A COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS BETWEEN SICKLE CELL ANAEMIA PATIENTS ON HYDROXYUREA AND HYDROXYUREA NAÏVE PATIENTS AT KENYATTA NATIONAL HOSPITAL, KENYA

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A Comparative Study of Haematological Parameters between Sickle Cell Anaemia Patients on Hydroxyurea and Hydroxyurea Naïve Patients at Kenyatta National Hospital, Kenya

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DECLARATION

This thesis is my original work and has not been presented a degree or any award in any university or institution.

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DEDICATION

I dedicate this work to my parents Mr and Mrs Kanyiri, my sister Brenda and my partner Peter for walking this journey with me and encouraging me all through.

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ACRONYMS AND ABBREVIATIONS

SCA	Sickle Cell Anaemia.
Hb	Haemoglobin.
HbF	Foetal haemoglobin.
HU	Hydroxyurea.
DNA	Deoxyribonucleic acid.
RR	Ribonucleotide reductase.
WBCs	White blood cells
RBCs	Red blood cells.
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
RDW-CV	Red cell width distribution
PLT	Platelets
MTD	Maximum tolerated dose.
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention

WHO World Health Organization

EDTA Ethylene Diamine Tetraacetic Acid

IQC Internal Quality Controls

DEFINITION OF TERMS

Hydroxyurea naïve patients This term refers to patients who have not been taking hydroxyurea at all for a period of at least three months prior to the study.

R state This refers to the oxygenated form of the red blood cell.

Sickle cell anaemia A genetic condition characterized by the deformity of red blood cells from their normal round shape to a sickle shape in hypoxic conditions.

Steady state This is when a sickle cell anaemia patient has not experienced painful crises or had any illness or blood transfusion for a period of at least three consecutive months.

T state This refers to the deoxygenated form of the red blood cell.

ABSTRACT

Sickle cell anaemia is an autosomal recessive blood disorder that has over the years been a big burden on healthcare in Kenya and Africa as a whole. The disease tends to be caused by a point mutation that occurs in the beta globin chain of red blood cells. The gene Adenine (A) is replaced by the gene Thyamine (T). This in turn causes an alteration of the amino acids from the hydrophilic Glutamic Acid to the hydrophobic Valine. This in turn causes the production of red blood cells that tend to sickle in hypoxic conditions, HbS. The production of this type of red blood cells is characterized by various complications. The symptoms include; anaemia, jaundice, stroke, bacterial infections, vaso occlusive crises, acute chest syndrome, splenic sequestration among others. Vaso occlusive crises is one of the major complications of the condition. This is whereby a patient experiences a substantial amount of pain in any body part. Most commonly in the back, chest or legs. This pain occurs when the sickled red blood cells block the flow of blood to certain tissues thus depriving them of oxygen. This in turn causes the body to initiate an inflammatory response so as to deal with the problem. The key strategy in the management of these patients is achieving a steady state. This refers to a situation whereby a patient has not experienced any illness or any vaso occlusive crises and has not received any blood transfusion in a duration of at least 3 consecutive months. In order to achieve this, sickle cell patients have to take a lifelong regimen of a drug named hydroxyurea (HU). This drug is not provided free and due to this and various other reasons, some patients within the Kenyan population have been found not to take this daily medication. Other various reasons such as a belief the drug would cause cancer, side effects felt from it and a lack of understanding of its purpose contributes to non-compliance to taking it. Sickle cell anaemia patients require routine laboratory analyses which include a full blood count. The full blood count is the test most utilized in the routine monitoring of these patients. However, there is no data that depicts the differences, if any, of the haematological parameters within the Kenyan sickle cell population and such data would lead to enhancing the management of these patients through hydroxyurea by both their clinicians and caregivers. The objective of this study was to compare the haematological parameters; haemoglobin levels (Hb), white blood cell (WBC) counts and Platelet (Plt) counts, of sickle cell patients in steady state, both those taking hydroxyurea and those not taking hydroxyurea. For this a comparative cross-sectional study was carried out at the Kenyatta National Hospital where 92 sickle cell patients in steady state were selected by systematic sampling. Of these, 46 patients were compliant to taking hydroxyurea and the other 46 patients were not taking hydroxyurea at all for a period not less than 3months. Upon giving consent, about 2ml of whole blood was collected from each participant and placed in vacutainers containing the anticoagulant Ethylene Diamine Tetra Acetic Acid. This blood was then evaluated for full blood counts, obtaining parameters including the white blood cell counts, haemoglobin levels and platelet counts. Demographic data was collected using a questionnaire, which was also used to collect data for screening of participants and collected data entered into a database. The student t test, two sample t test assuming unequal variance, was then used to assess the significance of difference between the

respective means of the groups. A p value of less than or equal to 0.05 was considered statistically significant in this comparison. The mean haemoglobin level of the group of patients on hydroxyurea was found to be significantly higher compared to the mean haemoglobin level of the hydroxyurea naïve group. On the other hand, the mean white blood cell count of the group of patients on hydroxyurea was found to be significantly lower compared to the mean white blood cell count of the hydroxyurea naïve group. The mean platelet count of the group of patients on hydroxyurea was also found to be significantly lower compared to the mean platelet count of the hydroxyurea naïve group. In conclusion, a statistically significant difference between the haemoglobin, white blood cell counts and platelet counts was noted between the two groups. This included a higher mean in Hb in the group of patients on hydroxyurea and a lower mean in white blood cell count and platelet count. These findings lead to the conclusion that hydroxyurea has a significant impact on the haematological parameters of sickle cell patients. This impact is positive and it consequently leads to an overall positive impact on health status for sickle cell patients. Studies such as this can form a basis for further research on the mechanism of action of hydroxyurea on the blood parameters.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Sickle cell anaemia (SCA) is an inherited blood disorder that has a proven a major health challenge in Kenya as well as other parts of the world. It affects millions of people worldwide and has been found to be more so among people of African, Mediterranean or Saudi Arabian ancestry (Maakaron *et al.,* 2016). According to the World Health Organization, of all the reported cases of SCA, up to 75% - 85% of these occur in Africa with 6-9 million children being born with the disease each year in the continent alone (WHO, 2010). Those who do not depict the disease state but possess the carrier state were found to attribute for 28% - 35% (Tenge, 2014). In Kenya, SCA is variably distributed among the various ethnic groups. Communities in the coastal region, have been found to have the highest prevalence for SCA. This is at 35%. The inhabitants of the Lake Victoria region come a close second with a prevalence observed to be at 20 - 30%. A study conducted in the western region of the country showed that about 4.5.% of children born here have sickle cell disease (Wanjiku *et al.,* 2019).

Sickle cell anaemia is an inherited genetic disorder that affects the haemoglobin molecule found in the red blood cells. It is not a contagious condition and thus not transmissible from one individual to another (Chakravorty *et al.,* 2015). Haemoglobin is the protein structure responsible for transporting oxygen and carbon dioxide molecules within the blood. An individual inherits two haemoglobin genes, one from each parent. When only one of these is a haemoglobin S gene, it results in the individual being a carrier, whereas if both the haemoglobin genes inherited by the individual are of haemoglobin S genotype, it results in sickle cell anaemia. Thus, sickle cell anaemia is said to be a recessive disorder (Piel *et al.,* 2013). People who are carriers of HbS are said to have sickle cell trait and do not exhibit any symptoms of sickle cell. They lead fairly

normal lives. Persons with sickle cell anaemia, however, exhibit various symptoms as a result of the condition, that affect their ever day lives (Mc Gann *et al*., 2016).

A point mutation within the DNA sequence is the primary cause of sickle cell anaemia. This mutation occurs in Beta- globin gene that is located on chromosome 11 where an Adenine (A) is replaced by a Thymine (T) (Chakravorty *et al.,* 2015). This leads to an alteration of the amino acid where the glutamic acid is replaced by valine. This change causes the body to produce HbS instead of the normal HbA. The resultant red blood cells possess the tendency to sickle in hypoxic conditions leading to a crescent shape and leading to various complications (Pace *et al.,* 2012). Normal red blood cells are disk shaped and flexible. However, sickle cell red blood cells are sickle shaped and rigid. They do not survive as long as normal red blood cells do. This causes them to have difficulty passing through blood vessels and stick to the walls of these. This hinders the flow of blood to various tissues and thus oxygen cannot reach these sites. This in turn leads to pain which is referred to as vaso occlusive crises (Piel *et al.,* 2013). The sickle cells do not last as long as normal red blood cells. Normal red blood cells can last for about 90 to 120 days in circulation whereas sickle cells may last only up to 10 to 20 days. Under normal circumstances, the body keeps on producing red blood cells to replace the old ones once they get destroyed but in sickle cell anaemia, the bone marrow is unable to keep up with the rapid rate of red blood cell destruction leading to anaemia. Other symptoms include high risk of serious infection, shortness of breath, acute chest syndrome, delayed growth and stroke (Maakaron *et al.,* 2016).

Hydroxyurea (HU) is currently one of the widely used drugs available for the management of sickle cell anaemia. It works by increasing the synthesis of HbF by the body. It also serves an inhibitory role on the polymerization of the sickle cells (Charache, 2017). The main aim in management of sickle cell patients is to achievement of steady state. Steady state is the situation whereby the patient is not undergoing hemolytic crisis, is not experiencing pain and does not depict any clinical illness for a period preceding 3 months (Juwah *et al.,* 2004). A sickle cell patient is said to be in steady state when they have not had an acute pain episode, a blood transfusion or any

illness for a continuous 3months (Ballas, 2011) .The use of hydroxyurea has been found to lead to an overall improvement in the quality of life for sickle cell patients by having a reduction in the number of hospitalizations, painful crises and episodes of acute chest syndrome suffered by the patients (Mulaku *et.al*, 2013). However, despite these positive effects depicted by the drug, HU continues to be highly underutilized. One of the reasons for this is the failure to maintain compliance by the patients or their caregivers as well as lack of experience with hydroxyurea among healthcare givers. This may be due to the cost of hydroxyurea which stands at about KSh 50 per tablet and resultantly about KSh 1500 per monthly dose (Mulaku *et.al*, 2013). Other reasons including the side effects, fear it causes cancer and the limited availability of HU also contributes to this non-compliance. In Kenya, HU has not been incorporation in the Kenya National Guidelines for use in children of under the age of 5years (Mc Gann et.al. 2016).

A number of sickle cell anaemia patients are not on hydroxyurea medication. Some of these are however able to lead fairly normal lives by just undertaking some measures to help avoid getting vaso occlusive crises. These include adequate hydration, staying warm and eating a balanced diet. As such there is a need to evaluate the haematological parameters of those taking hydroxyurea religiously in relation to the haematological parameters of those who are not on the medication.

The complete blood count (CBC) is a great tool used in the management of sickle cell anaemia. Understanding the haematological profiles of sickle cell patients in steady state can be a predictor of clinical outcome and help in devising management strategies for the patients.

1.2 Statement of the problem

Sickle cell anaemia is a public health burden affecting people worldwide especially people in the African continent. The main strategy in the management of sickle cell is the achievement and maintenance of steady state. Hydroxyurea (HU) is the key drug administered in order to achieve this. This drug is intended for use for the life time of

sickle cell anaemia patients. Studies conducted in India in the past have shown that hydroxyurea affects the red blood cells causing production of non-sickled Red Blood Cells (RBCs). However, the daily uptake of the drug is very important to maintain this since the bone marrow will continuously be programmed to make HbS due to the genetic mutation (Agrawal *et al.,* 2013). It has also been found to affect the other blood cells, WBC and Platelets in other studies conducted in the United States (McGann *et al.,* 2015). However, there is no data showing whether there is any difference between the haematological profiles of sickle cell patients in steady state taking hydroxyurea and those not taking hydroxyurea within the Kenyan population of sickle cell anaemia patients.

1.3 Justification

The aim of this study was to determine the blood count parameters of sickle cell anaemia patients who took their doses of hydroxyurea consistently for at least three months prior to the study and compare these with the blood count parameters of the sickle cell anaemia patients who did not take any hydroxyurea for a period of at least three months prior to the study. Understanding the complete blood counts of sickle cell patients in steady state will be able to provide baseline data on the effect of hydroxyurea among the sickle cell patients in Kenya. This data can also be used to create evidence-based guidelines for compliance to taking hydroxyurea among sickle cell patients. It can also help the Ministry of Health in their support or lack-there-of for the regimen.

1.4 Research Questions

i. Is there a difference in Hb levels between sickle cell anaemia patients taking hydroxurea and hydroxyurea naïve patients?
ii. Is there a difference in WBC counts between sickle cell anaemia patients taking hydroxurea and hydroxyurea naïve patients?
iii. Is there a difference in platelet counts between sickle cell anaemia patients taking hydroxurea and hydroxyurea naïve patients?

1.5 Objectives

1.5.1 Broad objective

To determine and compare the haematological parameters between sickle cell anaemia patients on hydroxyurea and hydroxyurea naïve patients.

1.5.2 Specific objectives

1. To determine and compare Hb levels between those steady state sickle cell patients on hydroxyurea and those not on hydroxyurea.
2. To determine and compare WBC count, between those steady state sickle cell patients on hydroxyurea and those not on hydroxyurea.
3. To determine and compare the platelet count, between those steady state sickle cell patients on hydroxyurea and those not on hydroxyurea.

1.6 Limitations of the study

There was no way to tell with certainty whether the participants were compliant with hydroxyurea or not. This study was dependent on an honesty system from the participants for this selection criteria and also whether or not they were in steady state at the time of the study. To counter this limitation, it was clearly explained to the participants that accuracy of the information they provided was required to achieve results that were as accurate as possible.

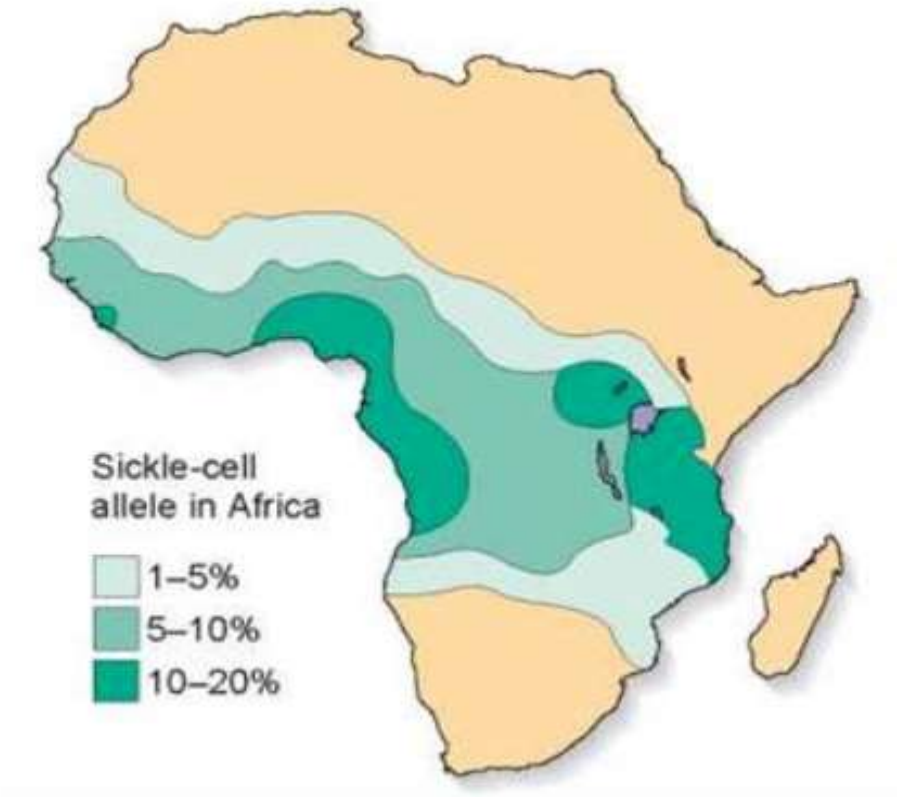
Since convenience sampling was utilized for the study, it was difficult to match the participants in the two groups. This made it difficult to be able to accurately compare the two groups I term of their demographics. However, this was mitigated by using patients of an age where their haematological parameters lie within the adult's reference range, that is, 12 years and above.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of Sickle cell anaemia

According to the Centers for Disease Control and Prevention (CDC), sickle cell anaemia affects millions of people in the world and more so people of African, Mediterranean, Saudi Arabian, Indian and Spanish descent (2018, para. 1). However, it is also a markedly neglected matter of public health in low income countries. The prevalence of sickle cell disease has been found to correlate directly to the prevalence of sickle cell trait. The World Health Organization (WHO, 2006) estimates that wherever there is a prevalence of sickle cell trait of more than 20%, the resultant rate of occurrence of sickle cell disease is at least 2%. The disorder is mainly found among people living in regions where malaria is or has been highly prevalent. Also noted, is that persons suffering from sickle cell trait are naturally protected against severe forms of malaria (Maakaron *et al.*, 2016). In the United States, about 8% of the Black Americans present with the sickle cell gene while the sickle cell trait has been found to have a prevalence of 30% in Africa. According to the United Nations, an estimated 20 to 25 million people worldwide have sickle cell disease with about 12 to 15 million of these being found in Africa (Aygun *et al.,* 2012). According to WHO, this has been observed in equatorial Africa where the prevalence of sickle cell trait is between 10% to 40% while the prevalence of the same in the north African coast is between 1% and 2% and less than 2% prevalence in South Africa (Figure 2.1). According to a study conducted in Kenya, about 4.5% of all children born in the western region of the country are born with sickle cell disease (Wanjiku *et al.,* 2019).



Retrieved from Forum, W. E. (2019)

Figure 2.1: Map showing the distribution of the sickle cell gene in Africa

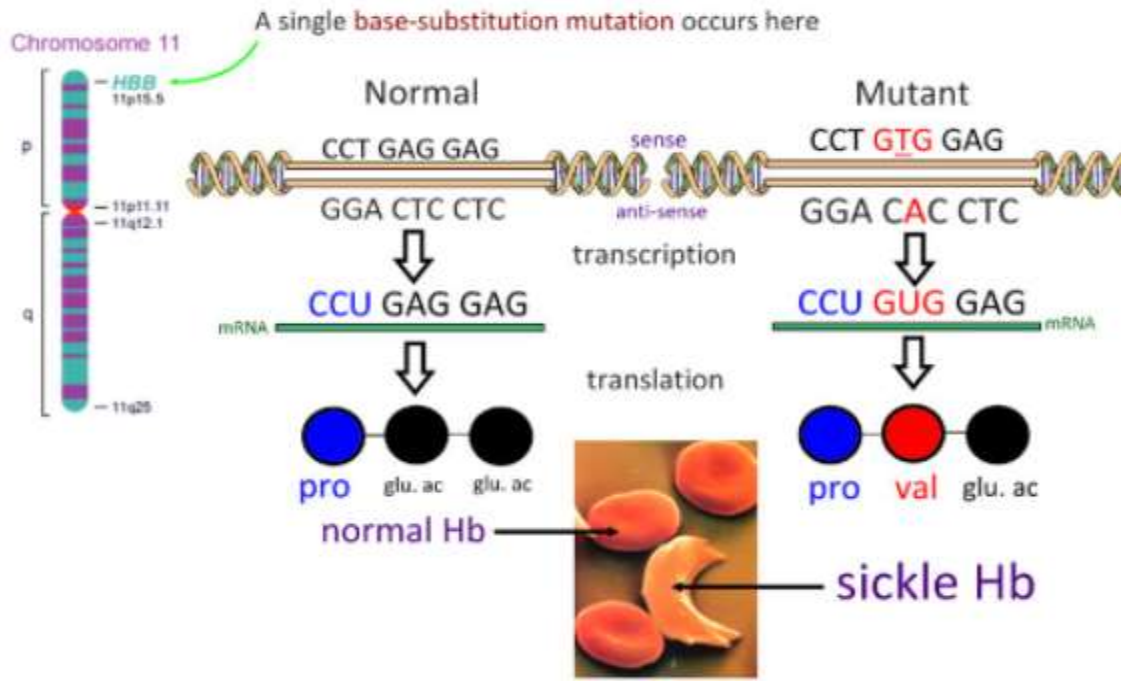
2.2 Pathophysiology of Sickle cell anaemia.

Sickle cell anaemia is one of the many haematological disorders that have been discovered to affect mankind. Sickle cell anaemias are a category of disorders that affect the haemoglobin component of red blood cells. Haemoglobin is the part or the red blood cells that is particularly responsible for the transport of oxygen from the lungs to the tissues as well as transport carbon dioxide from tissues, to the lungs for expulsion from the body (Bridges, 2002).

Haemoglobin is made up of two basic parts; the non-protein heme and the globin. The heme contains an iron (Fe^{2+}) ion that binds to oxygen. The protein-globin component is composed of four subunits; two alpha-globin chains and two beta-globin chains, in

adults. However, in fetal life gamma- globin chains operate instead of beta-globins in fetal haemoglobin. The HBA gene is responsible for the development of the alpha globins while the HBB gene controls the production of the beta globulins (Pace *et al.*, 2012).

Mutations during the development of the beta globin chains lead to the various types of sickle cell disorders. Mutations in the HBB gene cause alterations in the beta globulin chains produced. This occurs when the alteration results in a change of a DNA base from adenine (A) to thyamine (T). This causes the amino acid in position 6 to be substituted from glutamic acid to valine. The resultant haemoglobin is HbS (Bridges, 2002).



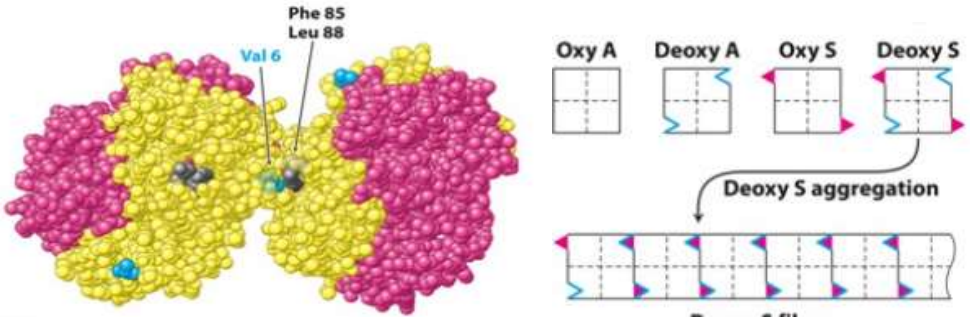
Retrieved from Microangiopathy Sickle Cell Lab

Figure 2.2: Image showing point mutation in sickle cell disease

Other variants of haemoglobin can occur due to mutation of the HBB gene including HbC, HbD and HbE. When only one of the beta globin subunits is replaced by HbS the

sickle cell trait occurs. When both beta globulin subunits are replaced by HbS then sickle cell anaemia occurs (Agrawal *et al.*, 2014). In other instances, one of the beta globulin segments is replaced by HbS while the other is replaced by a different variant of Hb such as HbC. This is referred to as sickle- haemoglobin C.A mutation can also occur that results in the reduction of the amount of beta globin chains produced. This is referred to as beta thalassemia. When an individual's HBB genes undergo mutations leading to both HbS and beta thalassemia, then this is referred to as haemoglobin S-beta thalassaemia. All these are categories of sickle cell disorders (Pace *et al.*, 2012).

In HbS red cells, the valine residue that was replaced glutamate rests on the surface of the deoxygenated molecule. This is referred to as the T state molecule. This creates a hydrophobic patch that needs to interact with another hydrophobic patch of another cell in order to aggregate. The second hydrophobic patch required is formed by Phe85 and Leu 88 on the beta chain of the neighboring molecule (Figure 2.3). A total of 14 chains of multiple haemoglobin molecules need to be interlinked in order to form one HbS fibre. These aggregates do not form in the oxygenated state that is the R state, of haemoglobin S since in the R state, the residues Phe 88 and Leu 88 are mostly buried inside the beta chain of their haemoglobin molecule assembly (Eaton, 2019).



Retrieved from *Hemoglobin, an Allosteric Protein Stryer Short Course*

Figure 2.3: Formation of a hydrophobic patch

When the resultant mutation leads to HbS, the red blood cells' haemoglobin molecules are able to form large fibrous aggregates that extend across the cell. This leads to a distortion of the red blood cells causing them to clog small capillaries and impair blood flow. This in turn causes painful swelling of the extremities and an increased risk of stroke or bacterial infections. The solubility of the deoxyhaemoglobin in HbS is substantially reduced and since these red blood cells do not last as long as normal red blood cells in circulation, anaemia occurs (Mc Gann *et al.,* 2016).

In addition to the red blood cells, sickle cell anaemia disease has been found to also have an impact on the white blood cell counts of sickle cell patients. Interestingly, the sickle cell patients have high WBC counts even without any obvious signs of infection, inflammation or pain crises. Possible reasons for this are functional asplenia or hyposplenia, an over active bone marrow due to the rapid need to produce RBCs and also sub clinical infection (Wun, 2000).

An association has been observed between high white blood cell counts and high rates of morbidity and mortality in these patients (Ahmed *et al.,* 2017). Specifically, with increased risk of vascular complications leading to stroke and also acute chest syndrome. Neutrophils in particular have been found to be in a constant state of activation in sickle cell patients. Sickled red blood cells are more adherent to these activated polymorphonuclear neutrophils, an adherence which is also promoted by the presence of IgG. The adherence stimulates a respiratory burst of the leucocytes leading to release of free oxygen radicals that cause an increase of adhesion molecules along the vascular endothelium leading to further adhesion of sickled red blood cells and consequently vaso occlusion and vascular injury (Okpala, 2004).

Monocytes are also activated upon binding with sickled red blood cells. The various types of white blood cells in the body adhere to the vascular endothelium thus leading to obstruction of the microvasculature. This stimulates the vascular endothelium to increase

the expression of ligands to the adhesion molecules on the blood cells. This results in tissue damage and inflammatory processes thus vaso occlusive crises (Okpala, 2004). The activation of vascular endothelial cells and the activation of white blood cells also plays a role in the activation of platelets. This is due to the release of tissue factor, expression of adhesion molecules, production of inflammatory mediators and the induction of innate immune responses (Conran, 2020).

Just as the red blood cells and white blood cells, platelets are also impacted in sickle cell disease development. These cells are the main regulators of inflammatory processes. Platelets are constantly in a state of activation in sickle cell disease patients and even more during vaso occlusive crisis. These activated platelets contribute to the adhesion of sickled red blood cells to the vascular endothelium by their secretion of thrombospondin. Thrombospondin is a family of glycoproteins that when secreted control cellular attachment and invasion. This in turn contributes to thrombosis and pulmonary hypertension in sickle cell patients (Zhang, 2016).

Another consequence of platelet activation is the adhesion of platelets and monocytes. This binding leads to activation of monocytes just as their binding to the sickled red blood cells. These platelet-monocyte aggregates also adhere to the vascular endothelium and contribute to vaso occlusion and painful crises (Wun, T., 2000). Platelets in activated state also bind to neutrophils to form platelet-neutrophil aggregates. This occurs in a P-selectin-dependent manner. This P-selectin glycoprotein ligand-1 transduced signals cause the directed movement of neutrophils so as to initiate inflammation. AKT2 in neutrophils, the serine/threonine kinase isoform also plays a role in the interaction between the platelet-neutrophil aggregates and the vascular endothelium (Zhang D., 2016). Hemolysis also plays a role in the activation of platelets. The release of adenosine 5’-diphosphate from the interior of the red blood cell during hemolysis causes the activation of P2Y1, P2Y12 and P2XI ADP receptors that are found on the surface of platelets (Conran, N., 2020).

Platelet activation is characterized by increased adhesion molecule activity, increased circulatory volume of platelet microparticles and platelet derived proteins. This activation is observed increasingly when sickle cell patients are experiencing vaso occlusive crisis. Increased activity and expression of adhesion molecules such as the glycoproteins and P-selectin on platelets facilitate the adhesion of these cells to the vascular endothelium hence increasing activation of the endothelium (Conran, 2020).

Elevated eosinophil counts are observed in sickle cell anaemia patients and have been found to also be in activated state. This may imply a contribution to vaso occlusive crisis although this is yet to be clearly determined. (Pallis *et al.,* 2010). Absolute lymphocyte count is also elevated in sickle cell anaemia. This is from high levels of circulating T lymphocytes and CD8 T cells (Azevedo *et al.,* 2020). Sickle cell patients have elevated white blood cell counts and platelet counts. The inflammatory processes triggered in sickle cell patients promote the activation of these cells causing increased adhesion between them and activated vascular endothelium. Thus, the increased levels of circulating white blood cell and platelet aggregates in the blood of sickle cell anaemia patients, correlates with the severity of disease (Okpala, 2004).

2.3 Clinical manifestation.

Whereas in most developed countries children are screened at birth for sickle cell anaemia, in developing countries such as Kenya, it is not until the symptoms appear that a child is known to have sickle cell anaemia. However, not all children will depict symptoms early in life, some develop symptoms much later into their adulthood. Symptoms also vary from one person to another (Mc Gann *et al.*, 2016).

Symptoms of sickle cell anaemia include painful swelling of the extremities, fatigue and jaundice. The most common sign of sickle cell is pain. Painful crises occur mostly in the chest, arms, abdomen, lower back and legs. This pain can be described as a sharp, intense, stabbing or throbbing pain. Chronic pain is also often felt by sickle cell patients (Pule *et al.*, 2014). This pain is also referred to as vaso occlusive crises since it occurs

due to obstruction of the vascular endothelium. This obstruction occurs as a result of the adhesion between sickled red blood cells, white blood cells, platelets, plasma proteins and the vascular endothelium (Okpala, 2004).

Another common feature in sickle cell anaemia is bacterial infections. These occur since the spleen tends to get damaged by the sickle cells. Common bacterial infections affecting them include. *Streptococcus pneumoniae, Neisseria meningitidis, Staphylococcus aureus* and *Salmonella.* They can lead to blood infections, bone infections, lung infections and even meningitis (Pule *et al.*, 2014). Functional asplenia in infancy causes some of these microorganisms to be virulent. However, the lungs tend to be most affected by infections in sickle cell patients. This is due to hyper activity in the bronchus, chronic inflammation in the airways and even asthma, which are highly prevalent in sickle cell patients. These conditions are characterized by inflammatory processes that render the linings prone to infections (Sundd *et al.*, 2019).

Acute chest syndrome is another symptom of sickle cell anaemia. It results when vaso-occlusion in the lungs lead to damage of the lining of the lungs as they cannot receive oxygen. It is a life-threatening condition. It portrays with chest pain, fever, shortness of breath and coughing (Green *et al.*, 2013).

Anaemia is another symptom observed in sickle cell anaemia patients. This occurs when the haemoglobin levels of the individual are below the normal ranges for their age and sex. The low HB levels occur due to the rapid destruction of sickle RBCs. This results in dizziness, fainting, rapid heart rate and headaches. Anaemia is treated by conducting blood transfusion on the patients. Persons suffering from sickle cell anaemia also tend to suffer strokes. These can also be referred to as transient ischemic attacks. These occur when vaso occlusion occurs in blood vessels that direct blood flow to the brain resulting in interrupted or blocked blood flow. It occurs suddenly and once a patient has their first stroke, the chances of having more are increased (Mc Gann *et al.*, 2016).

Jaundice is another common feature among sickle cell patients. This refers to the yellowing of the skin and eyes. Jaundice occurs as a result of the rapid destruction of sickle RBCs. When RBCs are broken down, bilirubin is formed as one of the by products for removal by the liver. In sickle cells disease, the rate of destruction of RBCs supersedes the rate of removal of bilirubin from the body by the liver. This in turn causes a buildup of bilirubin in the system leading to jaundice (Juwah *et al.*, 2014).

Splenic sequestration is also observed in sickle cell patients. This is the enlargement of the spleen that occurs as a result of the pooling of sickle RBCs. It is painful due to the increase in blood volume in the organ and can be life threatening if not treated immediately. If the spleen becomes sequestered frequently, this leads to scarring and permanent damage of the spleen. This may necessitate surgical removal of the spleen. Removal or damage of the spleen in turn leaves the patients prone to infections. Painful priapism is another complication observed in sickle cell patients. This occurs when blood vessels in the penis are blocked by the sickle cells. This can lead to impotence if left untreated (Green *et al.*, 2013).

2.4 Mode of action of hydroxyurea.

Hydroxyurea is the only known effective drug for management of sickle cell anaemia patients. While it is effective in managing the health state of sickle cell patients, it is not a cure for the disorder. It is an oral drug that can be in capsule or liquid form. It is administered once a day, every day for sickle cell patients. It works by increasing the amount of HbF produced in red blood cells. This is helpful in management of sickle cell since red cells containing HbF are less likely to sickle compared to those containing HbS thus reducing the incidences of complications associated with sickle cell anaemia (Charache, 2017).

Despite proving efficient in inducing the production of HbF the exact mode of action of HU is still not clearly understood. However, various mechanisms of action can be attributed to the drug. The most important one being that hydroxyurea works to inhibit

the production of ribonucleotide reductase (RR). Ribonucleotide reductase is an enzyme involved in DNA synthesis that works to convert ribonucleosides into deoxyribonucleosides which are the building blocks of DNA. This causes a reduction in DNA synthesis and eventually leads to cellular toxicity as well. This cellular toxicity is presumed to suppress erythroid progenitors as well as cell stress signaling and the production of neutrophils, reticulocytes and platelets. This eventually leads to a change in the kinetics and physiology of erythropoiesis leading to recruitment of erythroid progenitors with higher levels of HbF (Mulaku *et al.,* 2017).

The reduction in the production of WBCs is presumed to be therapeutic since raised levels have been found to be associated with morbidity and mortality in sickle cell anaemia. Neutrophils and reticulocytes promote vascular adhesion hence lead to vaso-occlusion. Thus, a reduction in their numbers by HU reduces the expression of surface adhesion receptors (Mulaku *et al.,* 2017). However, while a reduction in white blood cell counts reduces the occurrence of vaso occlusive crises, an impaired ability of white blood cells to kill microorganisms causes patients to be more prone to infections (Okpala, 2004).

On oral administration of hydroxyurea, the peak levels are attained within 1 to 4 hours and during the escalation of HU dose to its maximal tolerated dose (MTD), the red blood cells circulating in the peripheral system undergo a series of physiologic and morphologic changes that lead to increased mean cell hemoglobin (MCH), macrocytosis, increased hydration, less haemolysis and less sickled RBCs (Agrawal *et al.,* 2014).

However, the drug also causes some side effects on some patients. These include nausea, vomiting, diarrhea, loss of appetite, sores in the mouth and throat, rashes and headaches (Agrawal *et al*. 2014).

2.5 Prevention, management and treatment of Sickle cell anaemia

Since sickle cell anaemia is an inherited disorder, the most effective prevention strategy is screening of individuals. When a couple identifies that they are both carriers they can then be advised on the possible outcomes of their children in regards to sickle cell status. The screening can help avoid conception of children with sickle cell anaemia. Hydroxyurea remains to be the most effective and only available drug to deal with sickle cell anaemia crises. The drug is prescribed to sickle cell patients to help prevent sickle cell crises and acute chest syndrome (Juwah *et al.*, 2004).

Pain medication are also administered to help alleviate painful episodes among sickle cell patients. Opioid medications such as morphine are found to be necessary for painful crises in these patients. Drinking plenty of water to keep hydrated also helps to reduce chances of crises as well as alleviate the pain. This occurs by helping the red blood cells avoid sickling. At times intravenous infusion of normal saline may be necessary as well. Antibiotics and vaccinations for children are also administered to prevent and treat infections. Antibiotics such as penicillin can be administered for life. Blood transfusion can be administered to help treat the anaemia and reduce the chances of stroke as well. It also helps alleviate chronic pain, treat acute chest syndrome and splenic sequestration. It does so by diluting the sickle HbS haemoglobin with normal HbA haemoglobin. Folic acid can also be administered to help treat the anaemia (Green *et al.*, 2013).

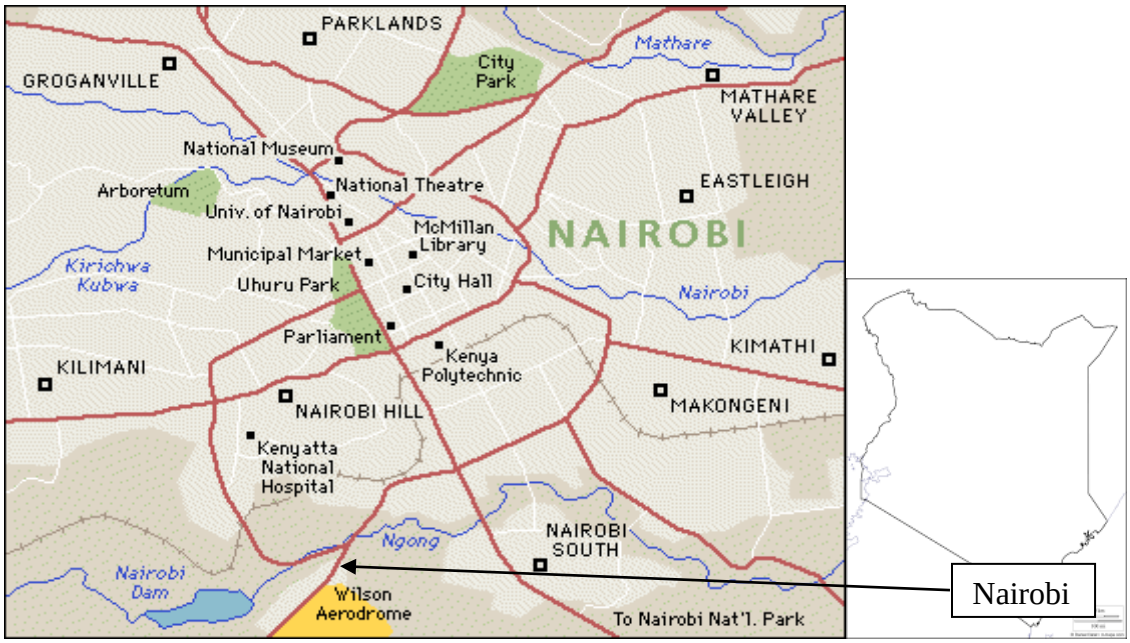
Bone marrow transplant can also be recommended for the sickle cell patients. This, however, depends on a number of factors. These include, the severity of the disease as well as the availability of a suitable bone marrow donor match. The bone marrow transplant is curative **(**Pule *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Haematology clinic of the Kenyatta National Hospital (KNH) which is located in Upper hill, Nairobi County (Figure 3.1). It is the referral hospital serving the whole country and has various departments that offer services to patients suffering from various ailments. These include the Haematology department under which the sickle cell clinic lies. The sickle cell clinic runs every Monday and is run by a team of 3 doctors assisted by 5 nurses, offering services to about 50 sickle cell patients per week.



Retrieved from http://kenya.rcbowen.com/cities/nairobi.html

Figure 3.1: Map of central Nairobi showing the sampling area. Source:

3.2 Study design

This study was a descriptive cross-sectional study.

3.3 Study population

The target population was sickle cell patients attending the sickle cell clinic at Kenyatta National Hospital, who were in steady state. They were categorized into two groups, those who had taken their doses of hydroxyurea consistently for at least 3 months prior to the study and those who hadn't taken any hydroxyurea for at least 3 months prior to the study.

3.3.1 Inclusion criteria

1. Patients confirmed to have sickle cell anaemia and aged 12years and above.
2. Patients in steady state, confirmed by thorough history.
3. Patients who had consistently taken their prescribed doses of hydroxyurea for at least 3 consecutive months prior to the study.
4. Patients who had not taken any doses of hydroxyurea for at least 3 consecutive months prior to the study.
5. Those patients who signed the consent forms and pediatric patients who sign assent forms or for whom parents signed consent forms.

3.3.2 Exclusion criteria

1. Patients who had no consistency in regard to taking or lack-there-of of hydroxyurea for the last 3 months prior to the study.
2. Sickle cell patients who have had a blood transfusion within the last 3 months.
3. Patients with conditions that can affect hematological values, such as renal disease.
4. Sickle cell carriers.
5. Pregnant females.

3.4 Sample size determination

The minimum sample size for each of the two groups in this study was determined using the formula shown below. Similar studies carried out in the past showed an average mean SD of +/-17.3 (Nnebe *et al.* 2018) between the group taking hydroxyurea and the hydroxyurea naïve group. The minimum sample size was determined to be 46 patients per group derived as shown below;

$$N = \frac{Z1\text{-}\alpha/2\ ^2 SD^2}{d^2}$$

Where:

- N = minimum sample size in each of the groups
- Z1- ∝/2. = 1.96, corresponds to 95% confidence interval on tables of standard normal distribution
- SD = Standard Deviation of variable, 17.3 (Nnebe *et al*. 2018)
- d = Precision

$$\mathbf{1.96^2\ (17.3^2)/5^2 = 46}$$

3.5 Sampling procedure

Convenience sampling method was applied to recruit participants for this study. Potential participants were approached on an individual basis as they attended the clinic and those found willing to take part were taken in for the selection interview. Those sickle cell patients in steady state were selected and divided into two clusters; of those taking hydroxyurea and those not taking hydroxyurea for at least three months prior to the study. In each of these two groups, a total of 46 subjects were randomly picked to participate in the study. The study was explained in detail by the trained research assistants. Those who met the inclusion criteria and were willing to participate in the study, filled the informed consent forms (APPENDIX I) and assent forms (APPENDIX

II) showing they fully understood and agreed to take part in the study. Only those who met the inclusion criteria and filled consent and assent forms were allowed to participate in the study.

3.6 Data collection tools

3.6.1 Qualitative data collection tools

A carefully designed questionnaire was used to collect qualitative data including the participants age, sex, last date of transfusion, any incidences of illnesses or surgery within the last three months and any history of kidney or liver disease. The questionnaires were filled with the assistance of well-trained research assistants who were well trained and supervised by the Principle Investigator.

3.6.2 Quantitative data collection tools

A well-structured table was incorporated at the end of each participant's questionnaire where haematological parameters acquired after running the sample on Sysmex XT-2000*i* were filled in.

3.6.3 Quality of data

In order to ensure the quality of both the quantitative and the qualitative data collected, the following measures were taken;

1. Internal quality controls were carried out every day patient samples were run and confirmed to have passed before running of patient samples.
2. Direct printouts of the haematological parameters were filed and crosschecking done for each parameter entered on the database before the end of each day.
3. The questionnaires were also translated into Kiswahili and both English and Kiswahili copies availed as per the participants' preferences.

4. The completeness of each questionnaire was confirmed before the end of the same day of completion.

3.7 Methodology

3.7.1 Questionnaire administration

A structured questionnaire (APPENDIX III) was translated into Kiswahili and administered as per each participant's language preference by the trained research assistants. The questionnaires provided demographic information as well as relevant clinical history such as if the participants had gotten any illnesses or blood transfusions within the last three months prior to the study. This data also provided information necessary to determine eligibility for enrollment.

3.7.2 Sample collection

Upon completion of filling of the questionnaires (APPENDIX III), the procedure of sample collection begun. 2ml of venous blood was collected into a 4ml vacutainer containing Ethylene Diamine Tetraacetic Acid (EDTA) vacutainer for each participant and immediately gently mixed to avoid clotting. Each sample tube was labeled with the unique participant's identifier and both the questionnaires and samples transported to the haematology laboratory for analysis.

3.7.3 Sample analysis

Each of the participant's samples were analyzed using Sysmex XT 2000*i* haematology analyzer upon ensuring the day's daily Internal Quality Controls (IQC) had passed. Each unique participant's identifier was keyed into the system, followed by their demographic information; age and sex. Following this, the open tube system was used for analysis. The sample was gently inverted for 5-7 times before uncapping and placing under the sample probe and pressing the start switch. The data from each of the samples was then printed and attached to the respective questionnaire. Whenever it was not possible to test

the samples within four hours, the samples were stored in a refrigerator at 2-8 degrees Celsius until it was possible to run them.

3.8 Data management

Data from each of the samples was filled into their respective questionnaires as a backup in the event of loss of the print out. All the data from the questionnaires and the haematological parameters data were then entered onto an Excel spreadsheet on a daily basis by the Principal Investigator. A regular back up of this data was also done on a flash disk to avoid any data loss. Data cleaning and validation was then carried out before the final copy was saved awaiting analysis. The questionnaires and haematological results were then arranged in a file and the file was then locked in a cabinet for safekeeping by the principal investigator to ensure privacy of the participants' information.

3.9 Data analysis

The data acquired was analyzed using the student t test to assess the significance of differences between the Hb, WBC and platelets among the group taking hydroxyurea and the group not taking hydroxyurea.

3.10 Ethical consideration

Ethical clearance was obtained from the Kenyatta National Hospital – University of Nairobi Ethical Research committee in line with the code of ethics for biomedical research that involves human subjects protocol No. P 289/04/2018(Appendix IV). Since the study population was made up of patients, they were allowed to first attend the clinic, after which they were approached and informed about the study. The patients were told the purpose of the study, the potential risks and benefits of participating. They were also informed that participation was voluntary and that they could exit from the study at any point. This was followed by filling of the written consent and assent forms.

The information of the study participants was safeguarded and no disclosure was done to persons not involved in the study. To ensure this, the interviews were carried out in a confidential manner and no identifying information was filled on the form. Also, all the patient questionnaires and patient samples were assigned a unique identifier. No questionnaires or blood sample vials had the names of the participants or any other information that could potentially be linked to them. Copies of the haematological reports were forwarded to the attending doctors for the purposes of the patients' routine checks while the originals were filed for the purposes of the study. The research data was kept under lock and key.

CHAPTER FOUR

RESULTS

4.1 Socio demographics of participants

4.1.1 Gender distribution of the study participants

A total of 92 study participants took part in this study. Of these, a total of 59 were female while 33 were male translating to 64% participation by female gender and a 36% participation by the male gender. The total population of study participants was divided into 2 groups; a group of sickle cell anaemia patients who took their doses of hydroxyurea consistently for at least three months prior to the study (On HU) and the other group comprised of sickle cell anaemia patients who did not take any hydroxyurea for a period of at least three months prior to the study (HU naïve) . A total of 46 people took part in each of the groups. In the group taking hydroxyurea, 32 of the participants were female while 14 were male, whereas 27 female and 19 male participants took part in the hydroxyurea naïve group. This translated to 70% females and 30% males in the group taking hydroxyurea and 59% females and 41% males in the hydroxyurea naïve group (Figure 4.1).

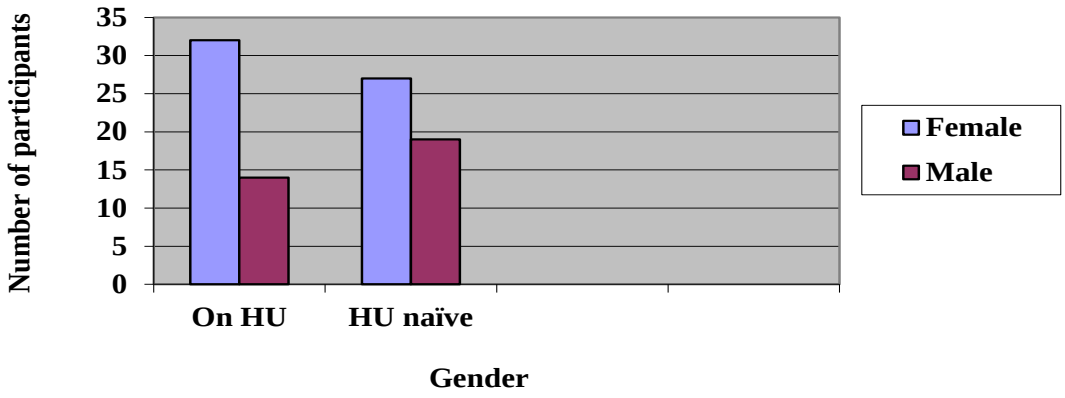


Figure 4.1: Bar graph showing gender distribution of participants in both study groups.

4.1.2 Age Distribution of the study participants

Of the total participants, 64 were of ages 18 years and above while 28 participants were below 18 years of age. In the group taking hydroxyurea, 33 of the participants were of age 18 years and above while 13 were children below 18 years. The adults in this group were of ages ranging from 18 to 64 years while the children ages ranged from 13 to 17 years. In the hydroxyurea naïve group, 31 were aged 18 years and above while 15 participants were below 18 years of age. The ages of the children ranged from 12 to 17 years while those of the adults ranged from 18 to 47 years (Figure 4.2).

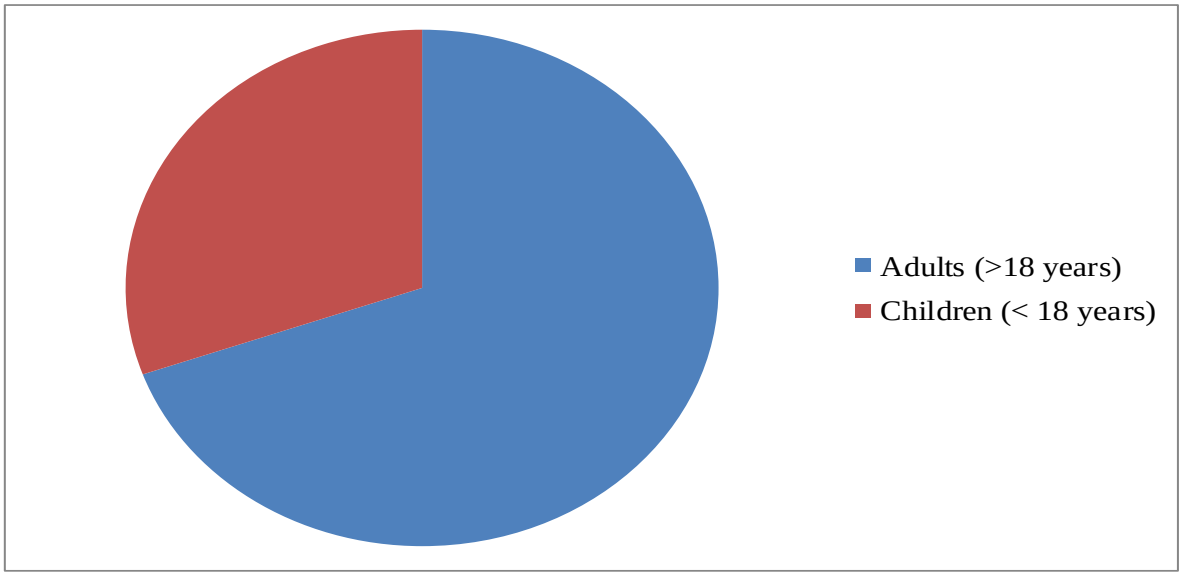


Figure 4.2: Pie chart showing age distribution of all participants

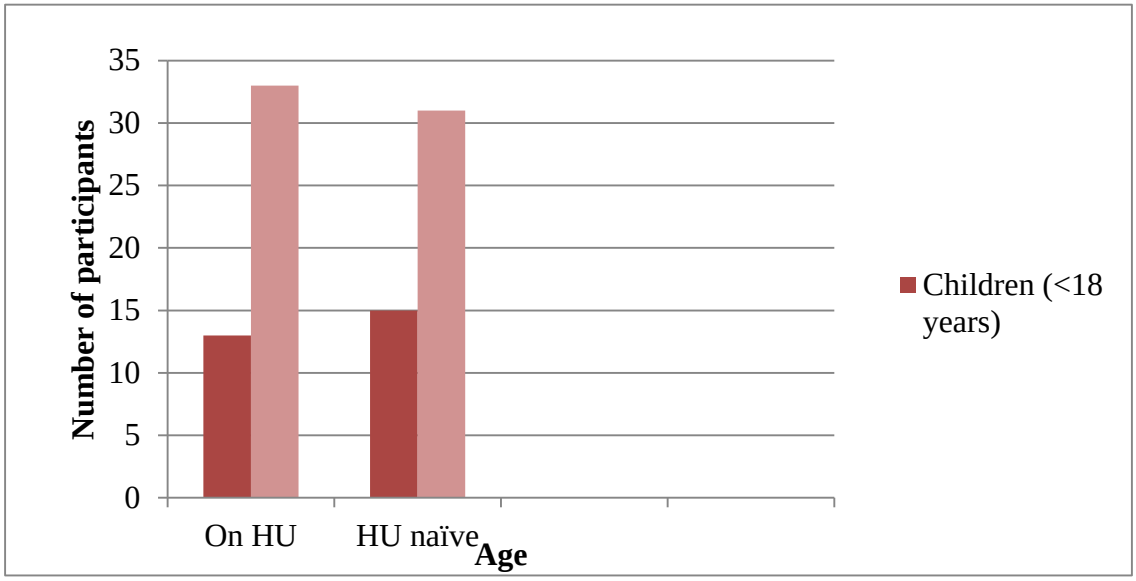


Figure 4.3: Bar graph showing age distribution of participants in both study groups

4.2 Comparison of Hb distribution between the study groups.

The mean Hb level of the group of study participants taking hydroxyurea was found to be 10.4 g/dl while the average Hb of the hydroxyurea naïve group of study participants was determined to be 9.0 g/dl (Figure4.4). This was determined to be a statistically significant difference (p=0.0108) upon statistical analysis (Table 4.1). The Hb levels of the group taking hydroxyurea ranged from 5.2 g/dl to 14.4 g/dl as depicted on the box plot. This gave a range of 9.2g/dl. On the other hand, the Hb levels of the hydroxyurea naïve group ranged from 6.5 g/dl to 14.4 g/dl as depicted on the box plot. This gave a range of 7.9g/dl (Figure 4.4). The results of Hb levels when evaluated within age groups showed consistently higher values in the group of participants taking hydroxyurea within all the age groups where data for both groups of participants was available (Figure 4.5).

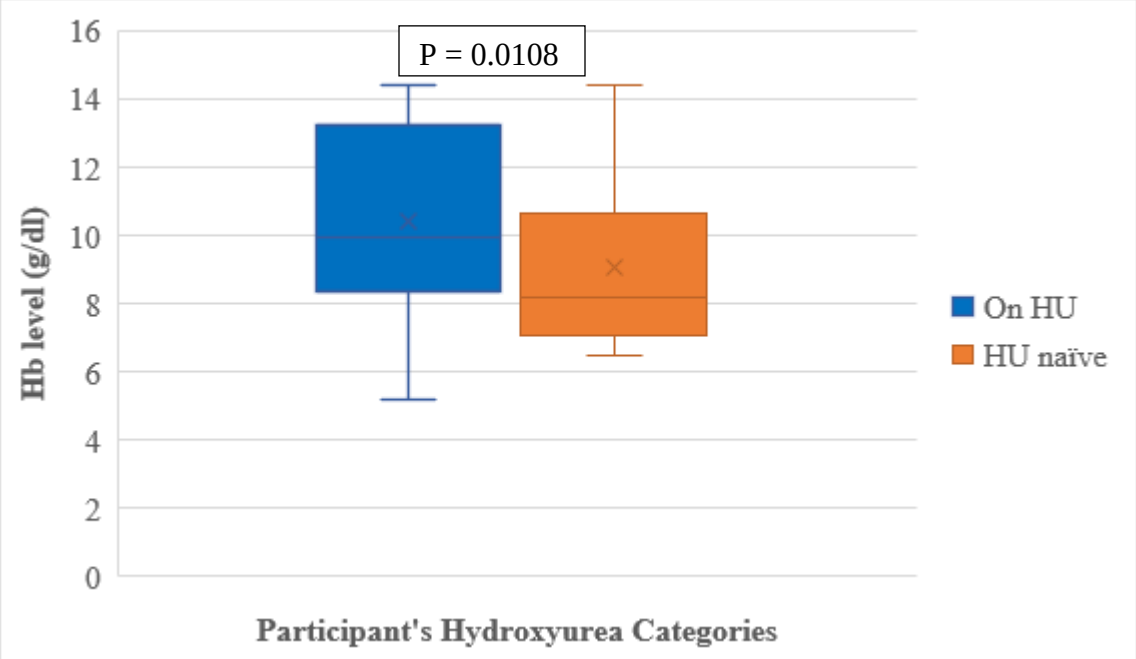
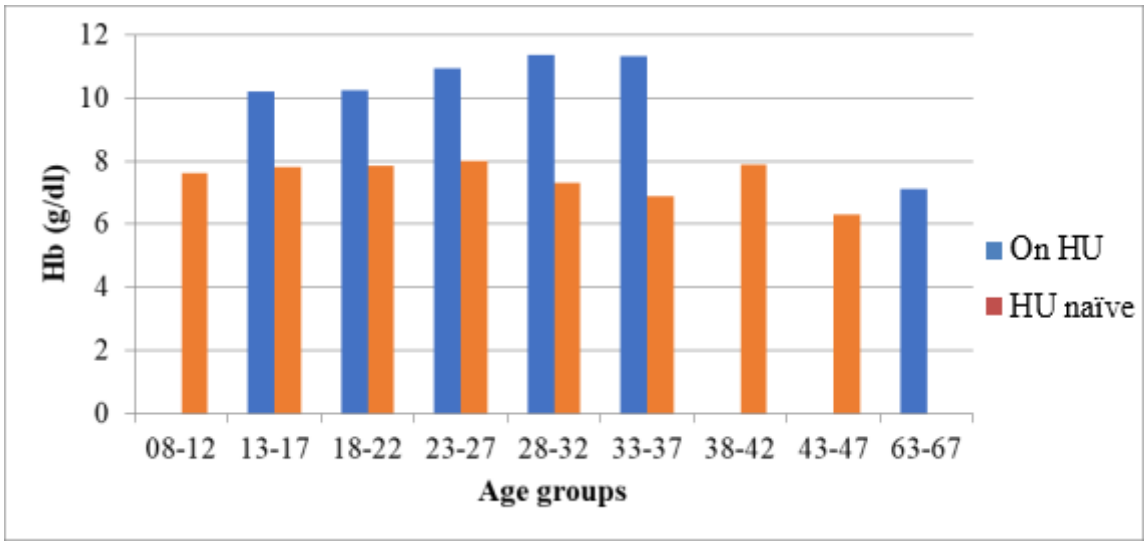


Figure 4.4: Box plot showing mean Hb levels of participants in both study groups.

Table 4.1: Distribution of Hb levels among participants

	On HU	*HU naïve*
Mean	10.4443	9.060
Variance	7.045	5.944
Observations	46	46
Hypothesized mean difference	0	
p value	0.0108	

Figure 4.5: Bar graph showing mean Hb levels among the study participants as per their age groups



4.3 Comparison of WBC distribution between the study groups.

The mean WBC of those on hydroxyurea was 11.0 x10^9/l and of those not on hydroxyurea was 14.7 x10^9/l (Figure 4.6). This translated to a statistically significant difference (p=0.00577) upon statistical analysis. (Table 4.2). The WBC counts of the group taking hydroxyurea ranged from 5.0 x10^9/l to 27.3 x10^9/l as depicted on the box plot. This gave a range of 22.3 x10^9/l. On the other hand, the WBC counts of the hydroxyurea naïve group ranged from 5.3 x10^9/l to 44.1 x10^9/l as depicted on the box plot. This gave a range of 38.8 x10^9/l. These high values were considered to be outliers since most of the results in the group on hydroxyurea were up to 19.3 x10^9/l while those

in the group not taking hydroxyurea were up to 16.5 x10^9/l, as shown. (Figure 4.6). The results of WBC levels when evaluated within age groups showed consistently lower values in the group of participants taking hydroxyurea within all the age groups where data for both groups of participants was available (Figure 4.7).

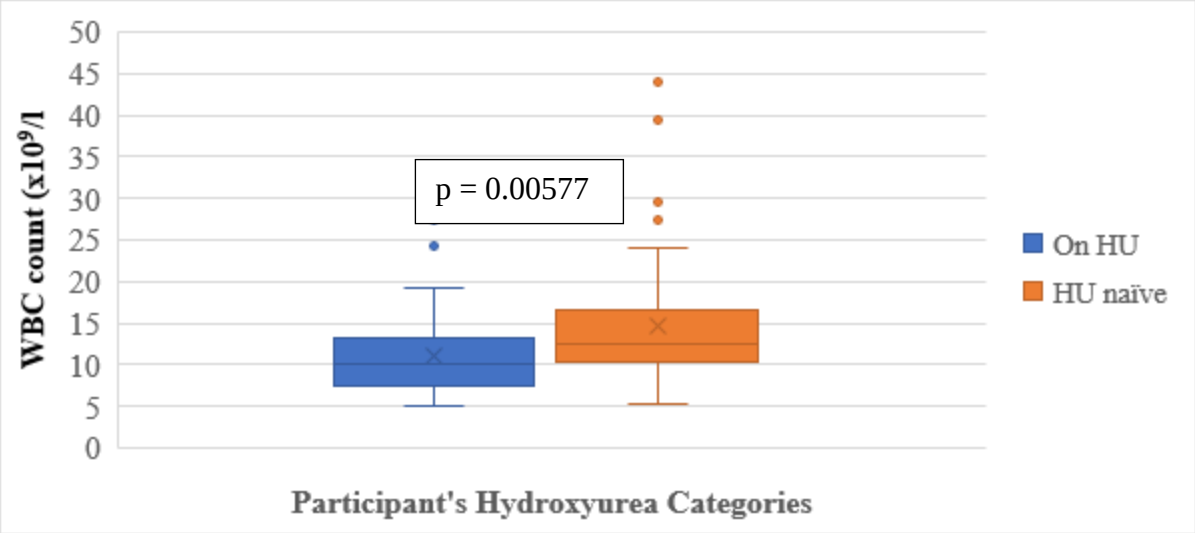


Figure 4.6: Box plot showing mean WBC levels of participants in both study groups

Table 4.2: Distribution of WBC levels among participants

	On HU	***HU naïve***
Mean	11.004	14.785
Variance	22.389	58.995
Observations	46	46
Hypothesized mean difference	0	
p value	0.00577	

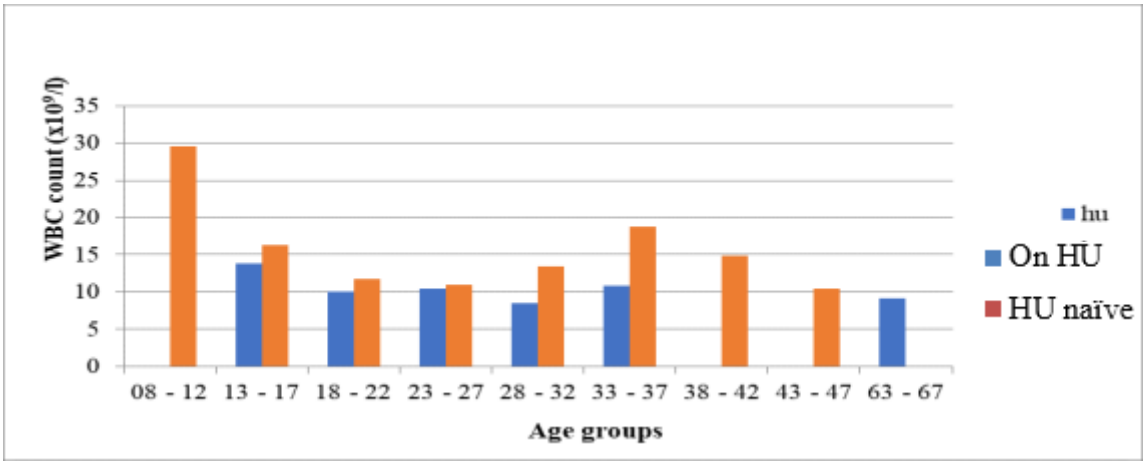


Figure 4.7: Bar graph showing mean WBC levels among the study participants as per their age groups

4.3.1 Comparison of neutrophil distribution between the study groups.

The mean neutrophil count of those on hydroxyurea was 5.1 $x10^9$/l and of those not on hydroxyurea was 6.0 $x10^9$/l (Figure 4.8). This translated to a statistically significant difference (p=0.05) upon statistical analysis. (Table 4.3). The neutrophil count of the group taking hydroxyurea ranged from 1.7 $x10^9$/l to 14.3 $x10^9$/l. However, most of the results ranged to 10.5 $x10^9$/l with only 2 values as outliers, as depicted on the box plot. This gave a range of 12.6 $x10^9$/l. On the other hand, the neutrophil count of the hydroxyurea naïve group ranged from 2.3 $x10^9$/l to 11.5 $x10^9$/l as depicted on the box plot. This gave a range of 9.2 $x10^9$/l. However, most values were up to 9.5 $x10^9$/l and the maximum value was therefore an outlier, as shown. (Figure 4.8).

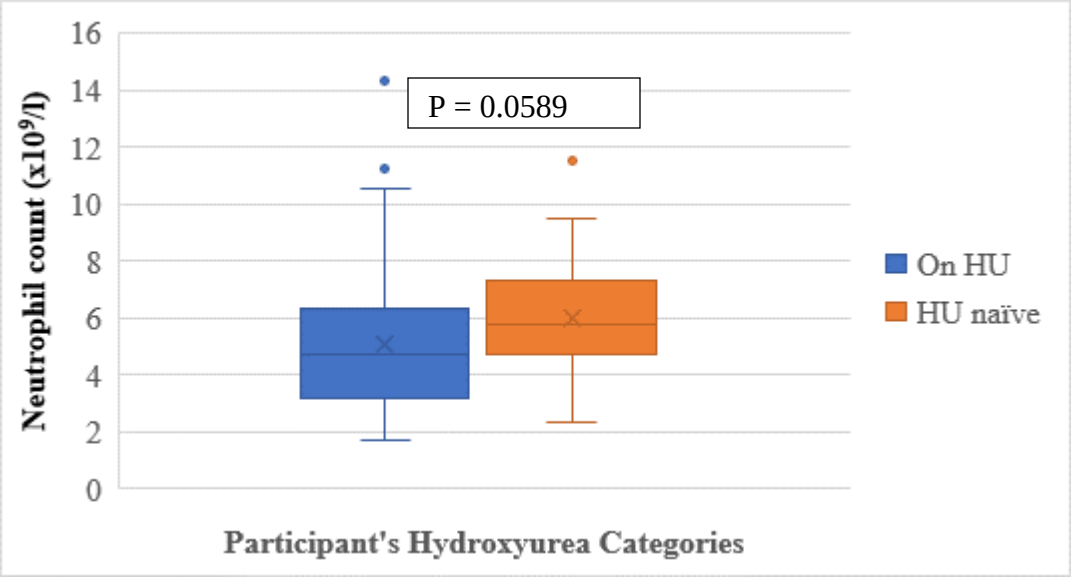


Figure 4.8: Box plot showing mean neutrophil levels of participants in both study groups

Table 4.3: Distribution of neutrophil levels among participants

	On HU	*HU naïve*
Mean	5.104	6.018
Variance	6.202	4.280
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.0589	

4.3.2 Comparison of eosinophil distribution between the study groups.

The mean eosinophil count of those on hydroxyurea was 0.21 $x10^9$/l and of those not on hydroxyurea was 0.25 $x10^9$/l (Figure 4.9). This translated to a statistically significant difference (p=0.05) upon statistical analysis. (Table 4.4). The eosinophil count of the group taking hydroxyurea ranged from 0.07$x10^9$/l to 0.45$x10^9$/l, as depicted on the box plot. This gave a range of 0.38 $x10^9$/l. On the other hand, the eosinophil count of the hydroxyurea naïve group ranged from 0.09$x10^9$/l to 0.48 $x10^9$/l as depicted on the box

plot. This gave a range of 0.39 x10^9/l. However, most values were up to 0.40 x10^9/l and one of the values was therefore an outlier, as shown. (Figure 4.9).

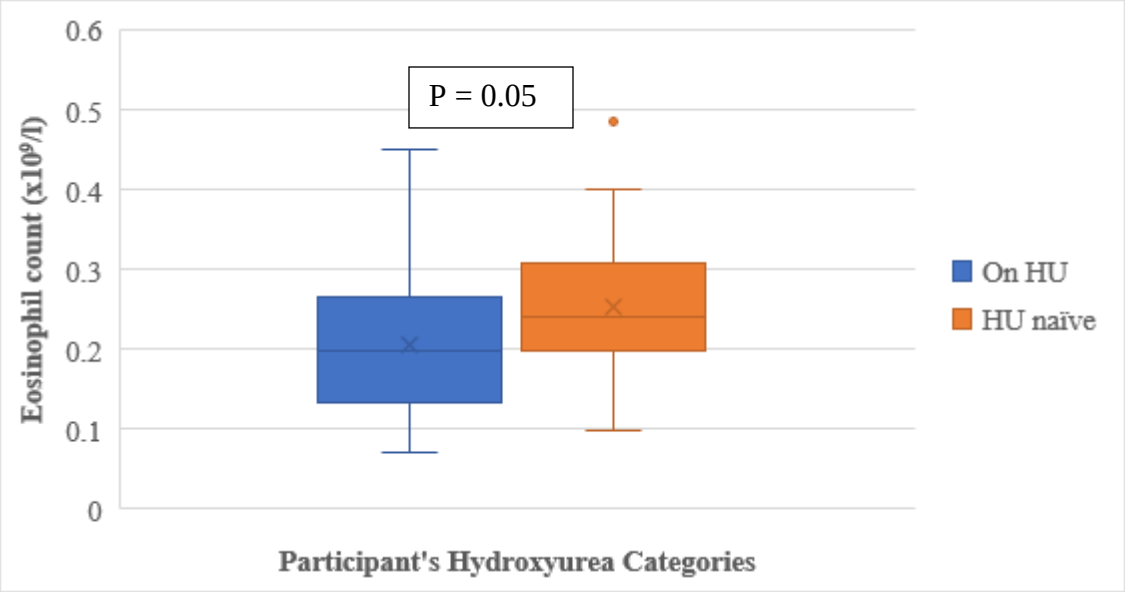


Figure 4.9: Box plot showing mean eosinophil levels of participants in both study groups

Table 4.4: Distribution of eosinophil levels among participants

	On HU	*HU naïve*
Mean	0.21492	0.253392
Variance	0.011	0.007589
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.0589	

4.3.3 Comparison of basophil distribution between the study groups.

The mean basophil count of those on hydroxyurea was 0.10 x10^9/l and of those not on hydroxyurea was 0.12 x10^9/l (Figure 4.10). This translated to a statistically significant difference (p=0.05) upon statistical analysis. (Table 4.5). The basophil count of the group taking hydroxyurea ranged from 0.02x10^9/l to 0.24x10^9/l. However, most of the

results ranged to 0.22 x10^9/l with only 1 value as an outlier, as depicted on the box plot. This gave a range of 0.22 x10^9/l. On the other hand, the basophil count of the hydroxyurea naïve group ranged from 0.04x10^9/l to 0.23 x10^9/l as depicted on the box plot. This gave a range of 0.19 x10^9/l. However, most values were up to 0.20 x10^9/l and one of the values was therefore an outlier, as shown. (Figure 4.10).

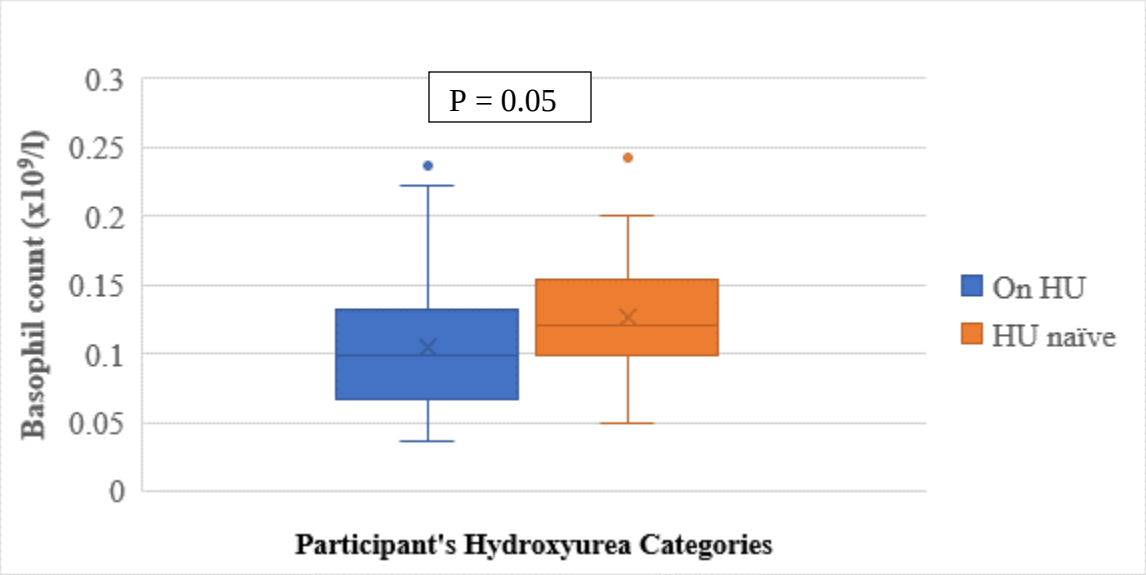


Figure 4.10: Box plot showing mean basophil levels of participants in both study groups

Table 4.5: Distribution of basophil levels among participants

	On HU	***HU naïve***
Mean	0.107	0.126
Variance	0.00274	0.00189
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.0589	

4.3.4 Comparison of lymphocyte distribution between the study groups.

The mean lymphocyte count of those on hydroxyurea was 4.4 x10^9/l and of those not on hydroxyurea was 6.9 x10^9/l (Figure 4.11). This translated to a statistically significant

difference (p=0.00845) upon statistical analysis. (Table 4.6). The lymphocyte count of the group taking hydroxyurea ranged from 1.1 x10^9/l to 9.1 x10^9/l as depicted on the box plot. This gave a range of 8.0 x10^9/l. On the other hand, the lymphocyte count of the hydroxyurea naïve group ranged from 1.0 x10^9/l to 29.7 x10^9/l as depicted on the box plot. This gave a range of 28.7 x10^9/l. These high values were considered to be outliers, with all but 5 results being above 11.6 x10^9/l as shown. (Figure 4.11).

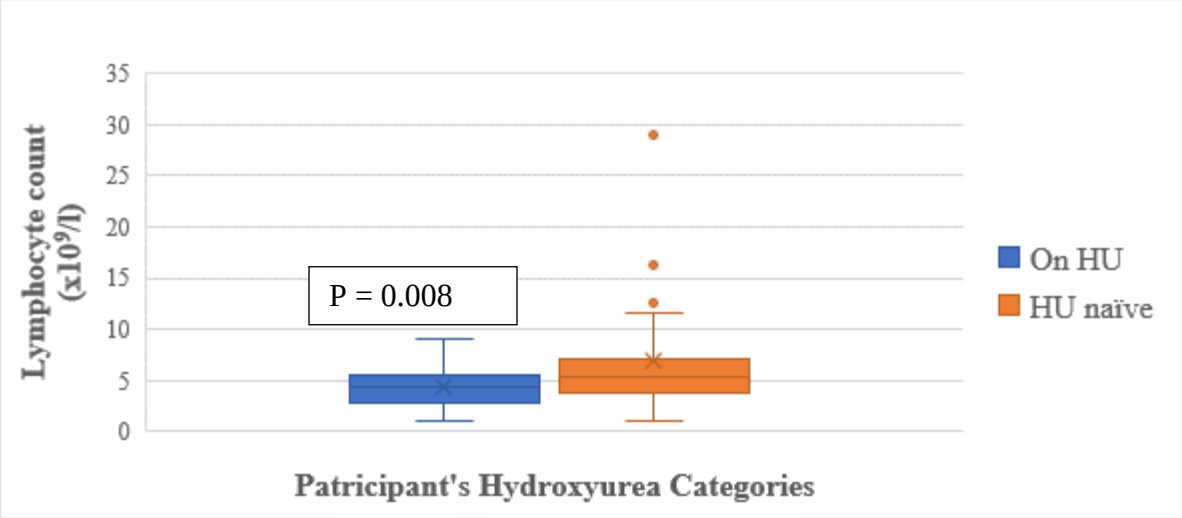


Figure 4.11: Box plot showing mean lymphocyte levels of participants in both study groups

Table 4.6: Distribution of lymphocyte levels among participants

	On HU	*HU naïve*
Mean	4.442	6.952
Variance	4.397	34.498
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.00845	

4.3.5 Comparison of monocyte distribution between the study groups.

The mean monocyte count of those on hydroxyurea was 1.19 x10^9/l and of those not on hydroxyurea was 1.4 x10^9/l (Figure 4.12). This translated to a lack of statistically significant difference (p=0.283) upon statistical analysis. (Table 4.7). The monocyte count of the group taking hydroxyurea ranged from 0.3 x10^9/l to 11.9. However, most results ranged to 1,9 x10^9/l with the last result as the only outlier in this group, as depicted on the box plot. This gave a range of 11.6 x10^9/l. On the other hand, the monocyte count of the hydroxyurea naïve group ranged from 0.4 x10^9/l to 5.1 x10^9/l as depicted on the box plot. This gave a range of 4.7 x10^9/l. However, most results were up to 2.4 x10^9/l and four results were outliers in this category, as shown. (Figure 4.12).

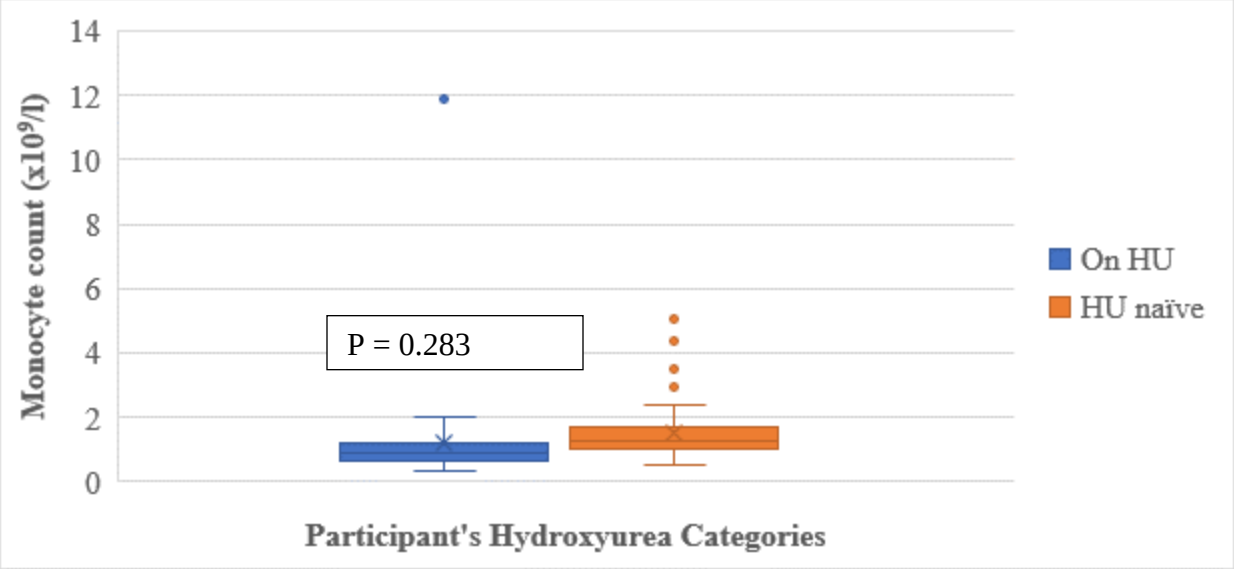


Figure 4.12: Box plot showing mean monocyte levels of participants in both study groups

Table 4.7: Distribution of monocyte levels among participants

	On HU	*HU naïve*
Mean	1.197	1.498
Variance	2.763	0.814
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.283	

4.4 Comparison of platelet distribution between the study groups.

The mean platelet count of those on hydroxyurea was 384 $x10^9$/l and that of those not on hydroxyurea and 485 $x10^9$/l (Figure 4.13). This was a statistically significant difference (p=0.0100) upon statistical analysis (Table 4.8). The platelet counts of the group taking hydroxyurea ranged from 122 $x10^9$/l to 746 $x10^9$/l as depicted on the box plot. This gave a range of 624 $x10^9$/l. On the other hand, the platelet counts of the hydroxyurea naïve group ranged from 222 $x10^9$/l to 1261 $x10^9$/l as depicted on the box plot. This gave a range of 1039 $x10^9$/l. These high values were considered to be outliers, as shown. (Figure 4.13). The results of platelet levels when evaluated within age groups showed consistently lower values in the group of participants taking hydroxyurea within all the age groups where data for both groups of participants was available (Figure 4.14).

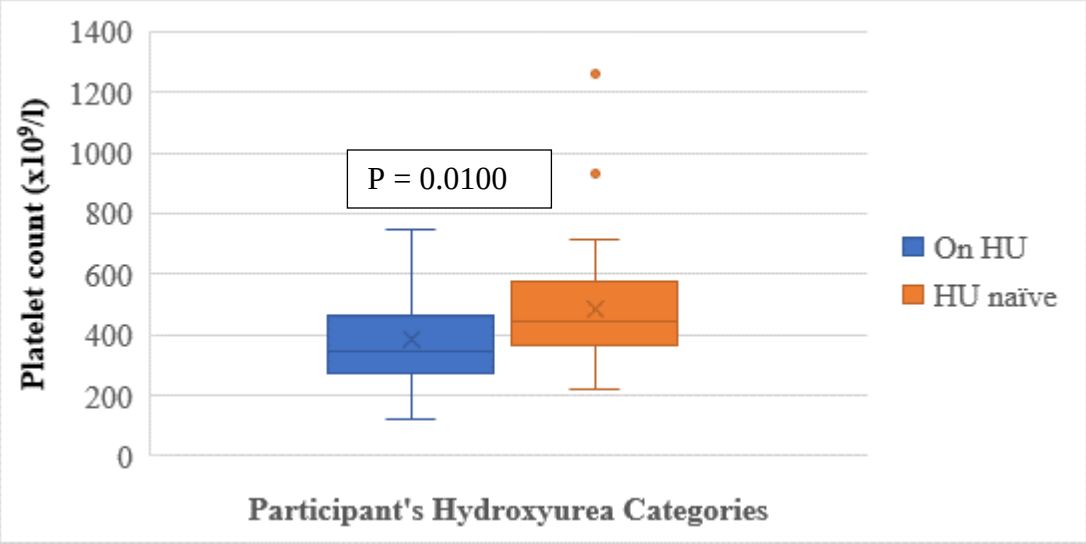


Figure 4.12: Box plot showing mean platelet counts of participants in both study groups

Table 4.8: Distribution of platelet counts among participants

	On HU	***HU naïve***
Mean	384.696	485.587
Variance	28032.394	39524.603
Observations	46	46
Hypothesized Mean Difference	0	
p value	0.0100	

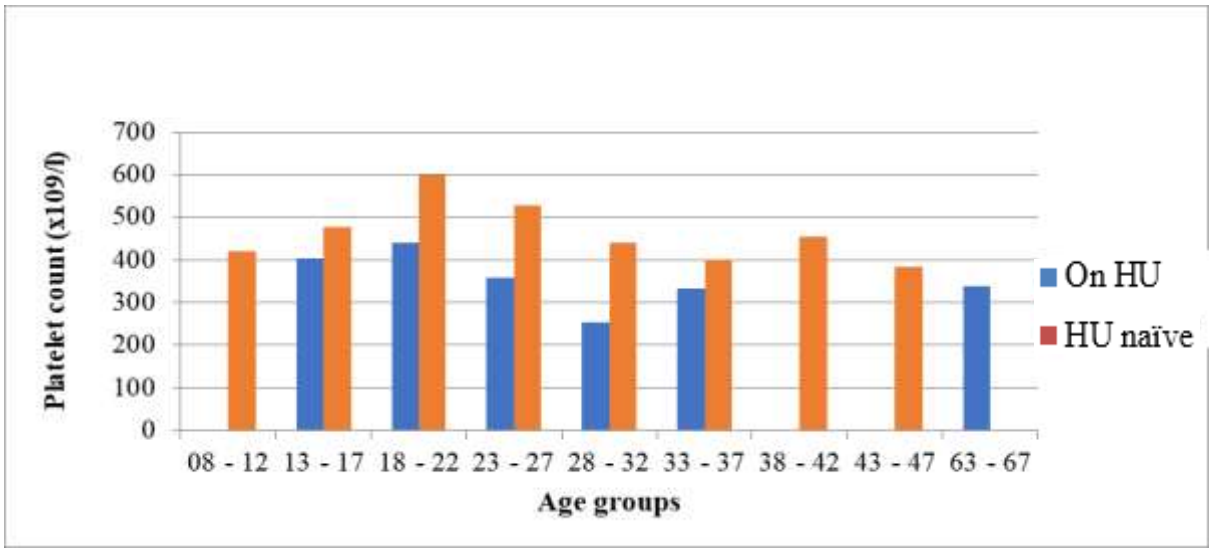


Figure 4.13: Bar graph showing mean platelet counts among the study participants as per their age groups

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Introduction

A number of studies have been carried out to determine the effect of hydroxyurea among sickle cell patients in various countries. No studies have been carried out in Kenya to determine the effects of hydroxyurea on the sickle cell population in this country. Also, most of these studies have been designed as prospective studies where haematological parameters were measured before the participants took hydroxyurea. They were then administered with specific doses of hydroxyurea and the haematological parameters evaluated again after a period of time (Shome *et al.*, 2016, Hassan A *et al.*, 2017). This study tried to determine if there any differences between haematological parameters of sickle cell patients on hydroxyurea and the hydroxyurea naïve patients among the Kenyan population using a cross sectional study.

5.1.2 Effect of hydroxyurea on Hb levels.

Our studies show that participants who had been on hydroxyurea consistently for a period of at least 3 months had a mean Hb level that was significantly higher compared their counterparts who were not on hydroxyurea for a period of at least 3 months. This is in line with a study conducted in Bahraini, where the mean Hb level of the study participants was significantly higher after taking hydroxyurea for a period of 12 months compared to the mean Hb level prior to taking hydroxyurea (Shome *et al.*, 2016). It also correlates to another study carried out in Brazil, where it was found that the mean Hb level of the participants after hydroxyurea therapy was significantly higher compared to their mean Hb level prior to taking hydroxyurea (Sètondji *et al.*, 2018). Similarly, in

another study carried out in India, where the effect of hydroxyurea on Hb was evaluated before and after administration of hydroxyurea, it was found that there was a significant higher level of Hb after hydroxyurea treatment compared to before treatment (Pradhan, 2018). This was also reflected in the Jain study of 2012 where the mean Hb of patients who were administered with hydroxyurea was found to be significantly higher compared to the placebo group (Jain, 2012). Our study included participants from the ages of 12 onwards as studies have shown that from the age of 12, the full blood count parameters remained largely unchanged through increasing age (Nah *et al.,* 2018). Despite the minimal difference in Hb observed in the sickle cell anaemia patients in the various studies, it was found to correlate to significant improvement in their clinical outcome as the patients experienced less events of vaso occlusive crisis. This is because, as per other studies, the main type of haemoglobin produced was HbF which doesn't tend to sickle (Charache, 2017).

5.1.3 Effect of hydroxyurea on WBC count.

In our study, a lower mean of total white blood cell counts was seen in the group of participants taking hydroxyurea compared to the mean in the group not taking hydroxyurea. Similar findings were seen in the study carried out in Nigeria, where a significantly lower mean white blood cell count was observed in the study participants after taking hydroxyurea for 12 months compared to their initial mean white blood cell count prior to administration of hydroxyurea (Hassan A *et al.,* 2017). This finding was also mimicked in the study carried out in Brazil where the mean white blood cell count of the participants after taking hydroxyurea was found to be significantly lower than the mean white blood cell count of the patients prior to taking hydroxyurea (Sètondji *et al*, 2018). This was replicated in the Jain study of 2012 where a significantly lower mean white blood cell count was seen in the group of patients taking hydroxyurea compared to the mean white blood cell count of the placebo group (Jain D., 2012). These findings were also portrayed in a study conducted in the North and Central regions of Nigeria where the total white blood cell counts of the study participants after administration of hydroxyurea were significantly higher compared to their mean white blood cell counts

before administration of hydroxyurea (Ofakunrin *et al.,* 2019). A similar study was conducted in larger scale in Sub-Saharan Africa and the findings were comparable with higher levels of mean white blood cell count after treatment with hydroxyurea when compared with the baseline mean white blood cell count (Tshilolo, L., 2019). The degree of margin of error for this data in our study may be attributed to the presence of outliers in our data pool. The observation of reduction in the production of WBCs is presumed to be therapeutic as raised levels have been found to be associated with morbidity and mortality in sickle cell anaemia patients. Neutrophils tend to promote vascular adhesion hence leading to vaso-occlusion. Thus, a reduction in their numbers by HU reduces the expression of surface adhesion receptors (Mulaku *et al*, 2017). However, the decrease in total WBC count could be detrimental to sickle cell patients since this translates into a reduction in the number of circulating immune response cells. Sickle cell patients are highly prone to infections from microorganisms such as *Staphylococcus aureus* (Pule *et al*, 2014).

5.1.4 Effect of hydroxyurea on differential count.

Our studies show that participants who had been on hydroxyurea consistently for a period of at least 3 months had significantly lower counts of differential white blood cells compared to their counterparts who were not on hydroxyurea for a period of at least 3 months. Lower mean cell counts were seen in the neutrophil counts, eosinophil counts, basophil counts, lymphocyte counts and monocyte counts. This is in line with a study conducted in Egypt where the differential counts were assessed on the participants prior to taking hydroxyurea and assessed again after hydroxyurea therapy. The neutrophil count was significantly lower post treatment with hydroxyurea compared to before. Similarly, a comparable observation was made in the comparison of the lymphocyte count where the mean lymphocyte count of the participants was lower after treatment with hydroxyurea compared to before treatment. A comparable observation was also made in the monocyte count. A lower mean lymphocyte count was observed post-treatment with hydroxyurea compared to the mean lymphocyte count before treatment (Zahran, A., 2020). These findings were also replicated in a study conducted

in the greater Sub-Saharan region where the mean absolute neutrophil count after administration of hydroxyurea to the participants was comparably lower compared to the mean absolute neutrophil count observed prior to hydroxyurea therapy (Tshilolo, L., 2019). This is also portrayed in a study in Abuja where the mean absolute neutrophil count was significantly lower after hydroxyurea was administered compared to the baseline mean (Nnebe., 2021). The comparable difference in our levels of monocyte counts between the groups can be attribute to a small sample size and the existence of outliers in one group (Shome *et al.*, 2016). This reduction in number of neutrophils and monocytes is presumed to be therapeutic since the reduction of the numbers of activated cells circulating in the blood reduces the number of surface adhesion molecules expressed hence reducing the chances of cell adhesion to vascular endothelium. Consequently, this leads to reduction of incidence of vaso occlusive crisis and vascular injury in the sickle cell patients who are on hydroxyurea (Wun, 2000). A reduced eosinophil count also reduces adhesion molecules available and also degranulation molecules that may be associated with complications of allergic reactions such as acute thoracic syndrome (Pallis *et al.*, 2010). The reduction in absolute lymphocyte count may lead to a reduction in inflammatory response thus reduced incidence of vaso occlusive crisis. However, it may also lead to increased risk of infection (Lederman *et al.*, 2014).

5.1.5 Effect of hydroxyurea on platelet count.

In our study, the mean platelet count for those who were on hydroxyurea was lower than the mean platelet count for those not on hydroxyurea and the difference was found to be statistically significant. This pattern is in agreement with the findings of the study carried out in Nigeria, where the mean platelet count of the participants found to be significantly lower after administration of hydroxyurea for 12 months compared to the mean platelet count at baseline (Hassan *et al.*, 2017). This was also the case in the study carried out in Brazil where a significantly lower mean platelet count was observed in the participants after administration of hydroxyurea compared to the mean prior to administration of the therapy (Sètondji *et al.*, 2018). Similarly, in the Jain study of 2012, the total platelet count of patients who had been on hydroxyurea for 18 months was

comparably lower than the mean platelet count of the placebo (Jain, 2012). In another study conducted in the larger Sub -Saharan Africa region, the total platelet count was also comparably lower after administration of hydroxyurea to the participants compare to their baseline mean platelet count (Tshilolo, 2019). This reduction in platelet count has also been found to be beneficial to sickle cell anaemia patients since they tend to be in a chronic state of haemostatic activation. Increased platelet activation is a component of this and is suspected to be part of the causes of chronic vascular complications (Vilagra *et al.*, 2001). Reduction in the total number of circulating platelets results in less platelets available to form aggregates by binding to neutrophils, monocytes and platelets. The reduced platelet-neutrophil and platelet-monocyte aggregates causes less molecules available to adhere to vascular endothelium and less aggregates to bind sickled red blood cells causing less events of vaso occlusive crisis (Zhang, 2016). Despite this, thrombocytopenia is a risk with the use of hydroxyurea and has been associated with disease severity as it has been found to be common in cases with multi organ failure as well as non survivors (Shome *et al.*, 2018)

5.2 Conclusion

The following conclusions can be made from this study;

1. Hydroxyuea therapy is associated with an increase in the overall haemoglobin levels of sickle cell anaemia patients within the Kenyan population.
2. Hydroxyurea causes is associated with a decrease in the total number of circulating white blood cells in the blood of sickle cell anaemia patients in the Kenyan population.
3. Hydroxyurea is associated with a decrease in the platelet counts of sickle anaemia patients in the Kenyan population.

The findings of this study lead to a conclusion that there is a difference between the haematolgical parameters of the sickle cell anaemia patients within the Kenyan population who take hydroxyurea and those who are hydroxyurea naïve.

5.3 Recommendations

The following recommendations can be supported by the findings of this study:

1. There is need for further studies to study levels of HbF production stimulated by hydroxyurea among the Kenyan sickle cell disease population.
2. There is need for further studies to understand the actual mechanism by which hydroxyurea causes a decrease in WBC levels among sickle cell patients.
3. There is a need for further studies on dose - dependent rate of reduction of platelet counts among the sickle cell disease population in Kenya.

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APPENDICES

Appendix I: Informed Consent Form

English version

PARTICIPANT INFORMATION AND CONSENT FORM

ADULT CONSENT

FOR ENROLLMENT IN THE STUDY

Title of Study: A COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS BETWEEN SICKLE CELL ANAEMIA PATIENTS ON HYDROXYUREA AND HYDROXYUREA NAÏVE PATIENTS.

Principal Investigator\and institutional affiliation: Eunice Wandia Kanyiri - JKUAT

Co-Investigators and institutional affiliation: Prof Cleophas Kyama – JKUAT

Dr Peter Maturi – UoN

Dr Fredrick Okinyi – UoN

Introduction:

I would like to inform you about a study being conducted by the researchers listed above. The purpose of this consent form is to offer you the information you ought to have to assist you to decide whether or not to be a participant within this study. Feel free to raise any questions on the aim of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything regarding the research or this form that is not clear. When we have answered all your inquiries to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you fully perceive and agree to participate in the study,

I will request you to sign your name on this form. You should fully understand the general principles which apply to all those taking part in this medical research: i) Your agreement to participate is entirely voluntary ii) You may withdraw from the study at any time without having to give a reason for your withdrawal.

Refusal to participate in the analysis will not affect the services you are entitled to within this health facility or other facilities. We will provide you a duplicate copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol No. P 289/04/2018.

WHAT IS THIS STUDY ABOUT?

The researchers listed above are interviewing individuals who suffer from sickle cell anaemia. The purpose of the interview is to find out the levels of haematological parameters among those taking hydroxyurea and those not taking hydroxyurea. Participants in this research study will be asked questions about their overall health within the last 3 months. Participants will also have the choice to undergo a full blood count.

There will be approximately 88 participants in this study carefully chosen. We are requesting your consent to consider taking part in this study.

HAPPEN IF YOU CHOOSE TO BE INCLUDED IN THIS RESEARCH STUDY?

If you decide to participate in this study, the following will happen:

You will be interviewed by a well - trained interviewer in a private area where you feel comfortable answering the questions asked. The interview will last approximately 10 minutes. The interview will cover topics such as any illnesses suffered within the last 3

months, any symptoms related to sickle cell that may have been suffered in the last 3 months, as well as any history of stroke and duration of taking hydroxyurea.

After the interview has finished and you qualify for the study, you will undergo a blood draw where you will sit comfortably on a chair. A tourniquet will then be applied to your arm and 1ml of blood drawn into a tube. A bandage will then be applied to your arm.

We will ask for a mobile number on which we can reach you if necessary. If you offer to provide your contact information, it will be used only by individuals working in this study and will never be shared with any other persons. The reasons we may need to contact you is in the case the need for further follow up arises.

ARE THERE ANY RISKS, HARMS, DISCOMFORTS ASSOCIATED WITH TAKING PART IN THIS STUDY?

Medical analysis has the potential to introduce psychological, social, emotional and physical risks. Effort should always be applied to reduce the risks. One potential risk of participating in the study is the loss of privacy. We will keep everything you tell us in the study as confidential as possible. We will use coded digits to identify you in a password-protected electronic database and will keep all our documented paper records in a locked file cabinet. However, no system of protecting your confidentiality can guarantee absolute security, so it is still possible that someone could find out you are participating in this study and could find out information about you.

Also, answering some questions in the interview may be somewhat uncomfortable for you. If there are any answers you do not want to divulge, you can skip them. You have the right to refuse any questions asked during the interview.

You may feel some discomfort when blood is drawn and you may have a small bruise or swelling in your arm at the site of blood draw. In case of any injury, illness or complications related to participating in this study, contact the study staff immediately

on the number provided at the end of this document. The study staff will treat you or refer you when necessary.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

You may benefit by receiving free health information in regard to your condition and the use of hydroxyurea .We will refer you to a hospital for care and support where necessary. Also, the information you provide will help us better understand the effect of hydroxyurea in sickle cell among the Kenyan population in regard to the blood counts. This information is a contribution to science and the well being of our sickle cell population.

WILL BEING IN THIS STUDY COST YOU ANYTHING?

No, taking part in this study will not cost you anything.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided below.

EUNICE W. KANYIRI Tel: 0722845612.

For any additional information concerning your rights as a research participant you may contact the following persons;

1.Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

2. Principal investigator

Eunice Wandia Kanyiri

Tel: +254722845612

3.Lead supervisor

Dr Peter Maturi

Tel: +254722400128

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to refuse participation in the study and you can withdraw from the study at any point without injustice or loss of any benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information in it read to me. I have had the opportunity to discuss this research study with a study counselor. I have had my questions answered in a language that I well understand. The risks and benefits have been fully explained to me. I understand that my participation in this study is completely voluntary and that I may choose to withdraw at any time. I freely agree to take part in this research study.

I understand that all efforts will be made to keep any information regarding my personal identity confidential.

By signing this consent form, I have not given up any of my legal rights as a participant in a research study.

I agree to take part in this research study:	**Yes**	**No**
I agree to have my blood preserved for later study:	Yes	No

I agree to provide my contact information for follow-up: Yes No

Participant printed name: _____

Participant signature / Thumb stamp _____ Date_____

Researcher's statement

I, the undersigned, have fully explained all the necessary details of this research study to the participant named above and I believe that the participant has fully understood and has willingly and freely given his/her consent.

Researcher's Name: Eunice W. Kanyiri **Date**: _____

Signature _____

Role in the study: Principal investigator.

For more information contact Eunice Kanyiri at KNH on Mondays from 8am to 9 pm

Witness Printed Name (*If witness is necessary, A witness is a person who is mutually acceptable to both the researcher and participant*)

Name _____Contact information _____

Signature /Thumb stamp: _____

Date:_____

Kiswahili version

FOMU YA IDHINI YA UANDIKISHAJI WATU WAZIMA KATIKA UTAFITI NA MAELEZO KWA MSHIRIKI

Jina la Utafiti: <u>UTAFITI LINGANISHI YA VIGEZO HAEMATOLOGICAL KATI WAGONJWA ANAEMIA YA SELI MUNDU WANAOTUMIA HYDROXYUREA NA WASIOTUMIA.</u>

Mchunguzi mkuu\ uhusiano wa taasisi: <u>Eunice Wandia Kanyiri - JKUAT</u>

Wachunguzi wengine\ uhusiano wa taasisi: Prof Cleophas Kyama – JKUAT

Dr Peter Maturi – UoN

Dr Fredrick Okinyi – UoN

Utangulizi:

Ningependa kukuambia kuhusu utafiti unaofanywa na watafiti waliotajwa. Madhumuni ya fomu hii ya idhini ni kukupa taarifa unayohitaji kukusaidia kuamua kama ungependa kuwa mshiriki katika utafiti huu. Jisikie huru kuuliza maswali yoyote kuhusu madhumuni ya utafiti, kile kinachotokea kama utakubali kushiriki katika utafiti, hatari ya uwezekano na manufaa, haki yako kama kujitolea, na kitu kingine chochote kuhusu utafiti au fomu hii ambayo si wazi. Wakati tumejibu maswali yako yote kwa kuridhika kwako, unaweza kuamua kuwa katika utafiti au la. Utaratibu huu unaitwa 'ridhaa'. Baada ya kuelewa na kukubali kuwa katika utafiti huu, nitakuomba uniandikie jina lako na utie sahihi kwenye fomu hii. Lazima uelewe kanuni za jumla zinazotumika kwa washiriki wote katika utafiti wa matibabu: i) Uamuzi wako wa kushiriki ni kwa hiari

yako mwenyewe ii) Unaweza kuondoka kutoka kwa utafiti huu wakati wowote bila kutoa sababu ya kujitoa kwako.

Kukataa kushiriki katika utafiti hakutaathiri huduma unayostahili kupata katika kituo hiki cha afya au vituo vingine. Tutakupa nakala ya fomu hii kwa kumbukumbu zako.

Naweza kuendelea? NDIO/ LA?

Utafiti huu umepitishwa na Kamati ya Maadili na Utafiti ya Hospitali kuu ya Kenyatta kushirikiana na Chuo Kikuu cha Nairobi itifaki nambari P 289/04/2018.

_____

UTAFITI HUU NI KUHUSU NINI?

Watafiti waliotajwa hapo juu watahoji watu ambao wanakabiliwa na anaemia selimundu. Madhumuni ya mahojiano ni kutafuta vigezo vya kihematolojia miongoni mwa wale wanaotumia dawa aina ya hydroxyurea na wale wasiotumia. Washiriki katika utafiti huu watatakiwa kujibu maswali kuhusu afya yao ndani ya miezi 3 iliyopita. Washiriki pia wana uhuru wa kuamua kufanyiwa uchunguzi wa damu.

Kutakuwa na takriban washiriki 113 waliochaguliwa kwa makini katika utafiti huu. Tunakuomba idhini yako kama ungetaka kushiriki katika utafiti huu.

ITAKUWAJE KAMA UTAAMUA KUWA KATIKA UTAFITI HUU?

Ukikubali kushiriki katika utafiti huu, mambo yafuatayo yatatokea:

Utahojiwa na mtaalamu katika eneo binafsi unapojisikia kuwa hauna wasiwasi wowote. mahojiano itadumu takribani dakika 10. mahojiano itaafikia mada kama vile magonjwa yoyote uliyopata ndani ya miezi 3 iliyopita, dalili zozote kuhusiana na kiini mundu ambavyo huenda ulipata katika miezi 3, pamoja na historia yoyote ya kiharusi na muda uliotumia hydroxyurea

Baada ya kumaliza mahojiano na uwe umetimiza masharti ya utafiti, utatolewa damu kama umestareheka kwa kiti. Utafungwa na kisongo mkononi kisha utatolewa 1mL ya damu itakayowekwa katika chupa. Kasha utafungwa bandeji mkononi mwako.

Tutakuomba nambari yako ya simu ndio tuweze kuwasiliana na wewe itapokuwa ni lazima.

Ukikubali kupatiana nambari yako, itatumika tu na watu wanaofanya kazi ya utafiti huu na kamwe haitapatiwa watu wengine. Sababu tunahitaji kuwasiliana na wewe ni kama kutakuwa na haja ya kufuatilia jambo lolote likijitokeza.

JE, KUNA HATARI YOYOTE INAYOHUSIANA NA UTAFITI HUU?

Utafiti wa matibabu una uwezo wa kuanzisha madhara ya kisaikolojia, kijamii, kihisia na kimwili. Juhudi lazima iwe imewekwa ili kupunguza hatari. Moja ya madhara yanayoweza kutokea katika utafiti ni kupoteza faragha. Tutaweka kila kitu unachotuambia kwa siri iwezekanavyo. Tutatumia nambari fiche kukutambua katika hifathi iliyolindwa ndani ya tarakilishi na pia tutaweka kumbukumbu zote zilizonakiliwa kwa karatasi kwenye faili na kuzifungia kwa kabati. Hata hivyo, hakuna mfumo wa kulinda usiri wako unaweza kuwa salama kabisa, hivyo kuna uwezekano mtu mwingine anaweza jua kuwa ulihusika katika utafiti huu.

Pia, kujibu maswali katika mahojiano inaweza kuwa na wasiwasi kwako. Kama kuna maswali yoyote hutaki kujibu, unaweza kuyaacha hayo. Una haki ya kukataa mahojiano au maswali yoyote utakayoulizwa wakati wa mahojiano.

Unaweza kusikia uchungu kidogo wakati damu inatolewa na unaweza kuwa na chubuko ndogo au uvimbe katika mkono wako katika mahali damu imetolewa. Ikiwa kuna jambo litakalosababisha kuumia, ugonjwa au matatizo kuhusiana na utafiti huu, wasiliana na wafanyakazi wa utafiti mara moja kutumia namba zilizotolewa katika mwisho wa waraka huu. wafanyakazi wa utafiti huu watakutibu kwa tatizo loote ndogo la kiafya litaloweza kutokea au wakutume kwa kituo chochote cha afya itakapobidi.

JE, KUNA FAIDA GANI KUWA KATIKA UTAFITI HUU?

Unaweza kufaidika kwa kupokea taarifa za afya kuhusu hali yako na matumizi ya hydroxyurea bure. Pia tutakuelekeza kwa hospitali ili upate msaada inapobidi. Halikadhalika utatusaidia kuelewa zaidi athari za hydroxyurea katika watu walio na shida ya kiini mundu nchini Kenya na kulinganisha na vipimo za damu

KUTAKUWA NA GHARAMA YOYOTE KUSHIRIKI KATIKA UTAFITI HUU?

La hasha, kushiriki katika utafiti huu hakuna gharama yoyote.

UNAWEZA KUWA NA MASWALI MENGINE BAADAYE?

Kama una maswali au matatizo kuhusu kushiriki katika utafiti huu, tafadhali piga simu au tuma ujumbe mfupi wa simu kwa wafanyakazi wa utafiti katika nambari iliyo hapa chini.

EUNICE W. KANYIRI Simu: 0722845612.

Kwa habari zaidi kuhusu haki zako kama mshiriki wa utafiti huu unaweza kuwasiliana na:

1.Katibu / Mwenyekiti, kamati ya maadili na utafiti Hospitali kuu ya Kenyatta na chuo kikuu cha Nairobi Nambari ya simu. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

MCHUNGUZI MKUU

2.Eunice Wandia Kanyiri

Nambari ya simu: +254722845612

3.MCHUNGUZI MWELEKEZI

Dr Peter Maturi

Nambari ya simu: +254722400128

CHAGUO LAKO LINGINGINE NI LIPI

Uamuzi wako wa kushiriki katika utafiti ni kwa hiari. Una haki ya kuacha kushiriki katika utafiti huu na unaweza kuondoka wakati wowote bila kudhalamiwa au kupoteza faida yoyote.

FOMU YA IDHINI (MAELEZO YA MAKUBALIANO)

Kauli ya mshiriki

Nimesoma fomu hii ya idhini au nikasomewa. Nimekuwa na nafasi ya kujadiliana kuhusu utafiti huu na mshauri wa utafiti. Maswali yangu yote yamejibiwa kwa lugha ninayoielewa.nimeelezwa mema na mabaya yanayoweza kunipata katika utafiti huu.Naelewa kwamba ushiriki wangu katika utafiti huu ni kwa hiari na naweza kujiondoa wakati wowote na nimekubali kushiriki kwa hiari yangu.

Naelewa kwamba juhudi zote zitafanywa ili kuweka maelezo kuhusu utambulisho wangu kibinafsi faraghani.

Kwa kutia sahihi idhini hii sijapatiana haki zozote za kisheria ninazonimiliki kama mshiriki katika utafiti huu

Nakubali kushiriki katika utafiti huu:	**Ndio**	**La**
Nakubali damu nitakayotolewa iwekwe kwa ajili ya utafiti baadaye	Ndio	La
Ninakubali kutoa taarifa za mawasiliano kwa minajili ya kufuatilia:	Ndio	La

Jina la mshiriki lililochapishwa:

_____

Sahihi ya mshiriki / Alama ya kidole _____ Tarehe _____

Kauli ya mtafiti:

Mimi , niliyetia sahihi hapanimepatiana maelezo ya utafiti huu kikamilifu kwa mshiriki aliyetajwa hapa juu na naamini kuwa mshiriki ameelewa na amepatiana ridhaa kwa hiari yake.

Jina la mtafiti: Eunice W. Kanyiri **Tarehe**: _____

Sahihi

Wajibu katika utafiti: Mtafiti mkuu

Kwa maelezo zaidi wasiliana na Eunice Kanyiri katika hospitali kuu ya Kenyatta, Jumamosi kutoka saa mbili asubuhi hadi saa kumi na moja jioni.

Jina la shahidi lililochapishwa (Kama shahidi muhimu, ni sharti mtafiti na mshiriki wakubaliane)

Jina _____Anwani _____

Sahihi/alama ya kidole:
_____**Tarehe**:_____

Appendix II: Assent form

English version

ASSENT FORM

Project Title: **A COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS BETWEEN SICKLE CELL ANAEMIA PATIENTS ON HYDROXYUREA AND HYDROXYUREA NAÏVE PATIENTS.**

Principal Investigator\and institutional affiliation: Eunice Wandia Kanyiri - JKUAT

Co-Investigators and institutional affiliation: Prof Cleophas Kyama – JKUAT

Dr Peter Maturi – UoN

Dr Fredrick Okinyi – UoN

We are doing a research study about how people with sickle cell anaemia like you are affected by taking the medication called hydroxyurea and comparing it with those who do not take the medication. This will be done by taking a little blood from you and looking at it to see what has been happening.

Permission has been granted to undertake this study by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee(KNH-UoN ERC Protocol No. protocol No. P 289/04/2018).

This research study is a way to learn more about people.

At least 20 children will be participating in this research study with you.

If you decide that you want to be part of this study, you will be asked to answer a few questions about your illness. Then if you qualify to take part in the study, a little blood will be taken from your arm for testing. All this should take about 20minutes.

There are some things about this study you should know. Your blood will be put in a machine to determine the number of the different kinds of cells in your blood. This will be compared to the values of the other sickle cell patients.

You may feel a little pain when a needle is put in your arm to draw some blood. This should go away quickly.

Not everyone who takes part in this study will benefit from it.

A benefit means that something good will happen to you.

We think these benefits might be that doctors will be able to know when you actually have an infection.

If you do not want to be included in this research study, we will understand.

When we are finished with carrying out this study we will write a report about what was learned.

This report will neither include your name nor that you were in the study.

If you decide to stop after we have begun, that's okay too.

Your parents also know about the study.

If you decide you want to be in this study, please sign your name.

I, _____, want to be in this research study.

_____ _____

(Signature/Thumb stamp) (Date)

Kiswahili version

FOMU YA IDHINI

Jina la Utafiti: UTAFITI LINGANISHI YA VIGEZO HAEMATOLOGICAL KATI WAGONJWA ANAEMIA YA SELI MUNDU WANAOTUMIA HYDROXYUREA NA WASIOTUMIA.

Mchunguzi mkuu\ uhusiano wa taasisi: Eunice Wandia Kanyiri - JKUAT

wachunguzi wengine\ uhusiano wa taasisi: Prof Cleophas Kyama – JKUAT

Dr Peter Maturi – UoN

Dr Fredrick Okinyi – UoN

Utangulizi

Tunafanya utafiti huu kuhusu watu walio na anaemia selimundu kama wewe wanaathirika na dawa inayoitwa hydroxyureana kulinganisha na wasioitumia hio dawa. Hili litafanyika kwa kutoa damu kidogo na kupima ili kudhibitisha yanayofanyika

Utafiti huu umeruhusiwa na Kamati ya Maadili na Utafiti ya Hospitali kuu ya Kenyatta kushirikiana na Chuo Kikuu cha Nairobi (nambari ya itifaki P 289/04/2018)

Utafiti huu ni njia mojawapo ya kujua mengi kuhusu watu.

Angalau watoto sabini watahusika katika utafiti huu.

Ukiamua kushiriki katika utafiti huu, utaulizwa maswali kidogo kuhusu ugonjwa wako. Kama utakubalika kushiriki damu kidogo itatolewa kutoka kwa mkono wako ili ipimwe. Hii yote ianfaa kuchukua dakika ishirini tu.

Kuna mambo unafaa ujue kuhusu utafiti huu. ya kwamba damu yako itawekwa kwa mashine kuangalia kama iko na seli mbalimbali na kulinganishwa na za wagonjwa wengine walio na seli mundu.

Unaweza hisi uchungu kidogo wakati unadungwa sindano kutoa damu. Uchungu huu unafaa kupotea upesi sana.

Si kila mtu anahusika katika utafiti huu atanufaika.

Manufaa yanamaanisha kitu mzuri itafanyika kwako

Tunafikiria kati ya manufaa itakuwa ya kwamba madaktari watajua kweli wakati unaadhirika na ugonjwa.

Tutakuelewa ukiamua kutohusushwa kwa utafiti huu.

Wakati tutakapomaliza utafiti huututaandika ripotiya yale tumesoma.

Ripoti hio haitakuwa na jina lako wala hatutataja ya kwamba ulihusika.

Si lazima uwe kwa utafiti huu kama hutaki kuwa.

Ukiamua kuacha kama ushaanza ni vema pia.

Wazazi wako wanajua kuhusu utafiti huu pia

Kama unaamua kuwa miongoni ya wale watakaoshiriki katika utafiti huu tafadhali tia sahihi hapa chini.

Mimi, _____, ningetaka kuwa katika utafiti huu._____ _____

(Sahihi/alama ya kidole) (Tarehe)

Appendix III: Questionnaire

English version

A COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS BETWEEN SICKLE CELL ANAEMIA PATIENTS ON HYDROXYUREA AND HYDROXYUREA NAÏVE PATIENTS.

QUESTIONNAIRE

Identifier:_____

Age:_____ Sex:_____

Address: _____

1. Do regularly go for medical check up?
   1. Yes 2. No

2. Have you suffered from any illness within the last 3 months?
   1.Yes 2. No

3. If yes above, how long ago was it?
   1.Less than 1 month ago.
   2. More than 1 month ago.
4. Did the illness require hospitalization?
   1.Yes 2. No
5. Have you had a blood transfusion within the last 3 months?
   1.Yes 2. No
6. Have you undergone surgery within the last3 months?
   1.Yes 2. No

7. Have you been diagnosed with any of the following?

   1.Kidney problem

   2.Liver disease

8. Have you ever been diagnosed to have suffered a stroke?

   1. Yes 2. No

9. Are you currently pregnant?

   1.Yes 2. No

10. Are you currently taking hydroxyurea?

    1.Yes 2. No

11. If yes, are you taking it daily?

    1.Yes 2. No

12. If yes, for how long have you been taking your daily dose of hydroxyurea?

    1. At least 3 months 2. Less than 3 months

13. If not taking hydroxyurea at present, how long has it been since you last took a dose?

    1. At least 3 months 2. Less than 3 months

I attest that I have honestly answered the above questions to the best of my knowledge.

Signature: _____ Date: _____

FOR OFFICIAL USE ONLY:

Parameter	Result	Reference ranges	Units
WBC		4.5 – 11.0	10^9/l
Neutrophils		1.8 – 7.8	10^9/l
Eosinophils		0 – 0.4	10^9/l
Basophils		0 – 0.2	10^9/l
Lymphocytes		1.0 – 4.8	10^9/l
Monocytes		0.2 – 0.8	10^9/l
HB		13.5 – 17.5	g/dl
PLT		150 - 450	10^9/l

Kiswahili version

HOJAJI

Jina:_____

Umri:_____Jinsia:_____

Anwani: _____

1. Je, wewe huenda kuangaliwa hali ya afya yako mara kwa mara?
   1.Ndio 2.La
2. Je, ulipata ugonjwa wowote ndani ya miezi 3 iliyopita?
   1.Ndio 2.La
3. Kama ni ndiyo hapo juu,ilikuwa kwa muda gani?
   1. Ndani yamwezi mmoja uliopita
   2. Zaidi ya mwezi mmoja uliopita
4. Je, ugonjwa ulihitaji wewe kulazwa hospitalini?
   1.Ndio 2.La
5. Je, umepewa damu ndani ya miezi 3 iliyopita?
   1.Ndio 2.La
6. Umefanyiwa upasuaji wowote ndani ya miezi tatu iliyoisha?
   1.Ndio 2.La
7. Je, umepewa matibabu yoyote kati ya haya?
   1. Ugonjwa wa figo
   2. Ugonjwa wa ini
8. Je, umewai kuteseka kiharusi kulingana na daktari wako?
   1.Ndio 2.La
9. Wewe ni mja mzito?
   1.Ndio 2.La

10. Je, unatumia dawa aina ya hydroxyurea?

    1.Ndio 2.La

11. Kama ni ndiyo hapo juu, umekuwa ukitumia kwa utaratibu?

    1.Ndio 2.La

12. Umewahi kuwa na ugonjwa kwa kupooza?

    1.Ndio 2.La

Nathibitisha ya kwamba nimejibu maswali iliyo hapo juu kwa uaminifu na ufahamu wangu.

Sahihi:_____ Tarehe: ___

KWA MATUMIZI YA OFISI PEKEE

Parameter	Result	Reference ranges	Units
WBC		4.5 – 11.0	10^9/l
Neutrophils		1.8 – 7.8	10^9/l
Eosinophils		0 – 0.45	10^9/l
Basophils		0 – 0.2	10^9/l
Lymphocytes		1.0 – 4.8	10^9/l
Monocytes		0 – 0.8	10^9/l
HB		13.5 – 17.5	g/dl
PLT		150 - 450	10^9/l

Appendix IV: Ethical Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/309

August 9, 2018

Eunice Wandia Kanyiri
Reg.No.HSB 331-2549/2016
Dept.of Biomedical Sciences
J.K.U.A.T

Dear Eunice

RESEARCH PROPOSAL – A COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS BETWEEN SICKLE CELL ANEMIA PATIENTS ON HYDROXYUREA AND HYDROXYUREA NAÏVE PATIENTS (P289/04/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 9th August 2018 – 8th August 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

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Appendix V: Publication

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Journal Of Medical Science And Clinical Research
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An Official Publication Of IGM Publication

A Comparative Study of Haematological Parameters between Sickle Cell Anemia Patients on Hydroxyurea and Hydroxyurea Naïve Patients

Authors
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[2]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
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*Corresponding Author
Eunice Wandia Kanyiri
College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Abstract
Sickle cell anemia is a genetic blood disorder that requires the patients to take a lifelong regimen of hydroxyurea drugs. Kenya, being a third world country, many of these patients are not able to afford to sustain their supply of the drug hence are off it most of the time. The primary aim of this study was to determine whether there were haematological differences between the sickle cell patients taking hydroxyurea and hydroxyurea naive patients. After obtaining consent and assent, a 2ml blood sample was collected from each study participant. A full blood count was run on the SYSMEX XT – 2000i and data entered into an Excel sheet. The parameters of interest were the hemoglobin, white blood cell count, and platelet count. A questionnaire was used to collect sociodemographic information and clinical history information. Ninety two sickle cell anemia patients participated in this study. Of these 46 were on hydroxyurea while the other 46 were off hydroxyurea. The mean Hb of those on hydroxyurea and those not on hydroxyurea was10.4 and 9.0 respectively (P value = 0.01). The mean WBC of those on hydroxyurea and those not on hydroxyurea was 11.0 and 14.7 respectively (P value =0.005). The mean Plt of those on hydroxyurea and those not on hydroxyurea was 384 and 485 respectively (P value= 0.01). A clinically significant difference between the Hb, WBC and platelet counts was noted between the 2 groups thus suggesting a positive impact of Hu on the haematological parameters of sickle cell patients. Studies such as this could help policy makers in devising strategies to make hydroxyurea more affordable to the Kenyan sickle cell population.

Background
Sickle cell anaemia (SCA) is an inherited blood disorder that has a proven a major health challenge in Kenya as well as other parts of the world[1,2,3]. It is an inherited genetic disorder that affects the haemoglobin molecule found in the red blood cells[4,5,6]. It is not a contagious condition and thus not transmissible from one individual to another. Haemoglobin is the protein structure responsible for transporting oxygen and carbon dioxide molecules within the blood[7,8,9]. An individual inherits two haemoglobin genes, one from each parent[4,5]. When only one of these is a haemoglobin S gene, it results in the individual

being a carrier, whereas if both the haemoglobin genes inherited by the individual are of haemoglobin S genotype, it results in sickle cell anemia[4,5,7]. Thus sickle cell anaemia is said to be a recessive disorder[4]. People who are carriers of HbS are said to have sickle cell trait and do not exhibit any symptoms of sickle cell. They lead fairly normal lives. Persons with sickle cell anaemia, however, exhibit various symptoms as a result of the condition, that affect their ever day lives[4,9].

A point mutation within the DNA sequence is the primary cause of sickle cell anaemia[8]. This mutation occurs in Beta- globin gene that is located on chromosome 11 where an Adenine (A) is replaced by a Thymine (T)[2,5,9,11]. This leads to an alteration of the amino acid where the glutamic acid is replaced by valine[2,5,6,9]. This change causes the body to produce HbS instead of the normal HbA[5,6,10]. The resultant red blood cells possess the tendency to sickle in hypoxic conditions leading to a crescent shape and leading to various complications[5,6,11]. Normal red blood cells are disk shaped and flexible. However, sickle cell red blood cells are sickle shaped and rigid[7,9] They do not survive as long as normal red blood cells do. This causes them to have difficulty passing through blood vessels and stick to the walls of these [7,10]. This hinders the flow of blood to various tissues and thus oxygen cannot reach these sites[7]. This in turn leads to pain which is referred to as vaso occlusive crises[12]. The sickle cells do not last as long as normal red blood cells[12,13]. Normal red blood cells can last for about 90 to 120 days in circulation whereas sickle cells may last only up to 10 to 20 days[7,14]. Under normal circumstances, the body keeps on producing red blood cells to replace the old ones once they get destroyed but in sickle cell anaemia, the bone marrow has a hard time keeping up with the rapid rate of destruction leading to anaemia[17]. Other symptoms include high risk of serious infection, shortness of breath, acute chest syndrome, delayed growth and stroke.

Hydroxyurea is currently one of the widely used drug available for the management of sickle cell anaemia[3]. It works by increasing the synthesis of HbF by the body[5,12]. It also serves an inhibitory role on the polymerization of the sickle cells[12,16]. The main aim in management of sickle cell patients is to achievement of steady state. Steady state is the situation whereby the patient is not undergoing hemolytic crisis, is not experiencing pain and does not depict any clinical illness for a period preceding 3 months[19]. A sickle cell patient is said to be in steady state when they have not had an acute pain episode, a blood transfusion or any illness for a continuous 3 months[20]. The use of hydroxyurea has been found to lead to an overall improvement in the quality of life for sickle cell patients by having a reduction in the number of hospitalizations, painful crises and episodes of acute chest syndrome suffered by the patients[6,8,12]. However, despite these positive effects depicted by the drug, HU continues to be highly underutilized[13]. One of the reasons for this is the failure to maintain compliance by the patients or their relatives as well as inexperience among healthcare givers[17]. The limited availability of HU also contributes to this non compliance as most patients are not able to afford the drug. In Kenya, HU has not been incorporation in the Kenya National Guidelines for use in children of under the age of 5years[2,12,13,15]. Understanding the haematological profiles of sickle cell patients in steady state can be a predictor of clinical outcome and help in devising management strategies for the patients. In light of this, it is important to know whether there is any clinically significant difference between the full blood counts of those steady state sickle cell patients taking hydroxyurea and those not taking hydroxyurea.

Methods

Study site and population

This cross – sectional study was carried out at the Kenyatta National Hospital from August 2018 to January 2019. The study population was

comprised of sickle cell anemia patients attending the hospital's hematology clinic. A total of forty six steady state sickle cell anemia patients taking hydroxyurea for at least 3 months were recruited as well as forty six steady state sickle cell anaemia patients who had not taken hydroxyurea in at least 3 months, were recruited into the study. Steady state was defined as having not had an acute pain episode, a blood transfusion or any illness for the last previous 3months. The patient's steady state was confirmed through their clinical notes. Patients diagnosed with acute renal disease were excluded from the study.

Sample collection and analysis

Interviewer administered questionnaires were used to obtain relevant information on socio-demographic and clinical history of the participants. Informed consent was obtained from all participants above 18years of age and assent gotten from the parents/guardians of the participants below 18 years of age. Two millilitres of venous blood were collected into ethylene diamine tetraacetic (EDTA) vacutainers for a full blood count. These were run on the SYSMEX XT – 2000*i* blood count machine and data on the Hb, WBC and platelet count gotten for each sample.

Statistical analysis

The Hb, WBC and platelet count data was the entered into Microsoft Excel, cleaned and validated. A two tailed t-test was used to compare the means of the Hb, WBC and Platelets between the 2 samples. A confidence interval of 95% was set at a *P*- values less than or equal to 0.05.

Ethical consideration

Ethical approval was obtained from the Kenyatta National Hospital – University of Nairobi Ethical Research Committee in accordance with the code of ethics for biomedical research involving human subjects (reference No. P 289/04/2018). Written informed consent and assent was obtained from all participants.

Results

A total of 92 subjects participated in the study. These included 46 patients on hydroxyurea and 46 hydroxyurea naïve patients. The mean Hb of those on hydroxyurea was 10.4g/dl while that of those not on hydroxyurea was 9.0g/dl (Table1 and the p value = 0.01). The mean WBC of those on hydroxyurea was 11.0 x10^3/uL while that of those not on hydroxyurea was 14.7 x10^3/uL (Table2, the P value =0.005). The mean Plt of those on hydroxyurea was 384.6 while that of those not on hydroxyurea was 485.5 (Table3, the P value= 0.01).

Table 1: t-Test: Two-Sample Assuming Unequal Variances for Hb levels

	HB	*HB-2*
Mean	10.44434783	9.060434783
Variance	7.044567343	5.944062029
Observations	46	46
Hypothesized Mean Difference	0	
P(T<=t) two-tail	0.010784954	

Table 2: t-Test: Two-Sample Assuming Unequal Variances for WBC count

	WBC	*WBC-2*
Mean	11.00434783	14.78478261
Variance	22.38931401	58.99509662
Observations	46	46
Hypothesized Mean Difference	0	
P(T<=t) two-tail	0.005768337	

Table 3: t-Test: Two-Sample Assuming Unequal Variances for platelet count

	PLT	*PLT-2*
Mean	384.6956522	485.5869565
Variance	28032.3942	39524.60338
Observations	46	46
Hypothesized Mean Difference	0	
P(T<=t) two-tail	0.010023191	

These results correspond with studies carried out in the USA where the WBC and platelet counts were found to decrease while the Hb levels increased in sickle cell anemia patients taking hydroxyurea compared to those who took a placebo drug[12]. Another study also showed

significant difference between the parameters with Hb levels rising from 8.3 g/dl before Hu to 9.0 g/dl after Hu,(P = 0.0003), reductions in the numbers of leukocytes from 10.0x10^3/uL before Hu to 5.7x10^3/uL after Hu (P < 0.0001) and platelet count reduction from 459 x10^3/uL to 373 x10^3/uL (P = 0.0002)[22].

Discussion

This study showed that there was a significant difference the Hb, WBC and platelet values between steady state sickle cell anaemia patients on Hu and steady state sickle cell anaemia patients not on Hu. The Hb was seen to be higher in patients taking Hu while the WBC and platelet counts were lower. The higher Hb values in those taking Hu is attributed to the action of Hu increasing HbF. The lower WBC nad platelet counts could be attributed to the cytoreductive action of the drug

Despite the documented evidence of the benefit of Hu to sickle cell patients, it is still widely underused. We hope this study together with others published in recent times, will continue to encourage physicians to emphasize on the need for sickle cell patients to use hydroxyurea.

Conclusion

The patients on hydroxyurea presented better values for their hemoglobin, white blood cell and platelet counts. Based on this, the authors suggest that policy makers and health providers improve health education among sickle cell patients on the importance of hydroxyurea and also make these drugs more affordable to all sickle cell patients.

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