N-TERMINAL PRO- BRAIN NATRIURETIC PEPTIDE AND HIGHLY SENSITIVE C-REACTIVE PROTEIN LEVELS IN SICKLE CELL DISEASE PATIENTS AT STEADY STATE

BY

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DECLARATION

I, JOYCE K. MAGANGA, declare that this dissertation is my original work and to my best of my knowledge has not been published elsewhere or presented for a degree program in any other university or forum

Signature …….

We certify that this dissertation has been submitted for examination to the University of Nairobi with our approval as supervisors

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To all Sickle cell Disease Patients in Kenya

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LIST OF ABBREVIATIONS

AHF- Acute heart failure

BMI -Body mass index

BP-Blood pressure

CSSCD-Cooperative study of sickle cell disease

CVD –Cardiovascular disease

HBAS -Haemoglobin A

HsCRP –Highly sensitive C - reactive protein

HBss -Haemoglobin s

HU-Hydroxyurea

LAE -Left atrial enlargement

LVDD -Left ventricular diastolic dysfunction

LVH -Left ventricular hypertrophy

LV -Left ventricle

KNH-Kenyatta national hospital

MDGs-Millennium development goals

NCD -Non-communicable diseases

NICE-National institute of health and care excellence

NT-Pro BNP - N-terminal pro brain natriuretic hormone

PAH-Pulmonary arterial hypertension

PH -Pulmonary hypertension

ROC-Receiver operating characteristics

SCD –Sickle cell disease

UON-University of Nairobi

VOC-Vaso-occlusive crisis

WBC -White blood cells

WHA-World health association

WHO -World health organization

IL -Interleukin

TNF-Tissue necrosis factor

ABSTRACT

Sickle cell disease is a non-communicable disease attracting global public health concern. Chronic effects of sustained hemolytic anemia drive the development of end-organ complications which include heart, brain, kidney, and bones as sickle cell disease patients live longer. Cardiovascular dysfunctions are on the rise leading to a large effect on morbidity and untimely morbidity. Cardiovascular dysfunctions are diagnosed mostly by use of ECHO which is expensive and not found in all the hospitals. Biochemical markers are affordable and can easily be done in all the hospitals. Cardiovascular risks in sickle cell disease patients using biochemical markers have not been documented locally here in Kenya

Objective

To determine serum NT- proBNP and HsCRP levels for prediction of cardiovascular risks (heart failure) in asymptomatic steady-state Sickle Cell Disease patients

Study design and setting

A cross-sectional descriptive study on prediction of cardiovascular risks in Sickle cell disease patients in asymptomatic steady-state in Kenyatta National Hospital and Baraka medical Centre in Mathare

Methodology

Sickle Cell Disease patients presenting at KNH Haematology clinic and Baraka Medical Centre after giving informed consent were sampled to identify those eligible to participate in the study. Targeted history was carried out using a screening proforma.NT-proBrain Natriuretic Peptide and Highly Sensitive C-Reactive Protein levels were measured using Flouroimmuno assay technique and Immunoturbidimetric techniques respectively in the Clinical chemistry laboratory University of Nairobi

Results

A total of 90 patients were recruited. The majority (50%) were in the age group 5-10yrs. There was no patient above the age of 40yrs. Females were more (55.6%) than males (44.4%). Those at high cardiovascular risk (n=20) were 22.2% where male (32.5%) were majority. A correlation was done between NT-proBNP and HsCRP(r=0.044) indicative of a weak but statistically

significant correlation. Further analysis was done thus making these biomarkers good predictors of cardiovascular events in Sickle cell disease patients in steady-state

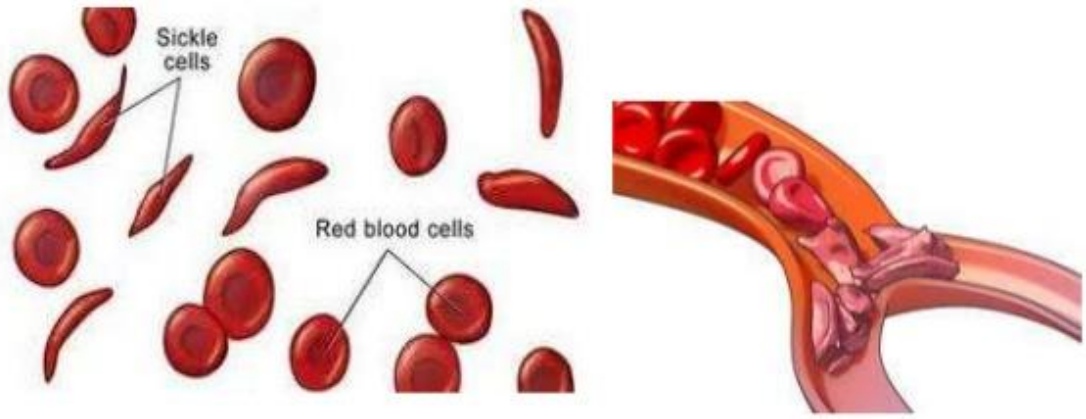
Conclusion

The findings of this research suggest that NT-proBNP is not a useful marker in determining the risk for heart failure in sickle cell disease patients at a steady state. HsCRP is non-conclusive because it is elevated in the majority of inflammatory processes

1.0 INTRODUCTION

A sickle-cell disease is a group of inherited disorders resulting from a missense mutation in the β-globin gene. The mutation causing sickle cell anemia is a single nucleotide substitution (A to T) in the codon for amino acid 6. The change converts a glutamic acid codon (GAG) to a valine codon (GTG). Sickle hemoglobin (HbS) which results after this substitution is a structural variant of normal adult hemoglobin (HbA) The underlying problem in sickle cell anemia is that the valine for glutamic acid substitution results in hemoglobin tetramers that aggregate into arrays upon deoxygenation in the tissues. This aggregation leads to the deformation of the red blood cell into a sickle-like shape making it relatively inflexible and unable to traverse the capillary beds. This structural alteration in the red blood cell can easily be seen under light microscopy and is the source of the name of this disease. Repeated cycles of oxygenation and deoxygenation can lead to irreversible sickling. (Chakravorty et al.,2015).SCD affects 20–25 million people globally, and 50–80% of infants born with SCD in Africa die before the age of 5 years (Aygun et al, 2012). It is estimated that 240,000 children are born with SCD annually in sub-Saharan Africa (Makani et al., 2011). Its mode of inheritance is autosomal recessive (Hoffman et al;2000) As Red blood cells get de-oxygenated; they undergo conformational change promoting intracellular polymerization. This leads to distortion of the normal biconcave shape to a crescent shape. This crescent shape makes it difficult for the red blood cells to maneuver through the blood vessels.SCD usually manifests in early childhood. For the first 6months of life, infants are protected because HbF is protective against clinical severity. As these patients live longer, the chronic effects of sustained hemolytic anemia and episodic vaso-occlusive events drive the development of end-organ complications which include the heart, brain, kidney, and bones.

Sickle cell disease- Biochemical Defect



The continuous formation and destruction of sickled cells contributes to severe hemolytic anemia. These rigid cells may initiate small vessel occlusions.

https://www..slideshare.net

Cardiovascular complications are on the increase, this is noted by pulmonary hypertension (PH), development of a progressive proliferative systemic vasculopathy, left ventricular diastolic dysfuntion . These complications have a large effect on morbidity and untimely mortality. Sickle cell anemia usually presents in infancy with milder cases manifesting later in life. The hallmark presentations are failure to thrive, repeated infections, and. Sickled hemoglobin forms stiff rods within the red cell, changing it into a crescent, or *sickle* shape. Due to their Sickle-shaped, red blood cells become inflexible and stick to vessel walls, causing a blockage that slows or stops the flow of blood thus obstructing oxygen to reach the tissues. The heart, therefore, has to pump blood harder through the narrowed vessels to reach all the body parts. The heart attempts to maintain cardiovascular homeostasis by releasing natriuretic peptides into circulation in response to increased wall tension. Over time, the heart muscle weakens and the heart pumps lesser blood gradually

Silent target organ damage causes sudden death with no signs of cardiac disease

The silent target organs include:

Left ventricular hypertrophy (LVH),

Left ventricular diastolic dysfunction (LVDD),

Left atrial enlargement (LAE).

This Silent target organ is evaluated by known biomarkers of the heart namely NT-Probrain Natriuretic Peptide (NT-proBNP) and Highly sensitive C-reactive proteins (HsCRP).

When the heart muscles are stretched, the left ventricle primarily produces a natriuretic hormone NT-ProBNP. The concentrations of NT-proBNP produced increase markedly indicating imminent heart failure.

2.0 LITERATURE REVIEW

2.1 SICKLE CELL DISEASE AND CARDIOVASCULAR DISEASE

Sickle cell disease which is a genetic disorder is characterized by recurrent episodes of ischemia-reperfusion injury to multiple vital organ systems and chronic hemolytic anemia. These contribute to progressive organ dysfunction. Chronic anemia in sickle cell disease leads to cardiac chamber dilation. The heart has a compensatory mechanism resulting in the increase in left ventricular mass accompanied by left ventricular diastolic dysfunction. This compensatory mechanism is a strong independent predictor of mortality in patients with sickle cell disease. Pulmonary Hypertension and diastolic dysfunction are marked abnormalities in SCD patients. Sudden death is an increasingly recognized problem, and further cardiac investigations are necessary to recognize and treat high-risk patients. Cardiovascular complications are noted with increased longevity which develops in proliferative systemic vasculopathy pulmonary hypertension and left ventricular diastolic dysfunction. High pulmonary pressures have been shown in autopsy studies as risk markers for mortality for these patients Autopsy (Gladwin et al., 2012)

2.2 EPIDEMIOLOGY

SCD is among the most common monogenetic disorders in the world and affects millions of people. The UN has recognized SCD as a global public health concern. WHO recommends that by 2020, 50% of member states will have established SCD control prognosis (WHO, 2006). The distribution of the sickle gene in Africa reflects a very close parallel with the endemicity of malaria, particularly that due to *Plasmodium falciparum*. The highest frequency has been traced to the low altitude, tropical areas with significant amounts of rainfall annually. (Grosse et al;2011) .It affects nearly 1 in 600 African Americans (Gladwin et al; 2008). It is common mostly in Spanish-speaking regions and those ancestors from Sub-Saharan Africa and in the Western. Sickle cell hemoglobinopathies are projected to increase by 2050. SCD leads to a substantial burden of disease, the greatest (75%) being in Sub-Saharan Africa. An estimated 1-4% of babies born in Sub-Saharan Africa have SCD (Aliyu et al., 2008. According to Machado et al 2006, 30% of patients with sickle cell disease develop pulmonary hypertension which is a major risk factor for death in this population. In Kenya, SCD is found mostly in Nyanza, Western

and Coastal regions. Prevalence of Sickle Cell Trait (SCTr) in Kenya was reported as 28 - 35 % (Aluoch et al 1993). In Uganda, the prevalence of SCTr and SCD in children was reported as 13·3% and 0·7% respectively (Ndeezi et al., 2016).SCD is one of the top ten NCDs in Nigeria. Attainment of MDGs is significantly undermined by morbidity and mortality. There has been significant improvement in the morbidity and mortality rates of children with SCD.Early diagnosis through newborn screening programs, prophylactic therapy, and comprehensive care programs like hydroxyurea and bone marrow transplant. in high resource countries such as the United States has reduced the mortality and morbidity rates in these countries.SCD burden in Africa can be reduced by these interventions through support training of health workers, research, and sharing of knowledge (Lucky et al; 2015)

Sickle cell disease-Frequency

- Sickle cell disease is most common in individuals of African descent but is seen in Hispanics, Arabians, Indians, and whites.
- In the United States the incidence is 1 in 625 live births to African-Americans.

2.2.1 CARDIOVASCULAR DISEASE IN GENERAL POPULATION

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. They include endocarditis diseases of the aorta and its branches, heart failure, arteriosclerosis, coronary artery disease, arrhythmia, hypertension, orthostatic hypotension, shock, disorders of the peripheral vascular system, and congenital disease. According to WHO, CVDs are the number one cause of death globally. More people die annually from CVDs than from any other cause.

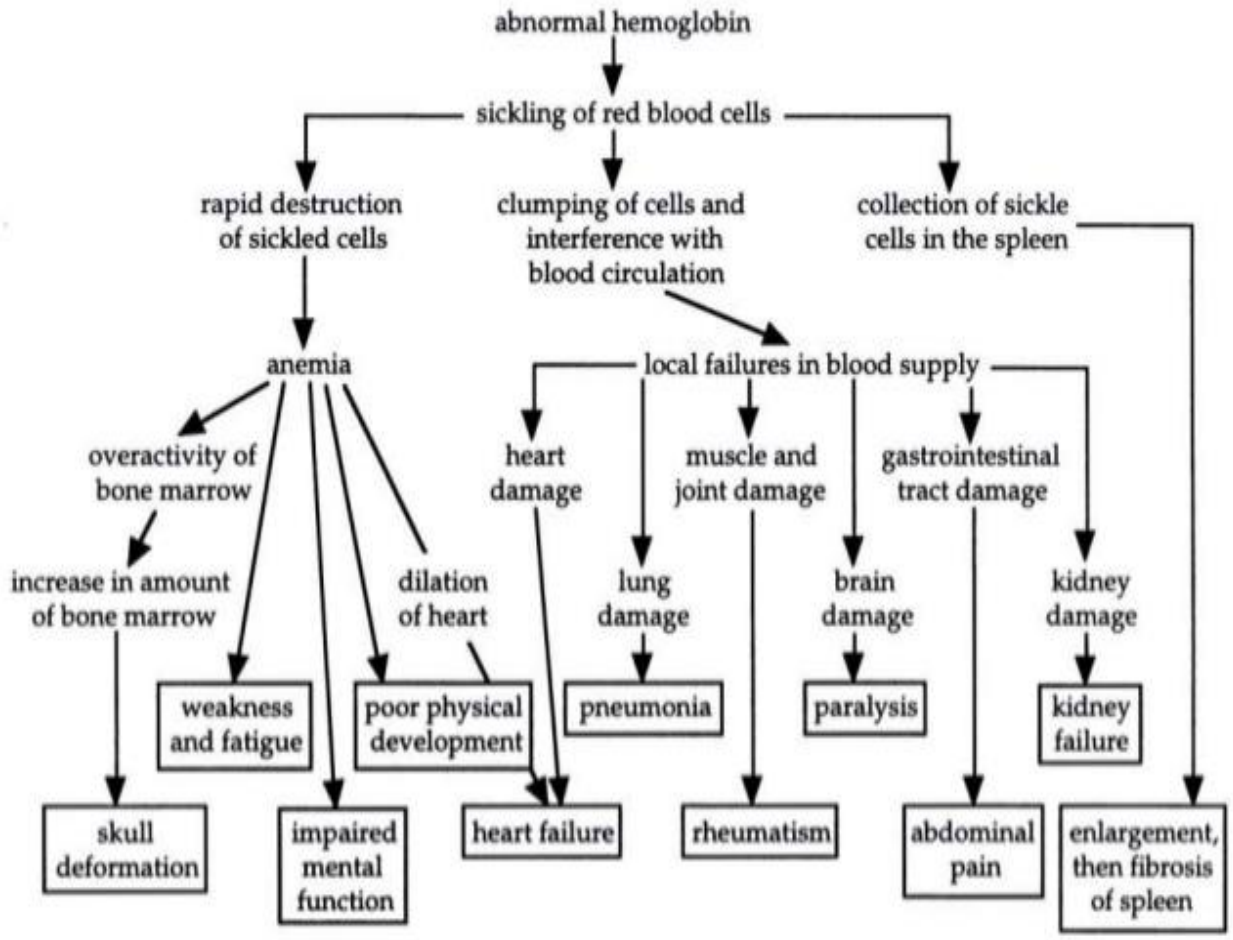
An estimated 17.7 million (31%) people died from CVDs globally in 2015. Of these deaths, an estimated 7.4 million (6.7%) were due to coronary heart disease and stroke respectively. Over three-quarters of CVD deaths take place in low- and middle-income countries. In 2015, out of the 17 million premature deaths (under the age of 70yrs) were due to non-communicable diseases.37% are caused by CVDs and 82% are in low- and middle-income countries.

In 2013, under the leadership of WHO, all member states (194 countries) agreed on global mechanisms to reduce the avoidable NCD burden. This included a Global action plan for the prevention and control of NCDs between 2013-2020. This plan aims to reduce the number of premature deaths from NCDs by 25% by 2025 through nine voluntary global targets two of which directly focus on preventing and controlling CVDS

2.2.2 LONG TERM COMPLICATIONS OF CVD

Long-term complications of SCD include stroke, acute chest syndrome, pulmonary arterial hypertension (PAH), and renal failure. PAH results from chronic hemolysis and can lead to heart failure. PAH affects 30% of patients with SCD in the U.S. with a mortality rate of 40% (Machado et al., 2006). Odero et al., 2009 in a study using echocardiography, reported a PAH prevalence of 49.4% in children with SCD at KNH

Sickle cell disease complications



Sickle-cell-anemia-example-of-a-beneficial-mutation

https://www. creationbc.org

2.2.3 CVD IN SCD POPULATION

SCD is associated with very high child mortality. It is found in tropical regions, particularly sub-Saharan Africa, the Middle-East and India and. (Weatherall et al., 2016). In Kenya, SCD is an endemic disease found mostly in the Western and Coastal regions

There are 225,000 children born per year with sickle cell anemia. 75% of birth occur in Sub-Saharan Africa. Approximately 50-80% dies before adulthood (Weatherall et al., 2016). Sickle cell disease (SCD) is associated with cardiovascular diseases such as stroke, kidney disease, and pulmonary hypertension. These are considered as proxies of severity in SCD. It vastly impacts the African continent and. Studies collectively show that stroke and kidney disease each affect ∼10% of SCD patients. Pulmonary hypertension displays a higher prevalence of 30% among adults with SCD.

In Nigeria, more than 15,000 children are born with the disease annually. About four million people are afflicted with the condition. Aliyu et al, 2008b reported a 25% prevalence of pulmonary arterial hypertension among sickle cell disease patients in Nigeria. This was based on an elevated echocardiogram-determine tricuspid regurgitation velocity.

A study done by (Mark et al., 2012) on cardiovascular abnormalities in sickle cell disease concluded that despite a significant increase in longevity for SCD patients over the last several decades the mortality rate from cardiovascular and pulmonary complications remains high. Multiple pathologic and clinical studies have shown that patients with PH and diastolic LV dysfunction represent a particularly high-risk subgroup. These cardiopulmonary complications contribute to a markedly low functional capacity and associated high risk of both sudden death and severe multi-organ dysfunction.

Odera et al 2009 on the prevalence of pulmonary arterial hypertension in children with sickle cell anemia in KNH found out that 49.4% had PAH and the majority of those affected (86%) had mild PAH using echocardiography. The patients recruited were between 6months and 5years.

2.2.4 NT-proBNP AND SCD

The single most clinically relevant use of NT-proBNP remains the diagnosis of heart failure as the prime cause of a patient's symptoms when the diagnosis is uncertain. Natriuretic peptides are

increasingly being studied to guide the adequacy of therapy in heart failure patients. (Rasekaba et al., 2009).

Machado,et al 2006 reported that NT-proBNP levels >160ng/l were associated with pulmonary hypertension in adults with sickle cell disease in the United States, with a positive predictive value of 78%.In that study, NT-proBNP levels were higher in patients with sickle cell pulmonary hypertension which correlated directly with Tricuspid regurgitant velocity($P<0.01$). He suggested that the risk of death in these patients was unrelated to vaso-occlusive pain episodes but it was largely determined by occult hemolytic anemia associated with pulmonary hypertension

A raised NT-proBNP (≥ 232ng/l) identified patients with a Left ventricular ejection fraction (LVEF) of ≤ 40% (n = 157) with a sensitivity of 73% and a specificity of 82%. The negative predictive value of having an NT-proBNP concentration below 232 ng/l was 98%. Concentrations of NT-proBNP increased with increasing age and with decreasing LVEF ($p < 0.05$) (Bay et al., 2009)

In Nigeria, Sickle cell disease patients have NT-proBNP a level elevated and is common among adults. It is associated with markers of anemia, inflammation, and iron status. In a cross-sectional study at Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria by Aliyu et al .,2010, 133 Nigerian sickle cell anemia patients aged 18-52 years at steady-state and 65 healthy controls were studied.26% percent of patients versus 5% of controls had NT-proBNP > or =160 ng/l ($P = 0.0006$). By logistic regression among the patients, lower hemoglobin levels predicted elevated NT-proBNP. He suggested that further studies need to be done in Africa to determine if NT-proBNP elevation predicts increased mortality. The present information supports the use of elevated NT-proBNP values to select potentially high-risk patients for inclusion in intervention trials in Africa (Aliyu, *et al*;2008). Cardiopulmonary organ dysfunction and chronic kidney injury have a large effect on morbidity and premature mortality, this accelerates in the second decade of life (Makani, et al;2007). These processes culminate in the development of pulmonary hypertension, left ventricular diastolic heart disease, dysrhythmia, and sudden death (Gladwin et al.,2016) An analysis of banked plasma samples from the CSSCD cohort revealed that an abnormally high N-terminal pro–B-type natriuretic peptide (NT-proBNP) level ≥160 pg/ml, a biomarker for PH in patients with SCD was present in 27.6% of adult SCD

patients, and high levels were associated with markers of hemolytic anemia such as a low hemoglobin level ($p < 0.001$). National Institute of health and care excellence (NICE) clinical guideline187 (2014) on diagnosing and managing acute heart failure recommend a single measurement of NT-pro BNP at presentation to rule out heart failure. The suggested threshold for excluding AHF is NT-proBNP <300ng/L. If these levels are exceeded, ECHO is recommended after 48hrs to guide in early specialized management

2.2.5 C-REACTIVE PROTEIN IN SICKLE CELL DISEASE

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to IL-1, TNF, and IL-6 and may serve as a useful global biomarker reflecting inflammatory activation in CHF development and progression (Wang et al 2003). It is a well-known marker for inflammation. Current evidence shows that inflammation plays a role in ischaemic heart disease. High-sensitivity C-reactive protein (HsCRP) is a strong independent risk factor for cardiovascular events.

Persistently elevated serum levels may indicate a bad prognosis for the patient's outcome. Slightly elevated levels are of diagnostic value as a risk indicator for atherosclerosis and coronary heart diseases. The symptoms and complications of sickle cell disease arise mainly from the crises (clinical and subclinical). Activation and damage of endothelial cells with activation of adhesion molecules lead to inflammation, the release of C-reactive protein (CRP) and other inflammatory mediators, and subsequent enhancement of ischemia (Manwani et al., 2013). This evidence suggests that SCD is associated with a chronic inflammatory state (Bundeira et al., 2014) in which inflammation, oxidative stress, and tissue oxidative damage occur, leading to various degrees of disease severity and end-organ dysfunction. The high-sensitivity CRP test measures low levels of CRP in the blood to identify low levels of inflammation that are associated with the risk of developing cardiovascular disease. Hs-CRP test accurately detects lower levels of the protein than the standard CRP test and is used to evaluate individuals for risk of CVD. It measures CRP in the range from 0.5 to 10 mg/L.It is now believed that a persistent low level of inflammation plays a major role in atherosclerosis, the narrowing of blood vessels due to the build-up of cholesterol and other lipids, which is often associated with CVD.

Okocha et al; 2012 in Nigeria showed that HbSS CRP levels were significantly raised (P=0.001) than the control group HbAS. CRP levels in the HbSS group showed a negative correlation with disease severity scores with *P*-value being close to significance (*P* = 0.17) WBC also showed a negative correlation with CRP levels in the same group of subjects (*P* = 0.73) He concluded that the biologic role of CRP concerning the inflammatory process in HbSS patients is a protective one. Fatima et.al., 2010 studied the relation of CRP in children with SCD. The study subjects comprised 104 SCA patients who experienced VOC event during the study period(VOC group) and 40 SCA patients who did not develop VOC for at least 9months before blood collection(steady-state group). It was found out that Hs-CRP levels were higher in VOC [median range = 31.3(1.14–363.0)] than steady-state [median range = 5(0.16–185.0)] groups (P < 0.001). Given evidence linking sickle cell anemia (SCA) with chronic inflammation, and given the role of high sensitivity C-reactive protein (Hs-CRP) as an inflammatory mediator, she hypothesized that SCA vaso-occlusive crisis (VOC) is associated with heightened Hs-CRP levels. Study subjects comprised 104 SCA patients who experienced VOC event during the study period (VOC group), and 40 SCA patients who did not develop VOC for at least 9 months before blood collection (Steady-state group). Hs-CRP determination was done by latex-enhanced nephelometry. Receiver-operating characteristic (ROC) analysis was employed in assessing the usefulness of Hs-CRP as a predictor of the frequency and severity of VOC. Spearman's correlation coefficient between Hs-CRP and VOC was 0.65 (P < 0.001) among unselected patients (0.71 in males and 0.59 in females). Hs-CRP area under ROC curves was 0.90 (95% CI = 0.85–0.94) among unselected patients, 0.94 (95% CI = 0.89–0.98) for males, and 0.85 (95% CI = 0.77–0.93) for females. Logistic regression analysis confirmed the positive association of increased Hs-CRP levels with VOC, which correlated positively with VOC frequency (P < 0.001), type (P < 0.001), pain (P < 0.001), and need for hospitalization (P = 0.024). These data support a strong association of increased Hs-CRP levels with VOC, which impacts VOC-related parameters and supports a role for Hs-CRP in VOC follow-up.

2.3 PATHOPHYSIOLOGY OF HEART FAILURE IN SCD

Sickle cell anemia is an inherited blood disorder primarily affecting groups with origins in endemic malarial areas, especially those of African descent. Polymerization of deoxygenated hemoglobin S within the erythrocyte ultimately reduces its flexibility and distorts its shape. This alters its rheological properties and reduces membrane fluidity in flowing blood. SCA

is characterized by chronic anemia and crises of red cell sickling and ischemia that are often painful and affect several organs and tissue types. SCA confers considerable disability, morbidity, and mortality. Annual mortality from SCA has been estimated at approximately 3%. As a significant number of these deaths are sudden, a cardiac cause has been suspected however, no cardiac mechanism of sudden death has been identified. Recently, it has been demonstrated that SCA patients with pulmonary hypertension (PH) have a higher incidence of sudden death than those with normal pulmonary pressures. In many patients, PH occurs in association with elevated pulmonary arterial wedge pressures and normal pulmonary arterial resistance, suggesting that the PH develops as the result of left ventricular (LV) abnormalities. In other conditions in which PAH develops, sudden death occurs only at pressures considerably higher than those observed in SCA. These factors suggest that PH in SCA is a surrogate marker for, rather than the cause of sudden death. Rather, an SCA cardiomyopathy process may provide a unifying mechanism that associates moderate degrees of PH and a high risk of sudden death from cardiac causes.

In SCD, several chronic complications appear to be related to hemolytic anemia while other complications are related to inflammation and vaso-occlusion (classic "sickling" events).

Vaso-occlusive pain crisis and acute chest syndrome are caused by vaso-occlusion by sickled and adhesive red cells and leukocytes In epidemiological studies the risk of developing these clinical manifestations are related to high steady-state hemoglobin levels (less hemolysis and higher viscosity), high white blood cell count (inflammation) of SCD.

Pulmonary hypertension in SCD is caused by pressure on the pulmonary artery and can lead to strain on the right ventricle hence the risk of heart failure. The heart is frequently enlarged in children with sickle cell anemia.

Rapid heart rates and murmurs are common. The heart muscle can also be injured by infarcts and iron depositing in the muscle as it leaks from the ruptured red blood cells. Over time, the heart muscle weakens and the heart pumps lesser blood gradually. Vascular complications have emerged as a major threat to the well-being of individuals with SCD. The cardiovascular marker NT-proBNP identifies patients with SCD at high risk of death. The NT-proBNP elevation is common in patients with SCD and is strongly associated with increased intensity of hemolytic anemia, age, renal insufficiency, and iron overload

2.4 CVD DIAGNOSIS AND RISK ASSESSMENT

Hypertension, hyperlipidemia, obesity, and a sedentary lifestyle are some of the risk factors leading to developing cardiovascular diseases.

Other risk factors include being of African-American ancestry, male, drinking excessive amounts of alcohol, having a lot of long-term stress, smoking, poor diet, and having a family history of a heart attack at an early age. Making a diagnosis of cardiovascular disease includes undergoing a complete medical evaluation and physical examination. Tests that may be used to diagnose a cardiovascular disease or the risk of cardiovascular disease include exercises stress testing, Electrocardiogram, echocardiographic assessment (ECHO) of the tricuspid valve regurgitation jet velocity, cardiac catheterization, Cardiac MRI, Cardiac CT scan, and Holter monitoring. Some of the biomarkers used include HsCRP, homocysteine; lipoprotein subclasses like lipoprotein A, Apo lipoprotein, A-fibrinogen WBC count NT-proBNP. Low hemoglobin levels (below 10g/dL) increase cardiac output and workload hence cardiomegaly. (Marie et.al., 2004). After hemolysis, Hb is released into plasma destroying Nitrite Oxide dependent vasodilatation (Reiter et al., 2002). CRP is traditionally measured down to concentrations of 3-5 mg/L.

According to the American Heart Association and U. S centers for disease control and prevention, Hs- CRP levels in the assessment of CVD risk have been categorized as follows:

- Low risk: less than 1.0 mg/L
- Average risk: 1.0 to 3.0 mg/L
- High risk: above 3.0 mg/L

Above 10 mg/L usually indicates acute inflammation

According to the National Institute of health and care excellence (NICE), a threshold of >300ng/L is recommended as a cut off value for NT-proBNP in diagnosing heart failure. Values above this are regarded as a CVD risk predictor.

2.5 THE ROLE OF HYDROXYUREA IN SICKLE CELL DISEASE

Hydroxyurea is a myelosuppressive agent. Its cytotoxic effects reduce marrow production of neutrophils, reticulocytes, and platelets which is an important mediator of inflammation. Neutrophils and reticulocytes promote vaso-occlusion HU reduces surface expression of adhesion receptors (Rahit et al., 2013)

It has been used for the treatment of SCD in the United States and Europe for over 25 years; with proven effectiveness in the reduction of acute painful episodes (Aygun et al., 2012). Hydroxyurea works by increasing fetal hemoglobin and increasing the water content of red blood cells resulting in less cell deformity (Aliyu et al., 2008). It also upregulates the intercellular molecule receptor for adhesion of malarial-infected red blood cells and thus theoretically its use could enhance replication of malaria cells. It is also believed to have antihemolytic properties (Stuart et al., 2004)

HU raises the level of HbF and Hb level hence reduces the need for blood transfusion. HbF is protective against clinical severity and reduces hemolysis.HU helps RBCs to stay round and flexible, inhibits the effect of HbF on polymerization of sickle cell Hb. The low percentage of HbF is associated with a high risk of developing vaso-occlusive complications, organ damage, and early death. High HbF levels are associated with lower vaso-occlusive rates (John Hopkins, 1997).HU reduces the frequency of painful episodes by 50%. Hydroxyurea also reduces hepatic sequestration and priapism reducing the need for blood transfusion; and lowers mortality from SCD related complications by 40% (Aliyu et al., 2008). In 2013, Aneni, et al, published a systematic review of strategies for reducing morbidity from malaria in SCD, including the use of hydroxyurea to reduce malaria-associated morbidity and mortality in SCD patients

3.0 PROBLEM STATEMENT

Cardiovascular dysfunction is an important cause of morbidity and mortality in sickle cell disease. This can be due to ischemia or heart failure.

The diagnosis of heart failure remains essentially clinical based on history, physical examination, and chest radiograph findings.

However, clinical findings and examination alone are often inadequate in diagnosing cardiovascular abnormalities. The Gold standard of cardiovascular diagnosis is imaging studies like echocardiography. Because of its cost and limited availability, echocardiography is not suitable as a primary diagnostic screening tool. This delays early intervention and appropriate management of the patient; therefore, new cost-effective diagnostic tools are needed. In this respect, neurohormonal markers could be useful in the diagnosis of left ventricular dysfunction.

In this setting, the existence of a biomarker that could accurately identify heart failure as the cause of the patient's symptoms would be extremely helpful in guiding the timely initiation of appropriate management.

Natriuretic peptide namely NT Pro BNP presents as such a marker. Together with CRP can be used as biomarkers specifically for the heart.NT-proBNP and Hs-CRP have not been evaluated in SCD locally

It is envisaged that the information generated by this study will further improve our knowledge on the predictors of heart failure and could as well have applications in the early detection, monitoring, and management of cardiovascular risks in SCD. The WHA has classified SCD as a public health problem calling nations to establish control programs

3.1 JUSTIFICATION

Sickle cell disease (SCD) is a 'silent' problem with devastating effects in many parts of Kenya mainly Nyanza and Coastal areas. There is often very low awareness of this condition amongst people, and not much attention is given to the problem by the Ministry of Health. SCD is not included in routine health statistics because there is no national treatment guideline. SCD causes high rates of serious health problems and ends organ complications including cardiovascular.

Many children die undiagnosed and more than 90% die before 5 years of age due to lack of recognition and comprehensive care. High school dropout rates have been observed among those diagnosed with the disease often due to prolonged ill-health. In countries where comprehensive care is available, many people with SCD live normal lives into their 40s and 50s, or longer than this. No other study has been done in Kenya concerning cardiovascular risk in SCD using biomarkers

4.0 STUDY QUESTIONS

1. Do serum NT-Pro BNP and HsCRP levels change in asymptomatic SCD patients at a steady-state?

2. Is there a correlation between NT-proBNP, Hs-CRP levels, and hemoglobin level?

3. Does the duration of Hydroxyurea use correlate with levels of Hs-CRP?

4.1 BROAD OBJECTIVE

To determine serum NT- proBNP and HsCRP levels for prediction of cardiovascular risks (heart failure) in asymptomatic SCD patients in steady-state

4.2 SPECIFIC OBJECTIVES

4.2.1 PRIMARY OBJECTIVE

1. To determine the levels of NT-proBNP and HsCRP in asymptomatic SCD patients at steady state

2. To estimate the proportion of cardiovascular risk based on NT-proBNP and HsCRP values

3. To correlate NT-proBNP and HsCRP levels with hemoglobin

4.2.2 SECONDARY OBJECTIVE

1. To correlate cardiovascular risk with disease duration
2. To correlate Hydroxyurea use with HsCRP levels

5.0 METHODOLOGY

5.1 STUDY DESIGN

Cross-sectional descriptive study

5.2 STUDY AREA

The study was conducted at Kenyatta National Hospital hematology clinic, Baraka Medical Centre in Mathare, Nairobi County, and Thematic Unit of clinical Chemistry Human Pathology Department University of Nairobi

KNH is a tertiary referral and teaching hospital that receives patients from Nairobi as well as other health facilities in the country and also outside the country. The hematology clinic runs every Monday from 8 a.m to 1. 00 pm. It serves both adults and children with the two groups seen in different sections.

Baraka Medical Centre is located in the Mathare area 4A in Nairobi County. This medical Centre is owned by German doctors who generally see different types of patients including Sickle cell disease patients. It has a sickle cell clinic which runs every Tuesday between 8 am-5 pm. It serves both adults and children. The clinical support was provided by a Clinical officer who attends to the sickle cell disease patients

The NT-proBNP was carried out in the Clinical Chemistry laboratory using Finecare Fluorescent immunoassay analyzer and highly sensitive CRP was done using Biolis 50i Chemistry analyzer in the KNH Biochemistry laboratory

5.3 STUDY POPULATION

Patients who had been diagnosed with sickle cell disease using Hb electrophoresis from 5yrs and above and met the inclusion criteria were recruited

5.4 Inclusion Criteria

1. Patients who have been diagnosed with SCD from 5yrs and above

2. In a steady-state

5.5 Exclusion Criteria

1. Obese patients (>35kg/m^2-using BMI

2. Children less than 5yrs of age

3. Smokers

4. Patients with Diabetes mellitus

5. Patients with no manifest of vaso-occlusive crisis for the least 4weeks after the last episode

6. History of heart disease

7. Three or more months after the last blood transfusion

8. No febrile episode for at least 2weeks

5.6 SAMPLE SIZE

The sample size was calculated using the Fishers formula

$N=Z^2p\ (1-p)/d^2$

Where N=Desired sample size

Z=Standard normal deviation value corresponding to 95% confidence interval (=1.96)

p=Estimated prevalence 49.4%) Odero et al

d=Degree of precision

N=96

5.7 SAMPLING METHOD

A simple random sampling procedure was used to recruit patients who met the inclusion criteria during the study period until the desired sample size was attained.

5.8 CASE DEFINITION

According to Okocha et al 2014, the asymptomatic steady-state condition is defined as:

- No manifest crisis for at least 4 weeks after the last episode,
- Three or more months after the last blood transfusion and
- No febrile episode for at least 2 weeks**.**

5.9 RECRUITMENT AND CONSENTING PROCEDURES

The researcher with the help of a trained nurse and physicians recruited patients from the KNH Hematology clinic. The nurse together with the physicians who were attending to the SCD patients helped in identifying suitable patients for the study and referred to the researcher. Relevant information about the study was given to the patient

In Baraka Medical Centre, patients were first seen by a clinical officer and sent to the laboratory for a full haemogram as their routine. The clinical officer identified suitable patients for the study and referred to the principal investigator. Relevant information was given to the patients. In both sites, consent of participation was sought from the parent/guardian of children below 5yrs, who signed the consent form on their behalf. Informed consent was given to the participants above 18yrs to sign. An assent form was given to those between 8-18yrs. The researcher aided the patients in filling the forms after being given relevant information about the study. A screening proforma was used to select the patients who met the selection criteria. A questionnaire was issued to obtain social characteristics. Blood was collected from those who meet the inclusion criteria and consented.

6.0 DATA COLLECTION

Clinical methods

A study questionnaire was used to obtain medical data and to assess the eligibility of participation in the study. A screening proforma was used to obtain demographic data from enrolled patients. The assisting nurse took measurements of their height and weight of the adult patients,

Height

Standing height was measured once to the nearest O.5cm barefoot, the back square against the wall tape, eyes looking straight ahead, with a set square resting on the scalp and against the wall.

Weight

Weight was measured once to the nearest 100 grams using a lever balance, barefoot, in light garments.

Body Mass Index

The body mass index (BMI) was calculated using the World Health Organization (WHO)criteria as weight (in kilograms) divided by height (in meters) squared

$$\text{BMI}=\frac{\text{Weight (in kgs)}}{\text{Height (in meters)}^2}$$

Laboratory methods

Specimen collection

A blood sample (3ml) for adults and 2ml for children was drawn into a plain vacutainer (red top)It was allowed to clot and transported to the University of Nairobi Clinical Chemistry Laboratory for the specimens collected from KNH. Blood was centrifuged at 1000 RPM for 1minute, serum separated, stored in serum vials.

Blood samples from Baraka clinic were separated in their laboratory and the serum was put in serum vials. A cooler box with ice packs at approximately 4^0 C (2-8 ^{0}C) was used for temporary storage and the facilitation of transport to the clinical chemistry laboratory. All the serum obtained from the two sites were labeled using the study number at stored at -20^0 C until the time of analysis

Specimen analysis

The NT-proBNP analysis was done in the clinical chemistry laboratory using Finecare Flour immunoassay analyzer

Principle of the test

Fine care NT- proBNP rapid quantitative test uses a sandwich immunodetection method. When the sample is added to the test cartridge, the fluorescent-labeled detector anti-NT-proBNP antibodies on the sample pad bind to NT-proBNP antigens in the serum and form immune complexes. As the complexes migrate on the nitrocellulose matrix of the test strip by capillary action, the complexes of detector antibodies and NT-proBNP are captured to anti-NT-proBNP antibodies that have been immobilized on the test strip. Thus, the more NT-proBNP antigens in the serum, the more the complexes accumulated on the test strip. The signal intensity of fluorescent detector antibodies reflect the amount of captured NT-proBNP

Hs-CRP levels were determined by chemistry analyzer Biolis 50i using the immunoturbidimetric principle.

Principle of the test

Human CRP in the patient specimen, standard, or control reacts with anti-human CRP antibodies. The resulting immune complexes generate turbidity which is proportional to the CRP concentration and can be measured photometrically. Results are calculated using the CRP standard which is to be used with each series.

6.1 RISK ASSESSMENT

CVD risk assessment was done using the American Heart Association and U.S centers for disease control and prevention criteria for the CRP levels. CVD risk using HS- CRP levels were categorized as follows

- Low risk: less than 1.0 mg/L

- Average risk: 1.0 to 3.0 mg/L
- High risk: above 3.0 mg/L

Above 10 mg/L usually indicates acute inflammation

Risk assessment using NT-proBNP levels was done using the National Institute of Care excellence threshold of < 300ng/L.

7.0 QUALITY ASSURANCE

Commercial reagent kits were used for all biochemical assays. All analyses were performed according to the manufacturer's specifications by the principal investigator

Commercial quality control materials were included in all analytical runs. Results were only accepted when the control material was within acceptable limits.

SOPs were used to minimize the pre-analytical, analytical, and post-analytical errors

8.0 ETHICAL CONSIDERATION

Authority to conduct the study was sought from the Kenyatta National Hospital/ University of Nairobi (KNH/UON) Ethics and Research committee

After approval, all respondents were given information on the purpose and procedure of the study before the study commenced

Approval was sought from Baraka Medical Centre in Mathare to use their facility as one of the study sites

All respondents were assured of confidentiality. The questionnaires were coded and did not include the respondent's name

They were sealed in an envelope and the information was accessible to the researcher, the supervisors, and the statistician only.

A consent and where applicable an assent form was issued to the participants who meet the inclusion criteria. They were informed that there will be no victimization or any consequences for not participating or for withdrawing from the study.

9.0 DATA MANAGEMENT AND STATISTICAL ANALYSIS

All data emanating from the study was verified, cleaned, and entered into data entry sheets. Statistical analysis was performed using Statistical Package for Social Sciences, version 10.0 software for windows. For confidentiality data entered into the computer was protected by the use of passwords. Categorical variables were presented as percentages and continuous data summarized into means (standard deviations) or medians (interquartile ranges)

Pearson's correlation was used to test for correlation and statistical significance). The prevalence of cardiovascular risk defined by high or low NT-proBNP and HsCRP levels was analyzed and presented as a proportion with a 95% confidence interval. Student T-test was used to compare the means of the two measurements. All statistical tests were performed at a 5% level of significance. The data summary was presented in tables and graphs.

10.0 WORKFLOW PLAN

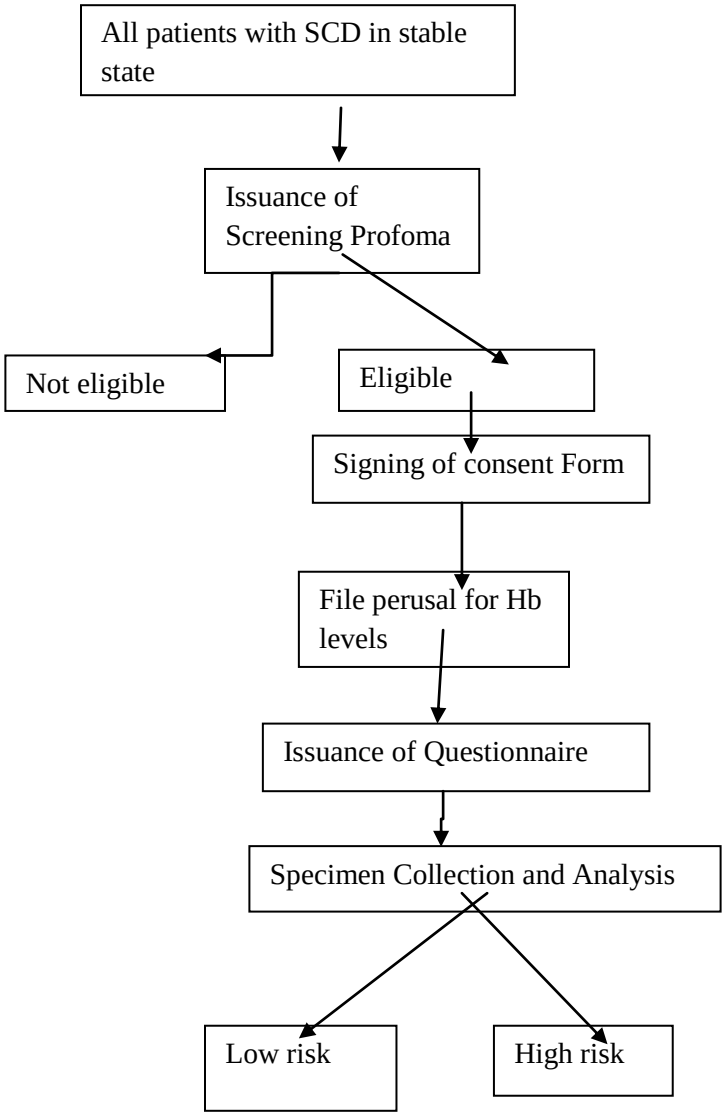


Figure 1: Work Flow Plan

11.0 RESULTS

During the sample collection period from Jan-March 2019, a total of 96 study subjects with confirmed sickle cell disease were enrolled in the study. However, during laboratory analysis six were excluded due to inadequate reagents so data analysis was done for 90 participants. Among the subjects recruited 12 were from KNH and 78 from Baraka Health Centre in Mathare. The numbers of females were 50 and male were 40. The high number of patients in the Baraka health center was due to its geographical location. It is located inside Mathare valley settlement where most of the patients live. Baraka health center is also a charitable organization where patients only pay a small fee hence, acts the low-income earners who cannot afford expensive healthcare centers. It also runs a purely sickle cell clinic once per week while KNH runs a combined haemato-oncology clinic.

The mean age of the patients was 12.1 (SD=6.8) years, while the median age was 10.5 (IQR=8) years. The minimum age was 5 years while the maximum age was 39 years.

Most of the patients (50%) were between 5 and 10 years of age. There were no subjects more than 40 years of age and only 2 patients were in the 36 - 40-year category as shown in Fig 2 below

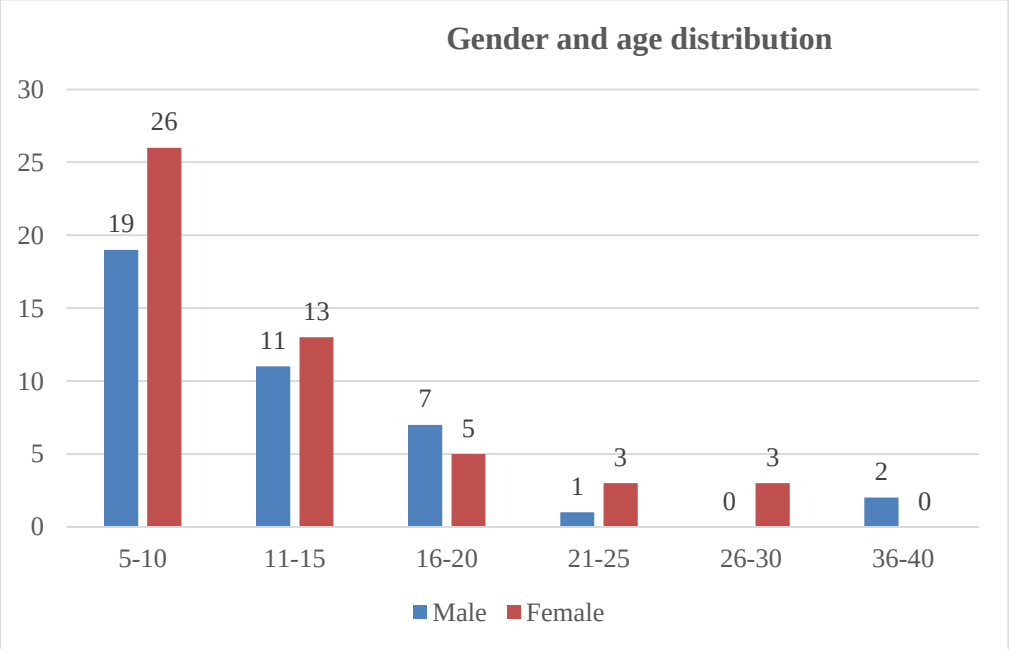


Figure 2: Gender and age Distribution of Study Participants

Levels of HsCRP

Serum HsCRP levels for study subjects were categorized according to the ACA risk categories. As shown in Table 1below, none of the subjects had CRP levels in the low-risk category. The majority (76%) had CRP values above 3.0mg, placing them in the high cardiovascular risk category. The mean HsCRP of the patients was 10.99 (SD=18.11), and the values ranged from 1.20mg/L to 118.9mg/L with a median of 4.6 (IQR=4.85).

Levels NT-proBNP

Serum NT-proBNP values were categorized according to AHA heart failure risk levels. The majority of subjects (64.4%) had normal Serum BNP values. The mean NT-proBNP of the patients was 354.4 (SD=644.96), while the median NT-proBNP was 253.1 (IQR=223.62). The minimum NT-proBNP was 18.0pg/ml while the maximum NT-proBNP was 5852.5pg/ml. This is shown in Table 1 below.

Table 1: Serum NT-proBNP and HsCRP Values

HsCRP (mg/L)	**Frequency**	**Percent**
Low risk: <1.0	0	0
Average risk: 1.0 to 3.0	22	24.4
High risk: > 3.0 mg/L	68	75.6
NT-proBNP (pg/ml)		
>300pg/ml	30	33.3
<300pg/ml	58	66.7

A Pearson correlation was done to determine the relationship between NT-proBNP and Hs-CRP levels. There was a negative correlation between the two parameters. The correlation was weak and not statistically significant. This is shown in figure 3 below.

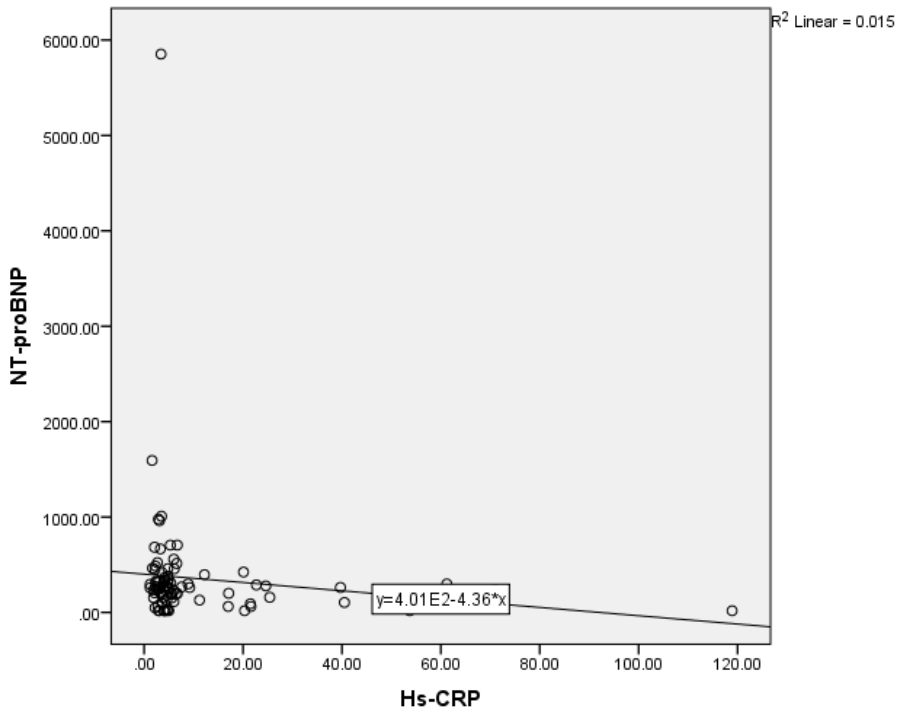


Figure 3: Relationship between NT-proBNP and HsCRP

Combined Cardiovascular Risk Groups

The study participants were categorized into cardiovascular risk groups based on both NT-proBNP and HsCRP values. A high-risk group was defined here as having both HsCRP levels above 3.0 and NT-proBNP >300pg/mL, while moderate risk were those with one of the two biomarkers elevated. Most of the study participants (77.8%) were in the moderate-risk group. This is shown in Table 2 below.

Table 2: Combined Cardiovascular Risk Groups

	Male Number (%)	Female Number (%)	P-value
High risk	13 (32.5)	7 (14.0)	0.044
Moderate risk	27 (67.5)	43 (86.0)	

Table 3: Risk of Heart Failure Sex Cross Tabulation

			Sex		
			Male	Female	Total
Risk of heart failure	At high risk	Count	13	7	20
		% within Sex	32.5%	14.0%	22.2%
	Average risk	Count	27	43	70
		% within	67.5%	86.0%	77.8%
Total		Count	40	50	90
		% within Sex	100.0%	100.0%	100.0%

Correlation of NT-proBNP and HsCRP levels with Hb levels

The mean hemoglobin (Hb) of the patients was 8.01 (SD=1.37). The minimum Hb was 4.10 while the maximum Hb was 13.2, with a median Hb of 8.0 (IQR=1.50). A statistically significant negative correlation was found between NT-proBNP and Hb level (p=0.003). This is shown in Table 5 and Figure 4 below. There was however no significant correlation between HsCRP and hemoglobin levels (Pearson correlation -0.132; p=0.215).

Table 4: NT-proBNP and Hb Levels

		NT-proBNP	Hb level
NT-proBNP	Pearson Correlation	1	-0315
	p-value		.003
	N	88	88

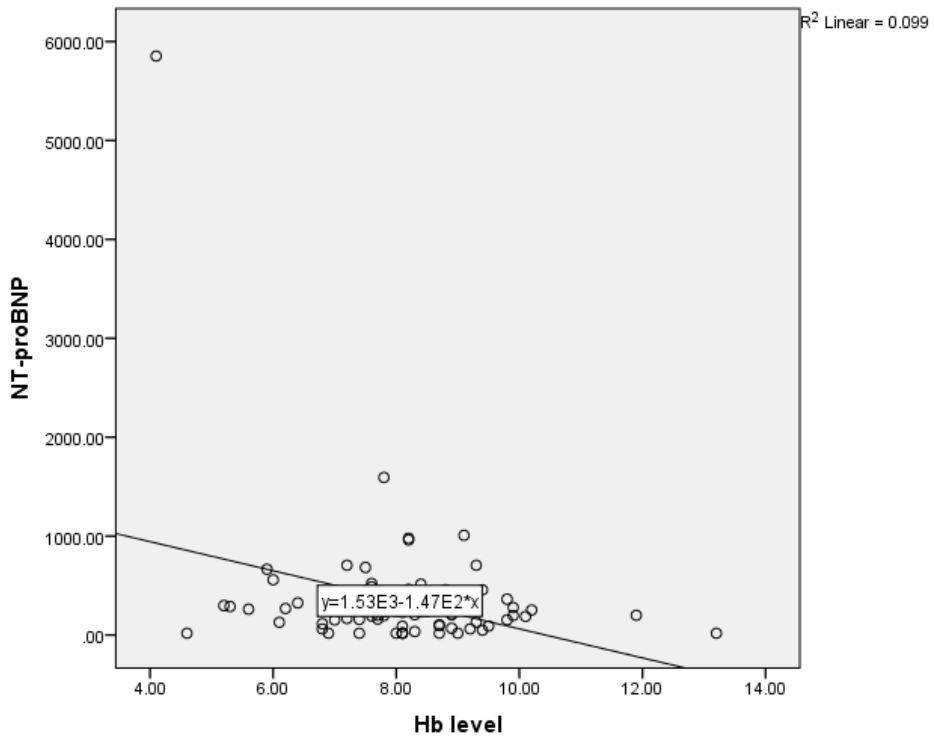


Figure 4: Correlation between NT-proBNP and Hb Levels

Correlation of Cardiovascular Risk and disease duration

The mean Disease duration of the patients was 8.07 years (SD=7.19). The minimum Disease Duration was 0.25years while the maximum was 39 years with a median of 6.0 years (IQR=7.50). An independent student's t-test was run to determine the differences in the mean disease duration among patients categorized as high risk and those of moderate risk. A mean difference of 2.8 years was obtained which was not statistically significant (p=0.280). This is shown in Table 5 below.

Table 5: Cardiovascular Risk and Disease Duration

	Cardiovascular Risk	N	Mean	Std. Deviation	p-value
Duration	At high risk	19	10.3	10.6	0.280
	Average risk	69	7.5	5.9	

Correlation of Hydroxyurea use with HsCRP Levels

The mean duration of hydroxyurea (HU) use among the patients was 32.83 months (SD=34.42), while the median HU use was 24.0 (IQR=37). The minimum HU use was 1month while the maximum was 216 months. A weak correlation was found between duration of HU use and serum HsCRP levels but it was not statistically significant (p=0.135) as shown in Table 8 below.

Table 6: Hydroxyurea Use and Hs CRP

		HU use (months)	Hs-CRP
HU use (months)	Pearson Correlation	1	-0.186
	p-value		0.135
	N	66	66

12.0 DISCUSSION

Among the 90 participants who were enrolled in this study, the majority of the patients were female n=50 compared to male n=40. The majority of the subjects fell in the age group of 5-10yrs (Fig 3). This coincided with a study done at KNH by Agai et al.2009 which showed that a high frequency between 5-9yrs. The explanations for the younger population in this study is that subjects were recruited from an outpatient facility during the normal school term, and older patients were in boarding school, secondly, it was a charity facility targeting mainly young subjects. The younger population could have been due to decreased life expectancy of sickle cell disease, male 42yrs, and female 48yrs (Orah et al., 1994).

The majority of the patients (66.7%) had NT-proBNP levels of less than 300pg/L which is the cut-off value of the risk of heart failure according to the National Institute of Health and care guidelines (NICE). This shows they were not in the risk category of heart failure (Table 1)

Some studies have shown risks at lower cut-off values. A study was done by Bay et al; 2009 showed that a raised NT-proBNP of >232ng/L identified patients with reduced Left ventricular ejection fraction of 40% (55-60%). From this study, NT-proBNP had a mean of 354.4, standard deviation 644.96, median 253.1, and IQR of 233. 62L.The majority of the patients (65%) had their NT-proBNp levels <300ng/L and (35%) had their levels >300ng/L.Machado et al 2006 found out that NT-proBNp levels of >160ng/L were associated with Pulmonary hypertension. This finding was similar to this study because patients who had NT-pro BNP levels raised were at a high risk of cardiovascular risk as their HsCRP levels were also raised. A possible explanation for difference includes lower sensitivity of biomarker or inappropriate cut-off for risk

A big number of the patients (76%) had their HsCRP levels above the high-risk category of >3.0mg/L with a median of 4.6 and IQR of 4.85. A study done by Fatuma et al 2010, showed HsCRP median of 5.0(0.16-1.85) with P=0.17 in sickle cell disease patients in a steady state. Okocha et al 2010 found that HsCRP levels were raised P=0.001 and it showed a negative predictive value with disease severity P=0.17. C-reactive protein is a well-known marker for inflammation. Increased levels of the inflammatory biomarker CRP at baseline are associated

with childhood VOC (Suba et al., 2010). This could explain the high percentage (75.6%) in the high-risk category.

In this study, a minority of the patients had (22.2%) were in the high-risk category of heart failure compared to 77.8% who were at average risk. The majority (32.5%) was male (Table 3). Those at high risk had both NT-proBNP and HsCRP raised. The maximum NT-proBNP level was 5852pg/ml. Highly sensitive C-reactive proteins are a strong independent risk factor for cardiovascular events this contrasts with the findings of this study because HsCRP was elevated in all the subjects This can be explained by the fact that CRP is a marker of any inflammation and thus nonspecific. The relationship between the two was a negative correlation

Another objective of this study was to correlate NT-proBNP and HS CRP levels with haemoglobin levels. Sickle cell disease patients normally have low Hb levels of between 6g/dl-8g/dl. This was similar to this study where we found the lowest level is 4.1g/dl and a median of 8.0. We found out that there was a negative correlation between the two parameters (Fig 3). This coincides with a study done by Aliyu et al .,2010 which found out that low Hb level related to high NT-proBNp levels

The relationship between HsCRP and Hb levels was weak r=-0.132 but statistically significant P=0.215(Table 6)

The lowest duration of disease in this study was 0.25 yrs and the highest 39yrs. The T-test carried out found a P-value of 0.28 which was not statistically significant. This could be due to adherence to the treatment given. Patients who adhered to the treatment given despite the duration they had the disease could not be at a high risk of a cardiovascular event.

The study also looked at the relationship between hydroxyurea use and HsCRP levels. It was found out that the minimum use was 1month and maximum 216months with a median of 24months.The p-value was -0.135 and r was-0.186(Table 6). This was a weak relation with no statistical significance. Hydroxyurea raises the level of HbF which helps the RBCs maintain their biconcave shape and to be flexible. The low percentage of HbF is associated with a high risk of developing vaso-occlusive complications, organ damage, and early death.

13.0 CONCLUSIONS

1. The mean NT-proBNP levels among stable asymptomatic sickle cell disease patients in this study was 354.4 (644.96), while the median NT-proBNP was 253.1 (IQR=223.62. The proportion of patients with high-risk category of heart failure based on NT-proBNP in this study was 33.3%.

2. HsCRP values among these patients ranged from 1.2mg/L to118.9mg/L. The proportion of patients with high-risk HsCRP values was 75.6%.

3. There was a significant negative correlation between NT-proBNP and Hb levels (p=0.003) among the study participants.

4. There was no correlation between Hs CRP and Hb levels (P=0.215) in this study.

14.0 RECOMMENDATION

Further studies should be done alongside ECHO to determine the sensitivity, specificity, and predictive value of NT-proBNP for heart failure detection in asymptomatic SCD patients in our population.

15.0 STUDY LIMITATIONS

This study did not capture older children who were in school during the data collection period

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17.0 APPENDEX 1: SCREENING PROFORMA

Do you smoke?

Yes ☐

No ☐

Do you suffer from Diabetes mellitus?

Yes ☐

No ☐

Have you ever been diagnosed with heart disease?

Yes ☐

No ☐

When was the last time you had a vaso-occlusive crisis

When was the last time you had blood transfusion?

BMI ☐

Hb level ☐

Are you on Hydroxyurea Yes ☐ No ☐

If Yes, for how long? ☐

FOR OFFICIAL USE

Recruited

Yes No ☐ ☐

Study number _____

17.1 APPENDIX 2: QUESTIONAIRE

Study no._____ ☐

Age (yrs)_____ ☐

Date of birth_____ ☐

Cell phone no._____

Year of diagnosis of SCD_____

Gender Male ☐ Female ☐

Marital status Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated ☐

Occupation

Self employed ☐

Employed ☐

Unemployed ☐

Retired ☐

Training/ student ☐

Level of education

Primary school ☐

Secondary school ☐

College ☐

Area of residence ☐

Others (specify)

LABORATORY RESULTS

	UNITS	RESULT	REF.RANGE
HsCRP	**Mmol/L**		**<1.0mg/L Low risk** **1.0-3.0mg/L Average risk** **>3.0mg/L High risk** **>10.0mg/L Acute inflammation**
NT-proBNP	**ng/L**		**>300ng/L at risk**
Hb level	**g/L**		**Male: 12.0-18.0g/L** **Female:12.0-16.0g/L**

17.2 APPENDIX 3: CONSENT FORM

Investigation statement

Investigator: Joyce Maganga

Position: Post graduate student, Department of Human pathology, University of Nairobi

Introduction

The investigator is conducting a research study to investigate cardiovascular risk (heart diseases) in patients with sickle cell disease. Sickle cell disease is a chronic disease and as you live, it can affect organs the heart, kidneys, bones and brain

Objective of the study

. The study will provide information as to the extent of this problem among sickle cell disease patients in our set up for future decision-making regarding detection, control and treatment. This will help improve the care of these patients including you.

Benefits

This study will identify whether you are at risk of developing heart condition or not. It will also help in early identification of persons with the condition to be referred to a physician for appropriate. The

information gathered will also help the clinicians in management of people with this condition.

Risks

You will not suffer any physical risks however there might be a hematoma after the specimen has been taken but this will disappear with time

Confidentiality

All tests carried out will be free of charge and the results will be analyzed in coded form with strict confidentiality. Your name will not appear anywhere in the information to be gathered from you.

Participation and compensation mechanism

Your participation in this study is entirely voluntary. No remuneration or compensation will be offered to participants of the study. You are free to decline to participate or withdraw from this research. Whether you choose to participate or not, all the services you receive in this clinic will continue and you will still be offered the treatment that you routinely get in this clinic. A questionnaire will be offered to gather more information from you. You will also have a right to confidentiality of the information gathered from you. You are also free to ask or make an enquiry to any area you do not understand for clarification

A consent form will be issued to the adult participants (above 18 yrs)

An informed assent form will be issued to children between 8yrs -18yrs

Parents/guardians of the children participants of less than 8yrs will sign a consent form on their behalf to allow them participate in the study

Type of specimen

3ml of blood will be drawn for the purpose of this study for the adult participants (above 18 yrs) and the children between 8-18yrs.2ml of blood specimen will be taken from children below 8yrs

Information on researchers

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may ask the following:

Name: Joyce Maganga (Primary Researcher)

Mobile no: 0722824521

Email: Joymaganga2017@gmail.com

Name: Prof.Angela Amayo (Supervisor)

Mobile no: 0733617678

Email: aamayo2@gmail.com

Name: Dr Fredrick Okinyi (Supervisor)

Mobile no: 0720325448

Email: fokinyi24@gmail.com

Name: Dr K.C Wafula (Supervisor)

Mobile no; 0722516690

Email: drwafula59@gmail.com

17.3 APPENDIX 4: CONSENT DECLARATION

(Above 18yrs)

I have read the information that has been presented to me and had the opportunity to ask questions about it. The questions that I have asked have been answered to my satisfaction. I therefore agree to participate in this study.

Name of participant_____

Signature of participant_____

Date _____

17.4 APPENDIX 5: INFORMED CONSENT DECLARATION

(Children 8-18yrs)

I_____parent/guardian to_____have been explained fully on this study. I was allowed to ask questions and clarifications which were answered to my satisfaction. I hereby give consent to agree my child to participate in this study.

17.5 APPENDIX 6: ASSENT FORM

Introduction

My name is Joyce Maganga from University of Nairobi. I am interested in carrying out a research on sickle cell disease that might help children with sickle cell disease live a better live. As a sickle cell disease patient lives there are possibilities of developing a heart complication. I therefore want to check if you are in danger of developing a heart complication. I am inviting you to participate in this research which will be of benefit to you. I have discussed with your parent /guardian and know that I am going to ask you for an agreement to participate from you.

You can choose whether to participate or not. I you agree to participate your parent/guardian also have to agree but if you do not wish to take part, you do not have to, even if your parents/guardian has agreed.

If there may be some words you don't understand or anything you want to be explained to, please ask I will take to explain to your satisfaction. If you decide not to be in the research it is okay nothing will change. This will remain to be your clinic and you will continue getting services as usual.

Benefits

In case you are found to have any heart condition, you will be referred to a physician for further management.

Risks

There will not be exposed to any danger in participating in this study.

Specimen collection

Blood specimen (2ml) with be drawn from you to carry out the tests that will determine if you have any heart complication.

17.6 APPENDIX 7: INFORMED ASSENT DECLARATION

(8-18yrs)

I_____parent/guardian of_____having been explained to about this study, was given an opportunity to ask questions and were answered satisfactorily.

I hereby agree my child to participate in this study

Signature of parent/guardian_____

Date_____

17.7 APPENDIX 8: FOMU YA IDHINI

Kauli ya uchunguzi

Mtafiti: Joyce Maganga

Cheo: Mwanafunzi wa shahada kuu katika chuo kikuu cha Nairobi idara ya Pathologia

Kwa mhusika,

Mtafiti mkuu angependa kufanya utafiti kuhusu ugonjwa wa moyo Kwa watu wenye ugonjwa wa Selimundu. Mgonjwa wa Selimundu anavyozidi kuishi huwa anaweza pata magonjwa mengine ya figo, akili, mifupa na moyo. Utafiti huu utaweza kugundua kama mgonjwa yuko kwa hatari ya haya magonjwa. Hii itasaidia kupata matibabu mapema kabla ugonjwa huu kuenea.

Faida na hatari za utafiti

Utafiti huu utachunguza kama wewe uko kwa hatari ya kupata matatizo ya moyo. Hakuna hatari yoyote itakayotarajiwa utakaposhiriki kwa utafiti huu

Matokeo ambayo yatapatikana kutoka kwa huu utafiti yata saidia sana madaktari kugundua mapema kama wagonjwa wa selimundu wako kwa hii hatari ili wawekwe kwa matibabu mapema iwezekanavyo.

Vipimo vyote zinafanywa bila malipo yoyote. Matokeo ya huu utafiti yatawekwa siri wala hayatapatiwa mtu yeyote asiyehusika na utafiti huu. Zaidi ya hayo badala ya jina la mhusika, nambari zitatumika kutambua mgonjwa. Matokeo yatazungumziwa kwa wasimamizi wangu pekee.

Kushiriki

Kushiriki kwa utafiti huu utakuwa kwa njia ya kujitolea. Hakuna malipo yoyote atakayolipwa mshiriki kwa utafiti. Iwapo ungependa kushiriki, uamuzi huu hautaadhiri kwa njia yoyote matibabu yako au utakavyo hudumiwa kwa cliniki hii

Iwapo utakubali kushiriki, wale wagonjwa wako na miaka kumi na nane (miaka 18) kuenda juu watapewa fomu wajaze. Wazazi ama walezi wa Watoto watajaza fomu badala ya watoto. Hii fomu itakuwa na maswali ambayo yatakuwa ni muhimu sana kwa huu utafiti. Baadaye damu ya kiasi cha mililita tano itatolewa kwa ajili ya huu utafiti.

.**RIDHAA TAMKO (miaka 18 kwenda juu)**

Nimesoma ujumbe nimeupewa na kipatiwa nafasi ya kuuliza maswali yoyote. Maswali ambayo nimeyauliza yamejibiwa na nikaridhika.

Nakubali basi kuuzishwa kwa huu utafiti

Jina la Mhusika _____

Sahihi ya mhusika _____

Tarehe _____

KUTIWA SAINI TAMKO

(Watoto chini ya miaka 8)

Mimi_____Mzazi/mlezi wa _____. Nimeelezewa kuhusu huu utafiti na nikapewa muda wa kuuliza maswali ambayo yamejibiwa kadri na matarajio yangu kwa njia ya kuridhisha. Kwa hio ningependa kupeana saini yangu na pia kujitolea kushiriki kwa utafiti huu.

Nakubali mtoto kuhusishwa kwa utafiti huu.

Sahihi ya mzazi/mlezi_____

RIDHAA TAMKO

(Kati yamiaka8 hadi 18)

Nimesoma ujumbe nimeupewa na kupatiwa nafasi ya kuuliza maswali yoyote. Maswali ambayo nimeyauliza yamejibiwa na nikaridhika.

Nakubali basi mtoto wangu kuuzishwa kwa huu utafiti

Jina la Mzazi/mlezi _____

Sahihi ya mhusika _____

Tarehe _____