5. >19yrs*	

21.	How often do you attend the Sickle Cell Clinic? *(please tick)*	*1.Every 2 weeks*	*2. Once a month*	*3. Every 3months*
		4. Twice a year	*5. Once a year*	
22.	When was the last time you had any crisis? *(please tick)*	*1.In the past week*	*2. >1-4wks*	*3.>1-6months*
		4. >6months		
23.	How many sickle cell crises have you had that required you to take pain medication(s) in the last year? *(please tick)*	*1.No Pain crises*	*2.1-2Pain crises*	*3. 3-5 Pain crises*
		4. 6-9 Pain crises	*5. >10 Pain Crises*	
24.	Where do you go for treatment of your pain crises? *(please tick)*	*1.Home treatment*	*2.Hospital ER*	*3. Clinic/GP*
		4.Other(Specify)		
25.	How do you manage your pain crises at Home (*please tick all applicable)*	*1. Drink Fluids*		*2. Massage*
		3.Heating Pad		*4. Rest/Sleep*
		5.NSAIDs		*6. Codeine*
		7.Multivitamin		*8.Traditional medications*
		9. Other (please specify)		
26.	Have you been hospitalized in the past 6 months?	*1. Yes*		*2. No*
27.	If Yes for how long? *(please tick)*	*1.<1week*	*2. >1-2wks*	*3.>2-4wks*
		4. >1months		
28.	How many blood or blood products have you received in the past 4 months? *(please tick)*	*1. 1-3*	*2. 4-6*	*3. >6*
29.	What is your steady state Hb? (If not known see below)			

PART 6: SICKLE CELL COMPLICATIONS (SICKLE CELL PATIENTS ONLY)				
30.	Have you ever had any of the following Sickle Cell Complications *(please tick which applies)*?	*1.Leg Ulcer*	*2.Large spleen (Splenic Sequestration*	*3.Pulmonary Hypertension*
		4.SCDx Kidney Disease	*5.Severe Sepsis (Not Malaria)*	*6. Others*
31.	Have you had a stroke?	*1. Yes*	*2. No*	
32.	If Yes how many episodes? *(please tick)*	*1.One*	*2. Two*	*3. Three*
		4.Four and above		
33.	Date of Stroke? (unknown-77/77/7777)		*DD/MM/YYYY*	
34.	Did you have seizures during the Stroke?	*1. Yes*		*2. No*

35.	Have you ever had a pneumonia or severe lung infection that required blood transfusion?	*1. Yes*		*2. No*
36.	If Yes *(please tick)*	*1. Don't Know*	*2. 1-3*	*3. 4-6*
		4. 7-9	*5. 10-12*	*6.>12*

PART 7: PHYSICAL MEASUREMENT		
37.	NIBP(mmHg)	38.Heart Rate (/min)
39.	Temperature (°C)	40.Height (m)
41.	Weight (kg)	42.BMI (kg/m^2)

If Steady Hb not known obtain past 3 steady state Hb's and document average

24 Hour Dietary Recall

Type Of Meal And Time Eaten	**Food Eaten**	**Handy Measure**	**Quantity (G)**
Breakfast			
Snack			
Lunch			
Snack			
Supper			
Snack			
Daily Water	**<500mls**	**500-2l**	**>2l**

Food Frequency Table

Food Item	**Consumption Frequency**				
	With every meal/ Daily	Often/4-6 times in a week	Not too often/1-3 times in a week	Rarely/1-2ce a month	Never
Add Table Salt/Rock Salt/ Iodated Salt					

Canned / Tin Tomatoes/ Vegetables					
Salted Snacks (Chips, nuts, salt popcorn etc)					
Koobi / Kako / Momone					
Processed Foods (meat (corned beef, canned fish etc), cheese, Indoomie etc)					
Ready-Prepared meals (soups, stock)					
Stock Cubes (Maggi, A1)					
Salted Beef (TOLO-BEEF)					
Salted Pork					
Spices/Mixed Spices (Adobo, Miss Cookie etc)					
Soy Sauce and Maggi sauce					
Margarine/ Butter					
Bread and Grain Products					
Food Vendors					

Labs

FBC		**BUE&Cr**		**Urine R/E**	
Hb		**Na**		**pH**	
WBC		**K**		**SG**	
PLT		**CL**		**Protein**	
		Mg		**Blood**	
Plasma Osm		**Ca**		**Nitirites**	
		Urea		**Leucocytes**	
Urine Osm		**Cr**			
		GFR			

Appendix 3: Personnel For Study

MPhil Student- Yaw Kusi-Mensah

Principal Supervisor- Rev. Charles Antwi-Bosiako (PhD)

2nd Supervisor- Dr E. Owusu-Darkwa

Data collection personnel- Yaw Kusi-Mensah (50%), Mc Clean and Derrick (40%) and Lim Kwawukume (10%)

Data analysis Personnel- Yaw Kusi-Mensah (40%), Gifty(20%), Robert (20%) Lim Abla Kwawukume (10%), Charles Hayfron-Benjamin (10%)

Lab technicians at Haematology Lab Korle Bu Teaching Hospital and Department of Physiology

Appendix 4: Ethical Clearance

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: CHS/EPRC/APRIL/2018 April 30, 2018

Yaw A. Kusi-Mensah
Department of Physiology
School of Biomedical and Applied Health Sciences
Korle- Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.8 – P2.13/2017-2018***

The College of Health Sciences Ethical and Protocol Review Committee on April 26, 2018 reviewed and unanimously approved your research proposal.

Title of Protocol: **"Factors affecting Osmolality in Sickle Cell Disease Patients at the Korle-Bu Teaching Hospital"**

Principal Investigator: **Yaw A. Kusi-Mensah**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till April 30, 2019.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

Cc: Provost, CHS
Dean, SBAHS
Head, Dept. of Physiology

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