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CHANGES IN HAEMATOLOGICAL AND CLINICAL PARAMETERS IN ADULT SICKLE CELL PATIENTS ON HYDROXYUREA: A BEFORE AND AFTER NON-EXPERIMENTAL RETROSPECTIVE STUDY AT THE SICKLE CELL CLINIC, KORLE-BU TEACHING HOSPITAL

BY

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(10875556)

THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MSc IN CLINICAL TRIALS DEGREE

MARCH, 2022

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DECLARATION

This dissertation, Changes in haematological and clinical parameters in adult sickle cell patients on hydroxyurea: a before and after non-experimental retrospective study at the sickle cell clinic, Korle-Bu Teaching Hospital is a study submitted to the Department of Epidemiology and Disease Control for the award Master of Science in Clinical Trial. This dissertation is the result of original research conducted by Akosua Yaago Asante-Offei, whereby paraphrases and quotations have been duly acknowledged.

This research was conducted under the supervision of the undersign supervisor.

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DEDICATION

To God Almighty, I say thank you for seeing me through this academic work. To my husband, Rev. Emmanuel Baiden Asare and the children; Christodea, Charis and Perez, thanks for your support and sacrifice.

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ABSTRACT

Introduction

Sickle cell disease (SCD) is a genetic blood disorder and major public health problem. The haemoglobin polymerizes at low oxygen and pH resulting in a sickled shape of the red cell causing a frequent painful vaso-occlusion crisis, anaemia, acute chest syndrome and organ damage. Hydroxyurea induces the production of haemoglobin F which inhibit sickling of red blood cell hence preventing these painful episodes with its associated complications. However, in Ghana, since the introduction of hydroxyurea, its effect on sickle cell patients have not been evaluated. Thus, this resaerch aims to investigate the changes in the haematological and clinical parameters of adult sickle cell patients on hydroxyurea.

Methodology: A pre and post non-experimental retrospective study was conducted on 105 SCD patients who are above 15 years. The data extraction tool, kobocollect was used to collect patients' socio-demographic information, clinical history and haematological findings from patients' folders. The primary outcome include changes in six haematological parameters and four clinical parameters 6 months before and after hydroxyurea therapy and the 12th month after being treated with 10-20mg/kg/day. Data analysis was employed using STATA 16/IC 16 software. Continuous variables such as age, Hb, HbF, MCV, WBC, platelet (Plt) and reticulocyte (Rct) count were summarized as mean and standard deviation (SD). Categorical data were summarized as percentages (%). Chi-square test was used to test for association between demographics and clinical parameters and the association between clinical manifestation and dosage. Paired t test was used to compare differences in means of continuous variables to determine changes in haematological parameters before and after hydroxyurea therapy.

Differences in proportions was estimated using z test of proportion. A significance level of 5% was adopted.

Results: There was a significant increase in haemoglobin (Hb) and mean corpuscular volume (MCV) at 6 months from 8.40±1.52 to 9.11±1.55 and 85.38±9.96 to 94.36± 11.87 respectively. Only Hb increased significantly at 12 months. A significant reduction was observed in white cell count (WBC), platelet count at the 6th month, but was not significant at the 12th month. An increase of 1.56% HbF was observed in one patient (7.4% to 8.96%). HU significantly reduced the frequency of vaso-occlusive crisis (65.4% to 19.6%, p<0.001) and hospitalization (36.5% to 9.6%, p< 0.001). Reduction in blood transfusion (6.7% to 2.9%, p= 0.195) and acute chest syndrome (3.9% to1%, p= 0.174) was not significant. Patients commenced HU on 10mg, 15mg and 20mg/kg/day which was not associated with vaso-occlusive crisis, acute chest syndrome, blood transfusion and hospitalization.

Conclusion: Hydroxyurea resulted in a beneficial reduction in WBC, PLT and RET and increases in Hb level, HbF and MCV. Clinically, HU reduced the frequency of vaso-occlusion crisis, acute chest syndrome, blood transfusion and hospitalization. There was no association observed between the dosages of HU and clinical parameters.

Recommendations: Based on the findings of this study, hydroxyurea is effective and improves the condition of sickle cell patients. Therefore, sickle cell patients should be encouraged to adopt the use of the medication. Clinicians should educate and recommend hydroxyurea to patients. More research should be conducted on the dosages of HU and clinical outcomes among adult sickle cell patients in Ghana.

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LIST OF ABBREVIATIONS

ACS	Acute chest syndrome
BT	Blood transfusion
HbF	Foetal haemoglobin
Hb	Haemoglobin
Hb S	Haemoglobin S
HbSC	Haemoglobin SC
HU	Hydroxyurea
MTD	Maximum Tolerated Dose
MCV	Mean corpuscular/cell volume
PLT	Platelet count
RET	Reticulocyte count
SCA	Sickle cell anaemia
SCD	Sickle cell disease
SD	Standard deviation
VOC	Vaso-occlussive crisis
WBC	White blood cell

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Globally, sickle cell disease (SCD) is a major public health problem (United Nations General Assembly,2009). In Africa, the burden of sickle cell is very high with about 200,000 children born annually with the disease (Diallo & Tchernia, 2002; Olowoyeye & Okwundu, 2020). In Ghana, it is estimated that 2% of newborns have sickle cell disease and about 25% to 30% are carriers (Edwin et al., 2011). Sickle cell disease (SCD) is one of the most predominant haemoglobinopathies in the world (Modell & Darlison, 2008; Weatherall, 2011)

Sickle cell disease (SCD) is a genetic blood disorder with a homozygous mutation of the β-chain of haemoglobin causing HbSS. Other haemoglobin mutation present in heterozygous form with a Hb S leads to other genotypes. These include Hb SC, sickle beta-plus-thalassaemia (HbS/β+) and sickle beta-zero-thalassaemia (HbS/β^0)(Manwani et al., 2014). This is a result of the oxygen-carrying haemoglobin molecule in the red blood cell undergoing polymerization at low oxygen concentration and pH.

Sickle cell disease is marked by frequent painful vaso-occlusive crisis, anaemia, jaundice and shortness of breath (Wankhade et al., 2013). Acute chest syndrome, avascular hip necrosis, leg ulcers, renal damage, retinopathy and stroke are some of the complications that are associated with the disease. These complications result in physical and psychosocial challenges to sickle cell patients (Pecker et al., 2019). Individuals with frequent painful crises tend to develop poor quality of life (Luzinete et al., 2018).

To reduce the severity and frequent pain episodes of people living with sickle cell, modifying drugs have become an urgent need. Through various research, hydroxyurea emerged as an effective disease-modifying therapy for sickle cell patients. The drug has been used for over 30 years in developed countries (Yawn et al., 2014).

In 1998, hydroxyurea was approved to treat adult sickle cell patients in the USA (Okam et al., 2014). Hydroxyurea enhances the production of haemoglobin F (Hb F) in the blood by inhibiting the intracellular haemoglobin S (HbS) polymerization, blocking sickling within erythrocytes and preventing vaso-occlusive crises and other complications. Most research studies have reported on the effectiveness of hydroxyurea (Lanzkron et al., 2010). Patients present with haematological improvement in haemoglobin F (HbF), haemoglobin level (Hb) and mean corpuscular volume (MCV) and a reduction in white cell count (WBC), platelet and reticulocyte counts (Adewoyin et al., 2017). Clinically, hydroxyurea improves the patient condition with reduction in vaso-occlusion crisis, frequency of hospital admissions, blood transfusion, and acute chest syndrome and organ damage (Silva-Pinto et al., 2013).

The effect of hydroxyurea is dose-dependent in both adults and children. Dosage is mostly commenced with 10mg/kg/day and escalated to reach a maximum tolerated dose (MTD) of 30-35mg/kg/day. Most studies reported variability in the response to hydroxyurea and some reported beneficial effects without attaining an MTD of 30-35mg/kg/day (Sethy et al., 2018).

Hydroxyurea has been used among children since 2014 but few SCD adults started using it in 2015. It became widely known when the Food and Drug Authority gave marketing authorization to Norvatis to supply free hydroxyurea for a year in October 2018. Since 2019, the adult Sickle Cell Clinic at the Korle-bu Teaching Hospital and

other clinics scattered across the country such as Police Hospital, Greater Accra Regional Hospital, Cape Coast Teaching Hospital, Komfo Anokye Teaching Hospital, and Ho Teaching Hospital among others have prescribed hydroxyurea routinely to sickle cell patients.

Despite the proven benefits of hydroxyurea among patients living with sickle cell disease, little is known about its effect on haematological and clinical parameters among adult sickle cell disease patients in Ghana. Therefore this retrospective study of patient's clinical records was designed to investigate the changes in haematological and clinical parameters associated with hydroxyurea use in adults living with SCD. This study also seeks to investigate the dosage that is associated with clinical manifestations in sickle cell disease.

1.2 Problem Statement

Sickle cell disease results in significant morbidity and early mortality. Hydroxyurea is an effective disease-modifying drug developed to decrease the severity and frequency of vaso-occlusive (VOC) episodes thus improving the patient's condition in developed countries (Charache et al., 1995). In Sub-Saharan Africa, hydroxyurea usage is low due to lack of accessibility, lack of adequate treatment guidelines and high cost (John et al., 2020; Power-Hays & Ware, 2020; Therrell et al., 2020). Young adult patients present with frequent painful episodes, acute chest syndrome and organ damage resulting in physical and psychosocial challenges. The onset of organ damage occurs early and progressively worsens usually resulting in early mortality (Ware, 2010). The clinical events associated with drug usage is important in understanding therapeutic success or failure as well as helping prevent adverse drug reactions. By determining, the clinically relevant haematological parameters associated with hydroxyurea usage can help

identify dose-dependent adverse drug reaction. Also these changes in haematological and clinical parameters are necessary in the effective management of sickle cell patients and also provides evidence of potential benefits for patients on hydroxyurea. Also in Ghana, there is little evidence on the changes in the haematological and clinical parameters of adult sickle patients on hydroxyurea. Due to population and genetic differences in the human race, it is therefore important to investigate these parameters of sickle cell patients being managed on hydroxyurea.

1.3 Conceptual Framework

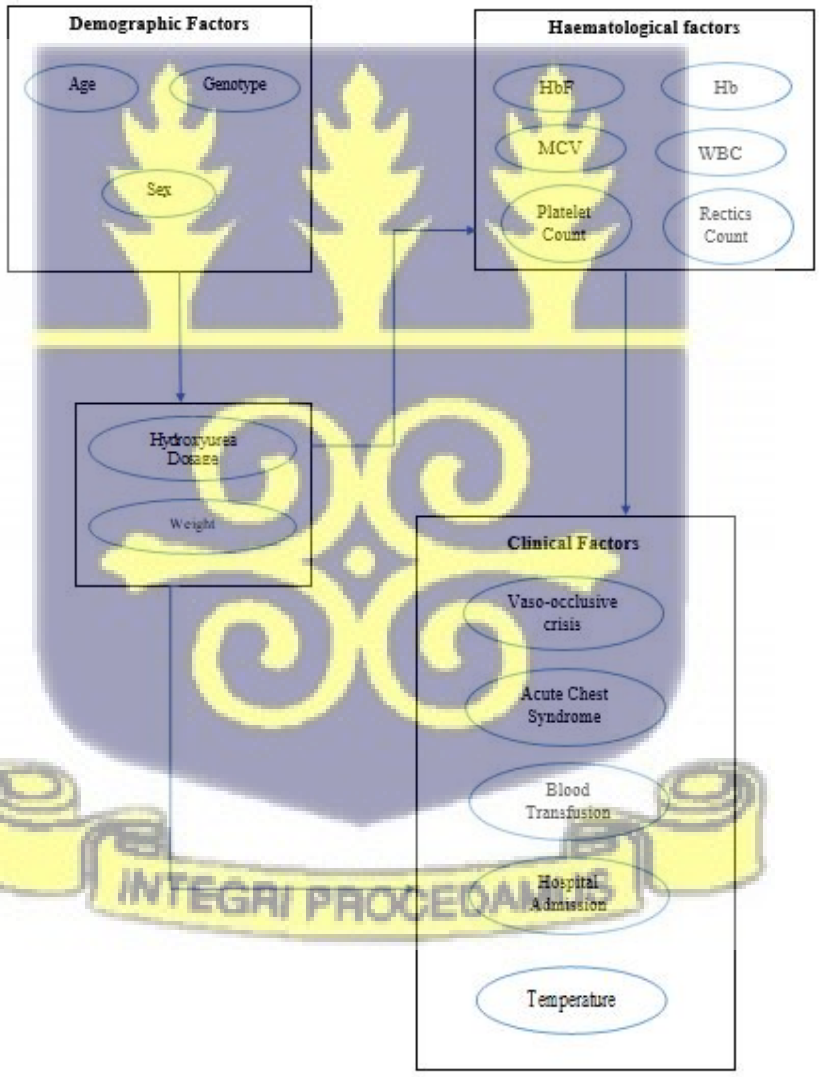


Fig 1. A conceptual framework of changes in haematological and clinical parameters of sickle cell patients on hydroxyurea.

1.3.1 Conceptual Framework Narrative of this Study.

This conceptual framework is based on evidence from literature. The various studies outlined the knowledge on the efficacy and effectiveness of hydroxyurea. They highlights the production of HbF in hydroxyurea. They describe factors associated with the usage of hydroxyurea. These factors are demographics, haematological factors, clinical factors and dosage Hydroxyurea use and effect depend on age, genotype, weight and dosage. Hydroxyurea effect a change in the haematological parameters by causing an increase in HbF, Hb, and MCV as well as a reduction in WBC, platelet and reticulocyte counts. Hydroxyurea induces foetal haemoglobin production, decreases intracellular HbS polymers within sickle erythrocytes, decreases red blood cell adhesion to endothelium and increases release of the potent vasodilator nitric oxide. These changes wihin the cells effect a response in the clinical parameters resulting in the decrease in the frequency of vaso-occlusive crisis, blood transfusion, acute chest syndrome and hospital admissions. Concerning the dosage, this is given based on the age and weight of the patient. This study seeks to identify any association with the frequency of clinical parameters.This is to investigate if the dose given is able to affect the frequency of the clinical events.

1.4 Justification

Adult sickle cell patients need a disease-modifying drug like hydroxyurea to improve their quality of life. The results from this study, will serve as evidence of the benefits of the drug and will be used to educate patients. This will alleviate the fear in most

patients who may feel skeptical of about a new drug. These haematological parameters are markers used to monitor the safety of the drug. Also it will also provide clinicians with in-dept knowledge to effectively manage SCD patients on hydroxyurea. The outcome of the study would provide evidence and add to the existing literature on adult patients managed on hydroxyurea in Ghana.

1.5 Research Questions

1. Is there any change in the haematological parameter(s) of sickle cell patients before and after treatment with hydroxyurea?
2. Is there a change in the frequency of clinical events among sickle cell patients on hydroxyurea therapy?
3. What is the association between the dosage of hydroxyurea and clinical events?

1.6 Study Objective

1.6.1 Primary Objective

To investigate the changes in clinical and haematological parameters associated with hydroxyurea usage in adult sickle cell patients.

1.6.2 Specific Objective

1. To determine changes in six haematological parameters (Hb, HbF, MCV, PLT, WBC and Reticulocyte count) before and after treatment with hydroxyurea.
2. To assess the frequency of four clinical manifestations(vaso-occlussive crisis, acute chest syndrome,blood transfusion and hospitalization) before and after hydroxyurea treatment.

3. To determine the association between four clinical manifestations and dosage of hydroxyurea intake among sickle cell patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Pathophysiology of Sickle Cell Disease

Sickle cell disease (SCD) is one of the most common haemoglobinopathies in the world. Sickle-cell disease is a genetic blood disorder with a homozygous mutation of the β-chain of haemoglobin causing HbSS. A compound of heterozygous mutation with a copy of HbS and an abnormal haemoglobin allele is also formed. Heterozygous groups include sickle-haemoglobin C disease (HbSC), sickle beta-plus-thalassaemia (HbS/β+) and sickle beta-zero-thalassaemia (HbS/β^0) (Manwani et al., 2014). This is a resultant of the oxygen-carrying haemoglobin molecule in the red blood cell undergoing polymerization at low oxygen and pH. In effect, the normal biconcave disc-like shape of the red cell is distorted giving rise to a long, thin and insoluble gel, resulting in the sickle red blood cell. Sickled red blood cells (RBCs) undergo constant haemolysis due to their fragile nature resulting in anaemia. The inability of the sickled RBC to return to its biconcave disc shape after recovering from low oxygen availability results in the occlusion of vessels and organ infarction.

2.2 Epidemiology of Sickle Cell

Globally, the population of sickle cell disease constitute 1%, with approximately 300,000 children born yearly. The United States of America, Jamaica, and United Kingdom are some countries with a well-documented sickle cell population. The burden of sickle cell disease is high with over 75% found in Sub-Saharan Africa. (World Health Organization, 2006, Diallo & Tehernia, 2002). Haemoglobin SS(Hb SS) is the most common form of sickle cell disease which account for 70% of all the

mutations (Modell & Darlison, 2008). The HbS is mostly common in Sub-Saharan Africa where malaria is common, while both HbC and ß-thalassemia is limited to parts of West Africa and North Africa. In these Tropical settings where malaria is common, the malaria parasite are able to thrive better in the Hb SS than the sickle cell trait (AS). This makes the trait a protective effect against malaria. From the Ghanaian population, studies indicate a carrier rate (those with a single S, example AS) of 25%-30% whereas 2% of newborns have sickle cell anaemia (Edwin et al., 2011).

2.3 Consequences of Sickle Cell Haemoglobin

The sickle red blood cells result in numerous clinical manifestations such as anaemia, fever, chest/joint pain, abdominal pain crisis, jaundice and shortness of breath (Wankhade et al., 2013). Acute chest syndrome, avascular hip necrosis, leg ulcers, renal damage, retinopathy and stroke are some of the complications associated with SCD. These complications result in physical and psychosocial challenges in sickle cell patients. The hallmark of this disease is the frequent occurrence of vaso-occlusive crisis resulting in pain. This accounts for most of its morbidity and mortality.

Vaso-occlusive episode is a severe pain that lasts for a least four hours and needs medical attention. It is the most common manifestation of SCD that results in frequent hospitalizations (Lanzkron et al., 2010). This pain is caused by ischemic tissue injury. This is due to the blocking or chocking of small blood vessels by a sickle cell thus impeding blood flow in the circulatory system. As a result, most organs and tissue are deprived of oxygen supply leading to damage over time with lifelong complications. The pain experienced by sickle cell patients can be acute and chronic. The extent of chronic pain is measured by how often it occurs within a given period and is measured by the frequency of pain episodes within a given year. The most common location of vaso-occlusive pain is the abdomen, chest, back, hip, waist, arm, and neck (Darbari et

al., 2020). Some factors that trigger vaso-occlusion episodes are extreme temperature, stress, infection, dehydration and menstrual cycle changes.

2.4 Supportive Treatment of Sickle Cell

Most of the treatment offered to the patients are supportive and does not actually change the pathophysiology of the disease. Sickle cell disease is been managed with analgesics, daily folic acid intake, blood transfusion and intake of penicillin to fight infections among children. Folic acid prevents deficiencies associated with chronically elevated erythropoiesis within the bone marrow. Patients are transfused to prevent stroke, acute chest syndrome and aplastic crisis. This aims at reducing the concentration of HbS to 30% or less. Patients are educated and always cautioned to hydrate by frequent water intake. This prevents vaso-occlusive crisis by increasing plasma volume and reducing blood viscosity when eight to ten glasses of water are taken daily. Patients are cautioned against adverse weather conditions. In search of a drug that modifies the pathophysiology of sickle cell disease, effective and less toxic, hydroxyurea was found and proven in this regard.

2.5 Hydroxyurea

Hydroxyurea (hydroxycarbamide) has been an effective, disease-modifying drug for sickle cell anemia patients over the past 30years (Yawn et al., 2014). In many studies, hydroxyurea has shown consistent improvement in the haematological parameters of sickle cell patients. Clinically, it reduces the frequency of acute painful vaso-occlusive crisis, acute chest syndrome, transfusions and hospitalization (Charache et al., 1995; Wang et al., 2011). Hydroxyurea improves survival for both adults and children with sickle cell disease (Steinberg et al., 2010; Voskaridou et al., 2010).

The clinical benefit of hydroxyurea is associated with haematological benefits. It improves haemoglobin level, mean cell volume, and HbF levels, while reducing white blood count (especially neutrophils), absolute reticulocyte count, and platelet count.

In Ghana, hydroxyurea have been used by some children for the past 7years while a few adult have been on it since 2017. However, the FDA granted marketing authorization to Novartis hydroxyurea in October 2018. A year later in November 6, 2019, through partnership to improve the diagnose and treatment of sickle cell disease in Ghana, a Memorandum of Understanding (MOU) was signed among the Ministry of Health, Ghana Health Service, the Sickle Cell Foundation and Novartis.

Under this MOU, 11 treatment centers were to be set up in all the regional capitals to have free access to the drug for one year. But only these centers, Komfo Anokye Teaching Hospital (KATH), Ho Regional Hospital, Accra Regional Hospital, Police Hospital, Korle-Bu Adult and Pediatric clinic and Tema General and Pediatric clinic, have been set up.

2.5.1 Mechanism of Action of Hydroxyurea

Hydroxyurea is primarily known for its ability to induce haemoglobin F level; it increases y-globin gene expression, which causes a shift in gene away from the ß-globin gene. This shift in gene expression results in an increased level of foetal haemoglobin. This drug manifests its clinical benefits through other mechanisms such as myelosuppressive effect, red cell interaction and rheological properties of Hb S. The main mechanism that results in increased HbF is called 'stress erythropoiesis or the production of red blood cell under stress. These cells develop more rapidly and make more HbF. Erythrocyte with more HbF are larger, that is have larger mean cell volume and more deformable (McGann & Ware, 2015). The drug by inhibiting DNA synthesis

for about six hours after intake slows red blood cell, as the drug wears off the red blood cell production accelerates and ends up making more Hb F.

Myelosuppressive effect of hydroxyurea is an important indicator of the reduction of symptoms of the disease condition. It reduces the production of neutrophils, reticulocytes, platelet count and lowering white cell count (WBC). Elevated white cell and platelet count results in chronic inflammation and associated with disease morbidity and mortality hence its reduction is of great benefit. Neutrophilia is known to cause severe disease (Platt et al., 1994). Neutrophils and reticulocytes promote vaso-occlusion thus hydroxyurea lowers their absolute numbers and reduces surface expression of adhesion receptor (Kumar et al., 2014). Another benefit is the increased levels of nitric oxide and cyclic nucleotides. These facilitate vascular dilation, induce HbF and have direct salutary effect on the vascular endothelium.

2.5.2 Dosage of hydroxyurea

Determining the effects of hydroxyurea is dependent on the dose. The optimal dose is a stable daily dose that causes mild mylosuppresion with absolute neutrophil count in the target range 2.0-3.0×10^9/L without any laboratory toxicity. This optimal level of hydroxyurea is reached when manifested in the clinical and laboratory improvement at a maximum tolerated dose . Maximum tolerated dose (MTD) is achieved when marrow suppression is achieved usually determined by absolute counts of neutrophil and reticulocytes.

Hydroxyurea is orally administered once daily. The recommended initial dose of hydroxyurea in sickle cell patient ranges from 10 – 20 mg/kg/day (Wong et al, 2014). Dose escalation is recommended at 2.5 to 5mg/kg every 4 weeks to 6 months (average 8 weeks), till the maximum tolerable dose is achieved. Patients are simultaneously monitored for clinical and haematological responses. Before and during treatment, liver

and renal function tests should be conducted every 4 to 8 weeks to assess for organ toxicities. Mostly, haemoglobin and mean corpuscular volume significantly increase at maximum tolerated dose in sickle cell patients. From most studies conducted, there is variability in the maximum tolerated dose and the HbF percentage which is an indicator of laboratory benefits (McGann & Ware, 2011,). Some patients can accept a high maximum dose level of 35 mg/kg/day before reaching myelosuppression, whereas others can only acecpt a dose of 15 mg/kg/day. Similarly, some patients can have more than 40% HbF levels, whereas others are not able to reach 20%. These can be attributed to differences in individual pharmacokinetics pharmacodynamics and pharmacogenomics. This differences contribute to variation of dosing and response to hydroxyurea among the various phenotypes (Mcgann et al., 2018).

2.5.3 Adverse Event of hydroxyurea

Hydroxyurea like any other drugs would have some side effects. Most studies have reported some side effects including gastrointestinal disorders involving abdominal pain, vomiting and diarrhoea. Colouration of skin, palm and nails are documented side effects. Leucopenia and thrombocytopenia are haematologic toxicities observed in most studies. Neutropenia is known to be the most common short-term toxicity, which is transient and reversible myelosuppression. The myelosuppressive effect of hydroxyurea is monitored by examining peripheral blood counts with dose adjustment.

2.5.4 Cost of hydroxyurea therapy

HbF was supposed to be performed prior to initiation of hydroxyurea therapy and at least once after therapy. But in this study, most patients were unable to bear the cost of GHc 110 to perform the test. Only one person was able to perform a pre and post test for HbF. This was also experienced in a previous study whereby only two patients

carried out the test prior to treatment. Reasons given was haemoglobin F assay not been more accessible and affordable (Adewoyin et al., 2017).

The cost of laboratory investigations needed to be done for a patient to enroll on hydroxyurea averagely cost GHc 500, while the medication cost GHC 2.5 per capsule. Majority of patients are unable to bear this cost.

2.6 Haematological Parameters of SCD patients on hydroxyurea

The haematological effects of hydroxyurea are essential for clinical benefits. These parameters are used for dose escalation to maximum tolerated dose as well as to monitor adherence of the medication.It has been found that hydroxyurea increases the level of HbF, total haemoglobin, mean corpuscular volume, while it reduces white blood cell , platelet and reticulocyte counts (Lanzkron et al., 2010).

2.6.1 Haemoglobin (Hb)

In the red blood cell is found haemoglobin, an iron-containing protein molecule which transport oxygen from the lungs to the body's tissues and back to the lungs. Normal red blood cell is spherical or disc-like in shape and survives for 120 days.

In sickle cell disease, the genetic mutation results in change of shape of the blood cell to half moon or sickled. This change in shape results in the cells becoming sticky, rigid and obstruct blood flow in the small vessel. Sickle cells are easily broken down, lives for a maximum of 10-20 days. This causes hyperheamolysis resulting in chronic haemolytic anaemia, a common feature in sickle cell patients (Kato et al., 2017). Anaemia generally reflect how severe sickle cell disease is. Most studies have reported on low haemoglobin levels in SCD patients compared to non- sickle cell patients (Akinsegun A. et al, 2012; Landry E et al, 2019). Low haemoglobin level poses

complications in SCD patients such as gall bladder disease, stroke, acute splenic sequestration and acute chest syndrome which results in a lots of deaths.

However hydroxyurea is the standard treatment to ameliorate these conditions. One of the beneficial effects of hydroxyurea is increase in heamoglobin level by extending the life span of the red blood cell which is evidence in most studies (Pradhan et al, 2018; Silva-Pinto et al, 2013; Singh et al, 2010). In this research, patients were monitored to avoid toxicity which results when Hb level falls below 20% of their baseline Hb level and < 4.5g/dl.

In India, a study by Pradhan et al among 69 adult patients, reported an increased Hb from 8.35±1.6gm% to 11.0±1.8gm% (31.7%). This increase was seen in the third month of initiation of hydroxyurea.

A retrospective study was conducted among thirty-seven sickle cell patients at Paulo, Brazil. This was to analyze the clinical and haematological effect during and after hydroxyurea treatment.The mean dose of Hu was 24.5±5.5mg/kg/day. The results indicated an increase in haemoglobin from 8.3g/dl to 9.0g/dl (p=0.0003)(Silva-Pinto et al, 2013).

A study was conducted in Italy among 652 peadiatric and adults of different descent and genotypes on hydroxyurea therapy.The mean age was 24.5 ± 15 years. From the study, mean total hemoglobin level increased significantly from 9.2 ±1.6 to 9.7 ±1.5 (+0.5 g/dL, p < 0.001). Among 44.5% patients, increase in haemoglobin level was ≥1.0 g/dL that is 125 out of 490 patients while a ≥2.0 g/dL increase in Hb was recorded in 19.0%, 218 out of 490 patients (Rigano et al, 2017).

Findings from Singh et al, 2010 revealed an increase of Hb level from 9.15g/dL to 9.98g/dL at the end of one year. The difference was not significant (p= 0.25).This was among 24 sickle cell anaemia patients with mean age 19.85.

2.6.2 Haemoglobin F (Hb F)

Haemoglobin F is a normal haemoglobin variant expressed by the fetus in the womb and during the first 3months of life. HbF is compose of 2 α-globin polypeptide chains and 2 γ-globin chains. This is in the red blood cell of the fetus which carries oxygen from the mother bloodstream to organs and tissues in the fetus. Studies report the persistence hereditary of Hb F in the red blood cell of infants in SCD of not showing any symptoms (Akinsheye I et al, 2011). The primary benefits of hydroxyurea is its ability to increase HbF. High levels of Hb F reduces the painful episodes of sickle cell patients, acute chest syndrome, leg ulcer, hip necrosis and reduce disease severity. Sickle red blood cells contain measurable amount of HbF (F cell) and non-F cells. These F cells are live longer and protect against sickle vaso-occlusion. HbF inhibit the polymerization of HbS by reducing its concentration, change the shape of the cells, making them less sticky and unable to get stuck in the blood vessels. Some studies have reported a significant increase in mean HbF level with hydroxyurea (Pradhan et al, 2018; Rigano et al, 2017:Singh et al., 2010).

Singh et al conducted a prospective study at Jagdalpur, India among twenty-seven sickle cell patients receiving hydroxyurea. The aim was to access the efficacy and effect of hydroxyurea on foetal haemoglobin and some haematological biomakers.The study observed an increase in mean HbF from 12.83% to 19.17%. A study by Silva-Pinto et al reported an increase in foetal haemoglobin from 2.6% to 19.8% among 37 sickle cell patients.

The baseline value for HbF was 19.3±6.0 and increased to 23.9±6.0, an increase of 23.8% in a study by Pradhan et al, 2018. Also findings from Rigano et a, 2017 revealed that, a significant increase in mean fetal hemoglobin level from 9.0 ±8.0 to 17.0 ±10.5 (+8.0%, $p < 0.001$) was observed with hydroxyurea intake. In another study in Nigeria, the median and inter quartile range of hemoglobin F was 4.0% and 2.6-6.6% at baseline and increased to 9.8% and 6.9-13.0%. Increased level of hemoglobin F from 8.0% to 26.4% was observed in a single patient (Tayo et al., 2019).

Another related prospective study was conducted in India. One- hundred and forty sickle cell patients on HU were followed for 24 months. At the end of the study, 118(84.3%) had complete follow-up and their data analysed. These patients were grouped into two: Group I having 27 pediatric SS patients, aged 3-14 years, consisting of 18 males and nine females (mean age of 9.3 ± 4.1 years). Group II consisting of 91 adult SS patients, aged 15-45 years, with 64 males and 27 females(mean age of 27.3±8.7 years). The control group comprised forty-five SS cases which was matched for age. Haematological parameters before and after 24months of HU were compared. The pretest Hb F level in Group 1 which ranged from 19.0 ± 6.9% increased to 22.5± 7.3% whereas 18.2 ± 6.4% increased to 22.5± 6.7% in Group II (Bishwal et al.,2012).

2.6.3 Mean corpuscular volume (MCV)

A mean corpuscular volume is part of the full blood count (FBC) indices and measures the average size of the red blood cell which may be used to monitor certain blood disorders. When the red blood cells are too small or too large, it indicates a sign of a blood disorder such as anaemia or other health condition. In sickle cell disease, MCV is used to monitor patients on hydroxyurea and to evaluate the biological action of HU and patient adherence to treatment. This results in an increase in volume and cellular

hydration of the red blood cell (Neves et al., 2012). The mean corpuscular volume of the red blood cell increases within 4-6 weeks of hydroxyurea treatment which results in clinical improvement which cause a decrease in vaso-occlusion crisis .

A study was conducted in Nigeria among 60 SCD patients with a mean dose of 10.61mg/kg at initiation. Hydroxyurea increased the mean corpuscular volume in patients who have complied with the drug with a mean of 103.6fl compared with non-users with 77.8fl. The mean MCV was significantly higher in consistent users, with a p value of 0.018 (Adewoyin et al., 2017).

A one-year prospective study was conducted on 95 patients on hydroxyurea to monitor the mean corpuscular volume values to determine if macrocytosis can be used as a marker of compliance with therapy. The study reported a significant change of the mean value of MCV from 92.76 to 99.77($p<0.001$). The Anderson and Gill(AG) model used indicated that, an increase of one unit of MCV implies a 5% reduction in the risk of visiting the emergency room ($p<0.001$)(Maria et al., 2013).

Sethy et al conducted a prospective study made up of 128 Hb SS patients (82 males and 46 females) from 2013 to 2016 in India. A minimum dose of 10 mg/kg/day was administered to all patients. After 12 months of HU usage, the low baseline MCV of 76.20 ± 8.20 fl increased to 90.78±6.59 fl. The low baseline mean value was attributed to the prevalent of iron-deficiency in the study population (Sethy et al, 2018).

In a study conducted in Brazil, 37 patients consisting of 26 SS and 11 SB-thal taking hydroxyurea were followed up after 12 months. Findings from the study showed an increase in MCV from 88.7 ± 13.5 to 104.8 ± 15 fl ($P = 0.001$) (Silva-Pinto et al, 2013).

2.6.4 Platelet counts.

Platelets, also known as thrombocytes, are a component of blood that functions with clotting factors to react to bleeding from blood vessel injury. Platelet adhere to the

injury site, aggregate with other platelet, release chemical compounds that stimulate aggregation of other platelets to initiate a clot to stop bleeding. In sickle cell disease, sickle blood cells interact with platelet, neutrophils, and endothelial cells to promote vaso-occlusion, which contributes to ischemia-reperfusion injury (Zhang, Manwani & Frenette, 2016). Hydroxyurea reduces the amount of platelet count in the circulating system. The platelet count is monitored in order not to fall below < 50-80 × 10^9 /L. Studies have identified low platelet count as a ricks factor of acute chest syndrome in SCD patients hospitalized with VOC. This is confirmed with results of CT scan and autopsy showing platelet aggregation as causing ACS (Anea et al, 2016; Chaturvedi S et al, 2016).

In a 7-year cohort study in Italy, hydroxyurea usage resulted in a significant decrease in platelet count (-44.9 × 10^9/L, P<0.001). Also platelet counts < 150 ×10^9/L was reported in 77 patients although 19(24.7%) of them already had counts < 150 ×10^9/L pre-hydroxyurea treatment (Rigano et al., 2017).

A study by Silva-Pinto et al reported a decrease in platelets from 459,000/µl to 373,000/µl (P = 0.0002). This reduction was seen after three months of therapy and lasted over the years.

2.6.5 White Blood cell count (WBC)

These are cells that fight against infections. In sickle cell disease, the spleen which filters the blood of infections is normally damaged by the sickled cell. Sickle cell patients are therefore prone to infections which is a common precipitant of sickle cell crisis resulting in early mortality. Leukocytosis is a risk factor of sickle cell disease predicting stroke, acute chest syndrome and early death (Elmariah et al., 2014). From

studies, neutrophil count strongly correlates with the clinical severity of sickle cell disease (Ahmed et al., 2017).

Hydroxyurea causes a reduction of high white blood cells (WBC) by myelosuppression and reducing ischemic damage in the microvasculature (Zhang et al, 2016; Ansari & Gavins, 2019).

A study conducted in Brazil among 37 SCD patients reported that patients on HU treatment presented with a significant reduction in WBC from 11,800/µl to 9,100/µl ($P < 0.0001$), after three months. They observed a drop in infections rate from 1.03 to 0.5 ($p=0.047$), which was not stated in other studies (Silva-Pinto et al., 2013).

Among twenty-four sickle cell patients who completed a one-year therapy on hydroxyurea, white cell count reduced from 9.62 $\times 10^9$ L to 8.33$\times 10^9$L. The reduction was non-significant (Singh et al., 2010).

2.6.6 Reticulocyte counts

Reticulocytes promote vaso-occlusion through vascular adhesion. Hydroxyurea lowers their absolute numbers and reduces its adhesive receptors. A study was conducted in Tanzania to investigate the effect of hydroxyurea on haemolysis in sickle cell patients. After 3 months of treatment, the mean absolute reticulocyte count decreased from 0.29(0.1) x 10^9/L at baseline to 0.17 (0.1) x 10^9/L (Gangji et al., 2021). Hydroxyurea induces formation of HbF which reduces sickling of HbS, which leads to a reduction in premature haemolysis of red blood cells found in SCD patients. This decreases the need for increased bone marrow erythropoiesis as a compensatory mechanism during haemolysis, thus the reduction in absolute reticulocyte counts. A study undertaken in Portugal reported a significant decrease in reticulocyte counts after HU therapy (Yahouédéhou et al., 2019).

In a case control study conducted in Brazil, treatment periods where between 7 and 72 months. The control group had an absolute reticulocyte count of 174.5 x 10^9/L, while the group on HU therapy had an absolute reticulocyte count of 74.9 x 10^9/L ($p = 0.0015$) (Borba et al, 2003). A study undertaken in Greece reported a decrease in reticulocyte counts at baseline 10% to 6% at the last follow up after 12 months. This was among 131 sickle patients on hydroxyurea therapy (Voskaridou et al., 2010).

2.7 Clinical Parameters of sickle cell patients on hydroxyurea

Hydroxyurea has proven to be effective and beneficial for sickle cell patients. With commencement of the drug, clinical improvement is noticed in the patients. Clinical improvement occurs as a result of reduction in the white blood cell counts, platelet counts, reticulocyte counts, surface adhesion of the sickle cell. Also, the induction of nitric oxide production cause clinical improvement in the four parameters under investigation (Gladwin et al, 2002).

2.7.1 Acute chest syndrome

Acute chest syndrome is the appearance of new infiltrate on chest x-ray, associated with chest pain, respiratory distress, fever and cough (Ballas et al, 2010). When the body is under stress from infection, fever or dehydration, the sickled cells stick together and block the flow of oxygen in the tiny vessels in the lungs. This is the second common cause of frequent hospitalizations and death in sickle cell patients. The factors that predisposes ACS is multifactorial but other identified factors includes thrombosis, infections, fat and pulmonary embolism (Tawfic et al, 2012). Early treatment of acute chest syndrome is crucial to prevent sudden respiratory failure and death. Most studies have reported the incidence of ACS to be higher in children of ages 2-4 years and very severe in adults, in which mortality rate is four times higher than in adults (Vichinsky et al, 2000).

The frequency of acute chest syndrome was reduced by patients on hydroxyurea therapy. A study by Rigano et al, 2017 reported a reduction of acute chest syndrome of 29.3% (p = 0.001). Findings from another study showed the frequency of acute chest syndrome reduced from 0.3 to 0.08 and this difference was significant.(Silva-Pinto et al., 2013).

A study conducted in Greece among SCD 133 patients on HU and 199 on standard treatment reported a reduction in acute chest syndrome from 6.1% to 0.8%, (p =0 .016).(Voskaridou et al., 2010).

A retrospective study was conducted with 140 patients among Caucasian Sickle Cell-Beta-Thalassemia patients in Italy. The patients were divided into three groups based on hydroxyurea use: patients without HU and those who stopped HU before the last visit were fifty (36%); those who took HU at<15mg/kg/day at the last visit were thirty(21%); or those on HU ≥15mg/kg/day at the last visit totaled sixty(43%). The mean number of acute chest episodes in both HU-group decreased from 0.7 to 0.23 and 1.1 to 0.32 respectively. The study concluded that patients with the phenotypes HbSS, HbS/ß^{0}–thal, and HbS/ß$^{+}$-thal response were similar with hydroxyurea use and better with a higher dosage (Maggio et al., 2018).

2.7.2 Vaso-occlusive crisis (VOC)

Vaso-occlusion is a pathophysiological feature of sickle cell disease resulting in frequent painful episodes (crisis). Vaso-occlusion crisis occurs when sickle red blood cell clump up and block small vessels carrying blood to muscles, bones and certain organs, resulting in ischemia, oedema, necrosis, pain and organ damage (De Montalembert,2002). Painful crisis can be defined as severe pain lasting more than two hours and parts of the body it affects includes chest, arm, legs, abdomen, waist, and

back. Infants and young children may experience painful swelling of the fingers and toes. This may be treated with analgesia and most often end in hospitalization. Vaso-occlusion crisis also results in various organ damage which can lead to disability, poor quality of life and early mortality (Manci et al, 2003). The pain can be acute or chronic due to its variability in intensity, location, quality and temporal patterns (Darbari et al, 2013). Extreme temperature, dehydration, infection, changes in menstrual cycle, alcohol, and emotional stress are the most common triggers of vaso-occlusion crisis, which is the most common cause of frequent hospitalization and emergency visit.

Previous studies have reported the reduction of vaso-occlusive crisis by the use of hydroxyurea. In a study, the baseline mean VOC decreased from 3.9±1.5 to 1.4±0.6, showing a reduction of 64.1% which was significant (Pradhan et al., 2018). Another study reported a reduction in the severity and frequency of vaso-occlusive crisis from a baseline 4.12 ± 0.9 to 0.23 ± 0.92 after 1 year with 10mg/kg/day (Sethy et al., 2018). Similarly in India, among 27 sickle cell patients, the mean number of vaso-occlusive crisis decreased from 3.63 to 1.67 at the end of 12 months (Singh et al., 2010).

The first placebo controlled randomized trial that reported on clinical efficacy of hydroxyurea was the Multi-Center Study of Hydroxyurea in Sickle Cell Anaemia (MSH). The study was made up of 299 adults sickle cell patients. The study was stopped early due to the significant reduction of median annual rate of vaso-occlusion crisis by 44% (Charache et al., 1995).

2.7.3 Blood Transfusion.

Blood transfusion is imperative in managing acute and chronic complications in sickle cell patients (Wahls, Quirolo 2009; Lee et al, 2006). Patients with chronic anaemia with a fall below their steady state haemoglobin requires emergency transfusion to

prevent complications which can result in early death. Heamoglobin AA blood type which is mostly administered increases the oxygen-capacity, reduces the proportion of HbS relative to Hb A, increases the haemoglobin level and prevent vaso-oclussion crisis and complications. Blood transfusion can be done by simple or exchange method, with simple transfusion been the most common method used. Contrary to these benefits, sickle cell patients are at risk of complications of multiple transfusions namely, alloimmunization, transfusion reactions, iron overload and infections. Alloimmunization which is red blood cell antibody formation resulting from an immune response against foreign RBC antigen is a major complication of chronic transfusion. Previous studies have reported the burden of red blood cell alloantibody in Sub-Saharan Africa with a pooled proportion of alloimmunization in SCD being 7.4 (Antwi Boateng, Ngoma, Bates, Schonewille, 2019).

Hydroxyurea therapy is proven in most studies to reduce the rate of transfusion among sickle cell disease patients. In a study conducted in India, seventy-eight percent of patients (60 out of 77) had no crisis after taking hydroxyurea. Before HU therapy forty-one patients usually received transfusion while 15 patients required 3 to 5 transfusions per year to maintain hemoglobin levels of greater than 7.0 g/dl. One patient was transfusion- dependent in group 1. After hydroxyurea therapy, 91% of patients did not require transfusion. The mean clinical scores were 12.6±1.8, 14±1.7 and 12.9±1.2 before and 7.2±0.9, 7.5±0.9 and 7.3±0.7 after hydroxyurea therapy in group I (29 adult SS cases) II (25 pediatric SS) and III(23 adult Sb-thal) respectively (P <0.001). A few weeks to the commencement of therapy clinical improvement was observed as bony pain and fatigue gradually disappeared (Italia et al., 2009).

A prospective trial was carried out in Greece by Voskaridou et al,(2010). Hydroxyurea was given to 133 patients whereas 199 patients were given the standard treatment. A

significant reduction in blood transfusion was from 1.5 ± 5.9/year before hydroxyurea reducing to almost zero after HU treatment which was significant.

2.7.4 HOSPITALIZATION

Studies have shown that hydroxyurea reduces hospitalization and mortality in sickle cell disease (Steinberg et al., 2010). In adult patients, the rate of hospital admissions generally decreased after treatment with hydroxyurea. In a study among Sicilians population, the annual number of days of hospital admissions decreased from 22.4 to 1.2 (SD, 2.3) (P< 0.001) (Rigano et al, 2001). A related study reported a reduction of 3.1 to 2.1 hospitalizations per year in 24 months (P= 0.04) (Fergueon RP et al,2002). A study was conducted to compare hydroxyurea with cognitive behavioral therapy. Findings from this study showed that hospitalization rates were similar among patients receiving hydroxyurea and those receiving therapy (Cummins et al, 2003).

A retrospective longitudinal study was done among 312 North Carolina Medicaid enrollees. This was to access patient's adherence to hydroxyurea. Authors reported a reduced risk of SCD-related hospitalizations in 12 months following initiation of HU (Candrilli et al, 2011).

In a study byNzouakou et al among 64 patients, the frequency of mean hospitalization decreased by 13.4 days after enrollment on hydroxyurea compared with before initiation (p<0.0001) (Nzouakou et al, 2011). Findings from Voskaridou et al study reported a dropped from 2.11± 2.96/year to 0.041± 0.018 in hospital admissions at the end of the study (p < .001). Also, authors in a prospective study consisting of 69 adults sickle cell patients reported a decrease of 83% in the rate of hospitalization at the end of 24 months. The decrease was from 1.52±1.2 to 0.25±0.5 (p=0.0001) (Pradhan B et al, 2018).

Summary of Literature Review

From the review of literature, several studies have reported an increase in Hb, HbF, MCV while platelet, white blood cell, and reticulocyte decrease with the use of hydroxyurea. These changes resulted in a reduction in frequency of the clinical parameters. Most of these studies were conducted in developed countries with very few studies in Ghana. Since hydroxyurea was accepted for use in Ghana, no study has been conducted on the changes of these parameters among adult sickle cell patients highlighting its effective use.

CHAPTER 3

METHODOLOGY

3.1 Study Design

The study was a retrospective cohort study with a pre and post evaluation. Designed data extraction tool, kobocollect **(Appendix 1)** was used to collect demographic characteristics, dosage, clinical and haematological parameters of sickle cell patients aged 15 years and older. Clinical records about treatment and outcome were reviewed on hydroxyurea from January 2018 to November 2021 at the Adult Sickle cell clinic, Korle-Bu Teaching Hospital. Six months baseline, 6months post-hydroxyurea and 12-months post-hydroxyurea records of haematological and clinical parameters were extracted from patient's folders

3.2 Study Location

The Ghana Institute of Clinical Genetics (Adult Sickle Cell Clinic) was established in 1974, located within Korle-Bu Teaching Hospital and serves as a major referral centre with an average daily attendance of almost 50 patients was the study site. The clinic is manned by both haematologist specialist and medical officiers. It is a day clinic with a bed capacity of 12 which renders treatment services to patients of ages 13years and above. The clinic provides outpatient services and has an emergency care unit where patients are detained and referred if not fully stabilised. It has over 25,000 registered patients. The clinic has a laboratory and a pharmacy unit. The laboratory investigations carried out includes haematology, chemistry, and urinalysis tests. These tests are performed by qualified biomedical scientist. The haematological tests are conducted using Horiba Micros ES60 analyzer with sixteen parameters. Patients are referred to a

specialist for further management at the main Teaching Hospital. Hydroxyurea was introduced to the clinic in 2018 after being approved by the Ghana Food and Drug Authority.

3.3 Study Variables

The variables are divided into dependent and independent variables.

3.3.1 Dependent Variables

The dependent variables are the haematological and clinical parameters. The haematological parameters are level in the blood (haemoglobin (Hb)), foetal haemoglobin (HbF), white blood cell count, mean corpuscular volume (MCV), platelet and reticulocyte count. The clinical parameters are vaso-occlusive crisis, acute chest syndrome, blood transfusion and hospitalization.

3.3.2 Independent Variables

These are sex, age, weight, temperature, genotype and dosage of 10, 15 and 20mg/kg/day.

Table 3.1 describe the dependent and independent variables, their operational definition, type and scale of measurement.

Table 3.1 Study variables, operational definition, type and scale of measurement

NO	Variable	Operational Definition	Type of Variable	Scale of Measurement
A	DEPENDENT VARIABLES			
	Haematological Parameters			
1.	HbF	Measuring the level of foetal haemoglobin in the blood in (%)	Continuous	Ratio
2.	Hb	Measurement of haemoglobin level in the blood in (g/dl)	Continuous	Ratio

3.	WBC	Measuring the total number of white blood cell count in the blood (L).	Continuous	Ratio
4.	MCV	Mean corpuscular volume that measures the average size and volume of red blood cell (fL).	Continuous	Ratio
5.	Platelet count	Measurement of the average number of platelets in the blood(mL)	Continuous	Ratio
6.	Reticulocyte count	Measurement of the number of reticulocyte in the blood (%).	Continuous	Ratio
		Clinical Parameters (Number of times occurred).		
1.	Vaso-occlussive crisis	Frequent episodes of pain experienced by sickle cell patients	Discrete	Interval
2.	Blood Transfusion	Process of transferring blood products into patient's circulation intravenously	Discrete	Interval
3.	Acute chest syndrome	A condition characterized by chest pain, cough, fever, hypoxia and lung infiltrates.	Discrete	Interval
4.	Hospitalization	Been on admission	Discrete	Interval
B	INDEPENDENT VARIABLE			
1.	Age	How old is the patient as at the time of data extraction (in years)	Continuous	Ratio
2.	Sex	What is the biological classification of a patient (male or female)	Binary	Nominal
3.	Genotype	Individual collection of genes, that is AA, SS, SC, Sb-thal	Categorical	Nominal
4.	Dosage	The prescribed administration of a specific amount of doses.	Continuous	Ratio
5.	Weight	A body's relative mass indicating the heaviness of a person.	Continuous	Ratio

6.	Temperature	The measure of hotness or coldness of the body expressed in terms of Celsius.	Continuous	Interval

3.4 Study Population

The study population comprised all sickle cell patients attending the adult sickle cell clinic, Korle-Bu Teaching Hospital enrolled on hydroxyurea for at least six months, aged 15 years and older

3.5 Inclusion Criteria

Patients living with SCD aged 15 years and older enrolled on hydroxyurea with at least six months of haematological and clinical records before starting hydroxyurea therapy and who had completed 12months of the therapy were included in the study.

3.6 Exclusion Criteria

Excluded from the study were patients who had no clinical records for at least six month in the clinic. Also excluded were patients from the peadiatric unit migrated to the adult clinic to continue the therapy. Most of these patients were enrolled on the therapy before this study duration.

3.6.1 Sample Size Determination

The sickle cell clinic has over 27,000 registered patients, and from records, about 461 patients have been enrolled on the hydroxyurea from January 2018-November 2021, but base on the study duration, that is from January 2018 to 2020, 270 patients had been enrolled hence this was obtained as the sample size. Patients who were enrolled

on hydroxyurea from January 2021 were excluded since their 12 month would have ended in 2022 by which time the MSc program would have ended. Also to obtain a good power for the study, patients within the study duration who had to end their 12 month within January to November 2021 were included. Since data collection was to end in November 2021. This explains the study duration and the sample size used.

3.6.2 Sampling Procedure

Total enumerative sampling was employed in the selection of the folders from the records departments.

3.7 Data Collection Technique

Print out of the names and folders numbers of patients were obtained from the records department. Folders were therefore removed from the pack of folders by research assistant. On a daily basis about fifteen folders were removed from the cabinet of folders. Data collection lasted for four weeks.

3.8 Data Quality Control

3.8.1 Training of Research Assistant and pretesting of data collection tools

One research assistant was trained to assist the investigator in the data extraction. The training focused on the purpose of the study and how to search for filed folders for easy identification for data to be extracted. The data extraction tool was pretested among 20 sickle cell patients on hydroxyurea at the Ghana Institute of Clinical Genetics (adult sickle cell clinic), KBTH. This was to identify any discrepancies in the tool. After pre-testing, identified gaps were modified and corrected before using the tool for actual data collection.

3.8.2 Data Handling

Data was cross-checked, inspected for errors with the patient's folder. Electronic Data files was password protected and access was limited to principal investigator and the supervisor only.

3.9 Data Processing

Data was extracted using tool designed with kobocollect, cleaned, cross-checked for completeness and imported into Stata version 16 for analysis.

3.10 Data Analysis

Data was analyzed using Stata version 16.0 statistical software. Continuous variables such as Hb, HbF, Wbc, MCV, platelet and reticulocyte counts were summarized as means and standard deviation (SD) and categorical data as percentages (%). Data was presented using frequency tables and charts as appropriate. Chi square test was used to test for association between demographics and clinical characteristics of study participants, and the association between clinical manifestation and dosing of hydroxyurea intake among study participants. Paired t-test was used to compare differences in means of the quantitative variables to determine changes in haematological parameters before and after treatment with hydroxyurea. Differences in proportions was estimated using z test of proportion . A significance level of 5% was adopted.

3.11 Ethical Consideration

The study was approved by the Korle-Bu Teaching Hospital Institutional Review Board (KBTH-IRB) with ID number STC/IRB/000102/2021 **(Appendix 2).** Permission for data collection was also sought from the Ghana Institute of Clinical Genetics (Sickle Cell Clinic) **(Appendix 3).**

3.12 Risk and Benefit of study

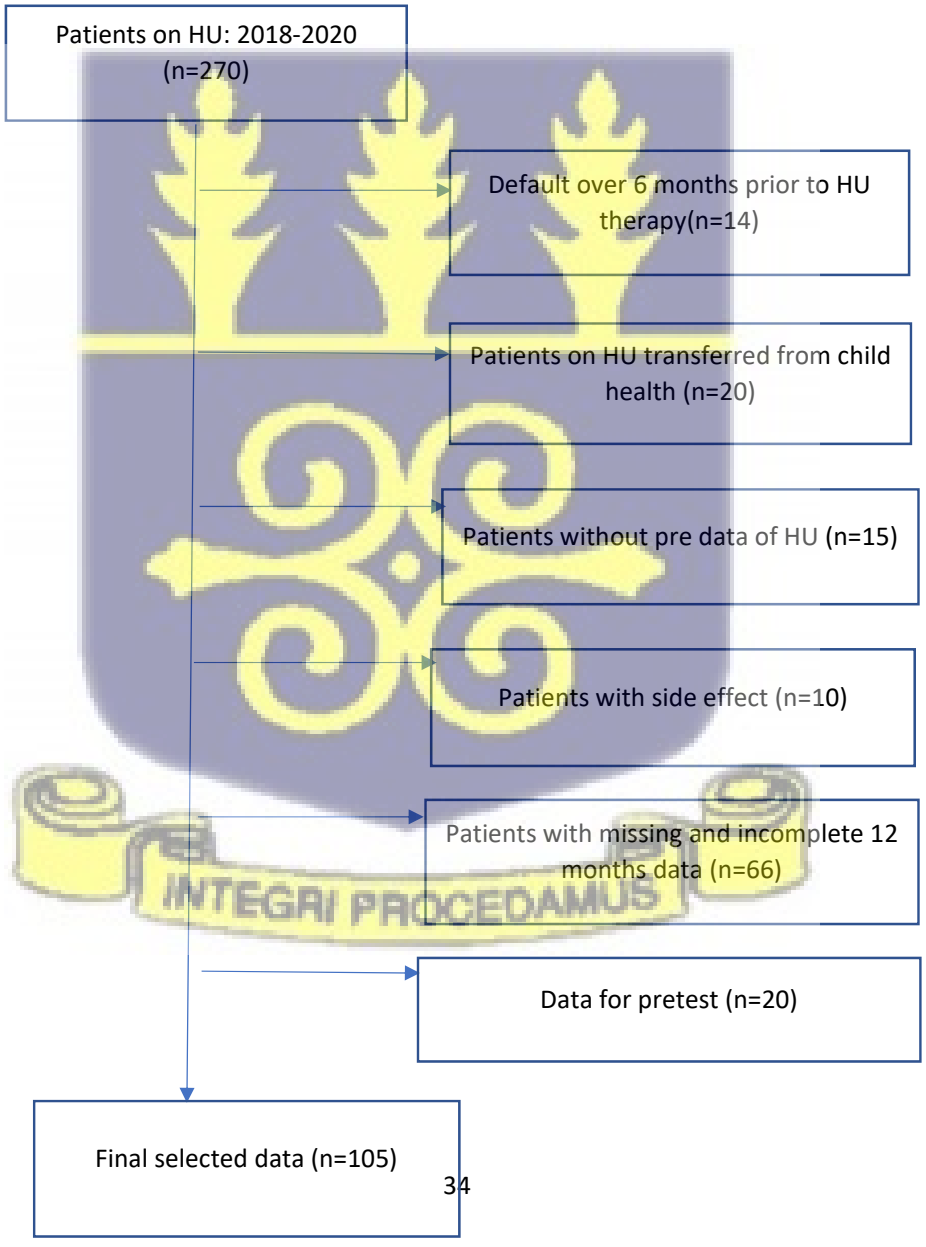
There was no risk, patient records were used for analysis. The names of patients were not extracted to ensure anonymity. The results of the study will be used to improve care in the management of sickle cell patients which is a benefit.

CHAPTER 4

RESULTS

Over the study period, there were 270 patients who had been put on Hydroxyurea. A total of 270 folders were assessed. However, 105 patients met the inclusion criteria. The study flowchart is presented in (Fig.2)

Fig.2



4.1 Sociodemographic characteristics of sickle cell patients at adult sickle cell clinic, KBTH

In table 4.1, out of the 105 patients, females were 56 (53.8%). The ages of the sickle cell patients were between 15 to 49 years with both median and inter quartile range of age being 11 years. Majority of the patients 66 (62.9%) were within the age group 20 to 39 years.

Regarding genotype of patients, 94 (89.5%) were SS. Majority of the patients 76 (72.4%) were normothermic with a median temperature of 36.3 ^{o}C. All the 105 folders had the weights recorded in them with a mean of 55.1 ± 12.5 kg. Details are showed in table 4.1.

Table 4.1: Demographic characteristics of sickle cell patients at Ghana Institute of Clinical Genetics.

Variables	Frequency	Percentage (%)
Age		
<20	32	30.4
20-39	66	62.9
40-59	7	6.7
Total	105	100
Sex		
Female	56	53.84
Male	49	47.15
		100
Total	105	
Genotype		
SB-THAL	2	1.9
SC	9	8.6
SS	94	89.5
Total	105	100

4.2 Changes in Mean Hb, WBC, MCV, PLT and RET after treatment with HU at different times

Table 4.2 depicts the changes in means of the heamatological parameters observed at baseline, 6 months and 12 months after hydroxyurea treatment.

The Hb increased by 0.71g/dl from the baseline to the sixth months which was statistically significant at ($p<0.001$). The white blood cell count decreased by -1.79 from the baseline to 6months post treatment. This drop was significant at $p<0.001$. However there was an increase in the WBC from the sixth months to 12months post treatment by 0.58 $10^3/mm^3$. This difference was however not statistically significant ($p=0.183$). Platelet count decrease from the baseline to sixth month by -32.91 $10^3/mm^3$. This change was significant ($p=0.031$). However, from sixth to twelfth month it showed an increase of 16.54 $10^3/mm^3$ which was not significant ($p=0.338$).

MCV increased from the baseline to the sixth month by 9.38 μm^3. This increment was statistically significant ($p<0.001$). From the sixth to twelfth month, there was a slight decrease of 0.40µm in MCV which was not statistically significant ($p=0.084$). Reticulocyte count decrease from baseline to sixth month by -1.71% which was statistically significant ($p<0.001$). But a drop of -0.34% was observed from 6months to 12 months which was not statitistically significant ($p= 0.112$)

Table 4.2: Changes in mean frequencies of haematological parameters of sickle cell patients at pretest, six and 12 months.

Variables	Pre-treatment (mean±SD)	6 M (mean±SD)	% Changes 0-6M	p-value	12 M (mean±SD)	% Changes 6-12M	p-value
Hb (g/dL)	8.40±1.52	9.11±1.55	0.71	**<0.001***	9.31±1.47	0.20	**<0.021***
WBC (10^3/mm^3)	11.48±3.92	9.68±4.13	-1.79	**<0.001***	10.26±5.50	0.58	0.183
MCV (μm^3)	85.38±9.96	94.77±12.41	9.38	**<0.001***	94.36±11.87	-0.40	0.612
PLT (10^3/mm^3)	426.28±158.82	393.36±162.74	-32.91	**0.031***	409.90±196.35	16.54	0.338
RET	8.76±5.43	7.05±2.77	-1.71	**<0.001***	6.70±2.69	-0.34	0.112

Note: *Significance at $p<0.05$

HbF at baseline was 7.4% and increased to 8.96% in one person. An increase of 1.56%.

4.3 Frequencies of clinical parameters of SCD patients before and after treatment with hydroxyurea

Table 4.3 outlines the frequency of the clinical parameters before and after introduction of hydroxyurea among the sickle cell patients presented at the adult clinic, KBTH.

Out of the 105 sickle cell patients' folders that were assessed, 68 (65.4%) experienced vaso occlusive crisis at baseline but this reduced to 20 (19.6%) after treatment with hydroxyurea. With regards to the acute chest syndrome, it reduced from 4 (3.9%) at baseline to 1 (1.0%) after post treatment. This change was not significant (p=0.174). The frequency of blood transfusion reduced from 7 (6.7%) at pre-intervention to 3 (2.9%) at post intervention. This was not significant (p=0.195). Hospitalization also reduced from 38 (36.5%) to 10 (9.6%). This change was statistically significant at P<0.001.

Table 4.3: Frequencies of clinical manifestations of sickle cell patients before and after treatment with hydroxyurea

Variables	Frequency		X^2	p-value
	Pre-treatment (%)	Post-treatment (%)		
Vaso occlusive crises			**44.10**	**<0.001***
No	36 (34.6)	82 (80.4)		
Yes	68 (65.4)	20 (19.6)		
Acute chest syndrome			1.84	0.174
No	100 (96.1)	103 (99.0)		
Yes	4 (3.9)	1 (1.0)		
Blood transfusion			1.68	0.195
No	97 (93.3)	101 (97.1)		
Yes	7 (6.7)	3 (2.9)		
Hospitalized			**21.23**	**<0.001***
No	66 (63.5)	94 (90.4)		

Yes	38 (36.5)	10 (9.6)

4.4 Association between clinical parameters of sickle cell patients with hydroxyurea dosage

This section describes the association between clinical parameters with the dosages commenced by the patients.

Among patients with reduced number of vaso-occlusive crisis, majority 16 (80%) were on 15mg compared to majority of patients 58 (72.55%) who did not experience vaso-occlusive crisis also on 15mg of hydroxyurea. There was no association between dosage (10mg, 15mg, and 20mg) and the occurrence of vaso occlusive crisis. One patient experienced acute chest syndrome at post-hydroxyurea on 15mg. There was no association between acute chest syndrome with hydroxyurea treatment dosages (X^2 = 0.36, p-value = 0.834).

With patients who were transfused after hydroxyurea treatment, majority 2 (66.7%) commenced on 15mg HU. There was no association between frequency of blood transfusion with hydroxyurea dosage (X^2 = 1.28, p = 0.527). At post-hydroxyurea in hospital admissions, majority7(70%) commenced on 15mg compared to the 10mg and 20mg. There was no association between hospital admission and the dosages.

Table 4.4: Association between post-treatment clinical parameters of sickle cell patients with dosage

Variables	Hydroxyurea treatment dosage			χ^2	p-value
	10mg	15mg	20mg		
Vaso-occlusive crisis				1.43	0.489
No	12 (15.0)	58 (72.5)	10 (12.5)		
Yes	1 (5.0)	16 (80.0)	3 (15.0)		
Acute chest syndrome				0.36	0.834
No	14 (13.9)	74 (73.2)	13 (12.9)		
Yes	0 (0.0)	1 (100.0)	0 (0.0)		
Blood transfusion				1.28	0.527
No	13 (13.1)	73 (73.7)	13 (13.1)		
Yes	1 (33.3)	2 (66.7)	0 (0.0)		
Hospital admission				0.59	0.745
No	13 (14.1)	68 (73.9)	11 (12.0)		
Yes	1 (10.0)	7 (70.0)	2 (20.0)		

Table 4.5 Frequencies and percentages of weight and temperature

Majority of the patients 76 (72.4%) were normothermic with a median temperature of 36.3 °C. All the 105 folders had the weights recorded in them with a mean of 55.1 ± 12.5 kg. The baseline weight and temperature recorded were values when the first dose was prescribed for them. Details are showed in table 4.5

Table 4.5 Frequencies and percentages of weight and temperature.

Variables (%)	Frequency	Percentages
Weight recorded in folder		
No	0	0
Yes	105	100.0
Weight (mean±SD)	55.1 ± 12.5	
Temperature recorded in folder		
No	0	0
Yes	105	100.0
Classification of temperature		
Hypothermia	29	27.6
Normothermia	76	72.4
Temperature (mean±SD)	36.3 ± 0.5	

CHAPTER 5

DISCUSSIONS

This study is one of the first studies to evaluate the effect of HU since it was approved for use among sickle cell patients in Ghana. The study assessed the effect of HU on clinical and haematological outcomes among 105 patients in the largest adult Sickle Cell Clinic in Ghana. The sample size employed is comparatively higher than most other studies on sickle cell disease and hydroxyurea with the same outcomes and sample sizes ranging from 24 to 128 (Italia et al, 2009; Singh et al, 2010; Neves et al, 2012; Silva-Pinto et al, 2013; Adewoyin et al, 2017; Pradhan et al, 2018; Sethy et al, 2018; Salles et al, 2018; Tayo et al, 2019).

This study demonstrates improvements in both heamatological and clinical parameters following hydroxyurea use. Hydroxyurea increased Hb level, MCV reduced WBC, Reticulocyte and Platelet count at 6 months and 12 months. This finding is similar to previous studies(Silva-Pinto et al, 2013; Pradhan et al, 2018) in which hydroxyurea significantly increased Hb level, MCV, reduced WBC, PLT count at 12 months. Most of these studies employed a pre and post design which is similar to this current study. Unlike Maggio et al, 2018 who used a control group while Adewoyin et al, 2017 did a cross-sectional survey.

At 6 months of hydroxyurea therapy, the patients presented with a significant increase in Hb, MCV, reduction in Wbc and platelet counts. These findings were similar to a study by Singh et al, (2010), Rigano et al, (2017) and Maggio et al, (2018). However, reticulocyte counts were not reported by these authors. Reticulocyte counts reduced significantly at 6 month but not significant at 12 months in this study. In contrary to

this result, Voskaridou et al, 2010 reported a significant reduction in reticulocyte counts at 6 month and 12 months.

There was no significant difference in the white cell count, platelet count and MCV at 6 and 12 months similar to a study by Singh et al, (2010), this is most likely due to the fact that most patients were maintained on the same dose of hydroxyurea.

At the end of 12 months, this study reported a significant increase in heamoglobin level which was similar to most studies (Silva-Pinto et al., 2013; Pradhan et al., 2018; Sethy et al., 2018). But Singh et al., (2010) reported a non-significant increase in Hb after a year with a sample size of 24 patients. In this study, MCV significantly increased at 6 months and decrease non-significantly at 12 months. However, Italia et al, (2009), Silva-Pinto et al, (2013), Sethy et al, (2018) and Maggio et al, (2018) reported a significant increase at 12 months. This could be probably be attributed to inconsistency in adhering to the medication. Some patients were cautioned by physicians on adherence based on their laboratory findings. It is possible that at 6 months when patients notice an improvement in their condition, they become reluctant in their follow-up visits, and miss their appointments. Compared to other studies, Voskaridou et al, (2018) study showed a highly significant increase in MCV and similar results was reported by Singh et al, 2010.

In this current study, only 68 out of the 105 patients were able to afford the cost and perform a pre and post laboratory test for Hb F. A report from a study in India (Singh et al., 2010) showed a good response to hydroxyurea even in the absence of a significant change in Hb F.

The frequency of vaso-occlusive and hospitalization was reduced significantly in this study. This was similar in a study by Singh et al, 2010 and Sethy et al, 2018. The

frequency of blood transfusion and acute chest syndrome was reduced by HU, but this reduction in both cases were not significant. This might be as a result of few number of patients who experienced acute chest syndrome and those who received transfusion.

Patients commenced HU treatment on 10mg, 15mg and 20mg/kg/day. The medication which was in 500mg was calculated based on the patient's weight and administered by the physician's discretion of the patient medical history. Majority of the patients were placed on 15 mg/kg/day. Majority of the patients 58 (72.5%) who did not experience vaso occlusive crisis commenced on 15mg of hydroxyurea. There was no association between the 15mg dosage and the occurrence of vaso occlusive crisis. This implies that the occurrence of vaso occlusive crisis in the sickle cell patients was independent of the hydroxyurea dosage. Acute chest syndrome was experienced by one patient on 15mg of hydroxyurea. There was no association between acute chest syndrome, blood transfusion and hospitalization with patients on 15mg even though it reduced its frequencies. None of the previous studies found the association between the clinical parameters and dosage. However, dosage administered to the patients at initiation and throughout the study was stated. What could have accounted for the findings in this study can be attributed to different weight of the patients. Some studies (Pradhan et al., 2018; Singh et al., 2010; Rigano et al., 2017) have demonstrated that a fixed low dose of hydroxyurea, was effective in reducing the rate of vaso-occlusive crisis, blood transfusion, acute chest syndrome and hospitalization.

The findings from this study implies that hydroxyurea is effective in improving haematological and clinical condition of sickle cell patients. To further improve the condition of sickle cell patients, researchers are obliged to conduct more studies on therapies to alleviate their pain and complications. For patients to be able to access quality medical care, policy makers and the right authorities needs to make the cost of hydroxyurea more affordable and accessible for patients use.

This study was conducted to have evidence to support the effective use of hydroxyurea among adult sickle cell patients in Ghana.

Study Limitation

Selection bias of patients' records may pose a limitation to this study since records were selected from only one facility which is a referral Centre.

Another limitation is the design of the study, due to the lack of a control group it is difficult to infer causality as to whether the change could be attributed to the treatment alone or any other factors.

Missed appointments, default in clinic attendance and lack of regular follow-up are some factors that affected the sample size of this study.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The study concluded that; Hydroxyurea resulted in a beneficial reduction in the white cell, platelet and reticulocyte counts and increases in haemoglobin level, mean cell volume.

Clinically, hydroxyurea reduced the frequency of vaso-occlusive crisis, hospital admissions, acute chest syndrome and blood transfusion.

There was no association observed between the dosages of HU and the clinical parameters.

6.1 Recommendations

Based on the findings of this study, hydroxyurea is effective and improves the condition of sickle cell patients. Therefore, sickle cell patients should be encouraged to adopt the use of the medication.

Clinicians should educate and recommend hydroxyurea to patients.

More research should be conducted on the dosages of HU and clinical outcomes among adult sickle cell patients in Ghana.

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APPENDIX 1

PATIENT DATA EXTRACTION TOOL

CHANGES IN HAEMATOLOGICAL AND CLINICAL PARAMETERS OF ADULT SICKLE CELL PATIENTS ON HYDROXYUREA: A PRE- AND POST-TEST NON-EXPERIMENTAL RETROSPECTIVE STUDY AT THE SICKLE CELL CLINIC, KORLE-BU TEACHING HOSPITAL

Patient's ID: _____ [*PatID*] Date: __ __ / __ __ / __ __ __ __ [*Date*]

Facility: _____ [*facility*]

SECTION A: COLLECT DATA FROM PATIENT FOLDER ONLY

Q1. **Patient Folder Number**		*Q1foldno*
Q2. **Is the patient's age recorded in the folder?** Yes (1) No (2)		*Q2age*
Q3a. **If yes, what is the patient's age in years according to the folder?** ***Write NA if Age is not recorded in Folder/Card***		Q3aage
Q3b. **Record Date of Birth in Folder/Card** ***Write NA if Date of Birth is not recorded in Folder/Card***		*Q3bdob*
Q3c. **Sex** Male (1) Female (2)		*Q3csex*
Q4. **Patient Genotype**		*Q4genotype*
Q5. **Is the patient's weight recorded in the folder?** Yes (1) No (2)		*Q5weight*
Q6. **If yes, what is the patient's weight in kg** (1 decimal place) **according to the folder?** ***Write NA if patient's weight is not recorded in Folder***		*Q6weight*

Q7. **Is the patient's temperature recorded in the folder?** Yes (1) No (2)		*Q7temp*
Q8. **If yes, what is the patient's temperature recorded in folder?** ***Write NA if patient's temperature is not recorded in Folder***	◦C	*Q8temp*

SECTION B: HAEMATOLOGICAL PARAMETERS

Q9. **Baseline**

	M1	M2	M3	M4	M5	M6	
Date Taken							*Q9datetaken 1-6*
a) Hb							*Q9ahb m1-m6*
b) WBC							*Q9bwbc m1-m6*
c) MCV							*Q9cmcv m1-m6*
d) Rectics count							*Q9dreccnt m1-m6*
e) Platelet count							*Q9eplatecnt m1-m6*
f) HbF							*Q9fhbf m1-m6*

POST	M1	M2	M3	M4	M5	M6	
Date Taken							*Q10datetaken 1-6,12*
a) Hb							*Q10ahb m1-m6,12*
b) WBC							*Q10bwbc m1-m6,12*
c) MCV							*Q10cmcv m1-m6,12*
d) Rectics count							*Q10dreccnt m1-m6,12*
e) Platelet count							*Q10eplatecnt m1-m6,12*
f) HbF							*Q10fhbf m1-m6,12*
Q11. **Dosage**							*Q11dosage*

SECTION C: CLINICAL PARAMETERS

Q12. **Pre hydroxyurea**

	M1	M2	M3	M4	M5	M6	
Date Taken							*Q12datetaken m1-m6*
a) VOC							*Q12avoc m1-m6*
b) ACS							*Q12bacs m1-m6*
c) Blood Transfusion							*Q12cbloodt m1-m6*
d) Hospital Admission							*Q12dhospadm m1-m6*

Q13. **Post hydroxyurea**

	M1	M2	M3	M4	M5	M6	M12	

Date Taken								*Q13datetaken m1-m6,12*
a) VOC								*Q13avoc m1-m6,12*
b) ACS								*Q13bacs m1-m6,12*
c) Blood Transfusion								*Q13cbloodt m1-m6,12*
d) Hospital Admission								*Q13dhospadm m1-m6,12*

Q14. **Any of the following adverse events recorded in patient's folder?**

a) Headache	☐	*Q14aheadache*
b) Dizziness	☐	*Q14bdizziness*
c) Skin rash	☐	*Q14cskinrash*
d) Fever	☐	*Q14dfever*
e) Leg ulcer	☐	*Q14elegulcer*
f) Vomiting	☐	*Q14fvomiting*
g) Drowsiness	☐	*Q14gdrowsiness*
h) Leucopenia	☐	*Q14hleucopenia*
i) Nausea	☐	*Q14inausea*
j) Constipation	☐	*Q14jconstipation*
k) Diarrhoea	☐	*Q14kdiarhoea*
l) Nail discolouration	☐	*Q14lnaildiscolour*
m) Hepatic disorder	☐	*Q14mhepaticdisorder*
n) Seizure	☐	*Q14nseizure*
o) Gastrointestinal disorder	☐	*Q14ogastrointesorder*
p) Others (specify)	☐	*Q14pothers*

Q15. **Any Extra Information** *Q15extrainfo*

1.
2.
3.
4.

APPENDIX 2

In case of reply the number
And the date of this
Letter should be quoted

My Ref. No. KBTH/MD/G3/21

Your Ref. No......

KORLE BU TEACHING HOSPITAL
P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

13th October, 2021

AKOSUA ASANTE-OFFEI
SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA, LEGON

INSTITUTIONAL APPROVAL: KORLE BU TEACHING HOSPITAL-SCIENTIFIC AND TECHNICAL COMMITTEE/INSTITUTIONAL REVIEW BOARD (KBTH-STC/IRB/000102/2021

Following approval of your study entitled "Changes in Haematological and Clinical Parameters in Adult Sickle Cell Patients on Hydroxyurea: A Before and After Non-Experimental Retrospective Study at the Sickle Cell Clinic, Korle-Bu Teaching Hospital" by the Korle Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board.

I am pleased to inform you that institutional approval has been granted for the conduct of your study in Korle Bu Teaching Hospital.

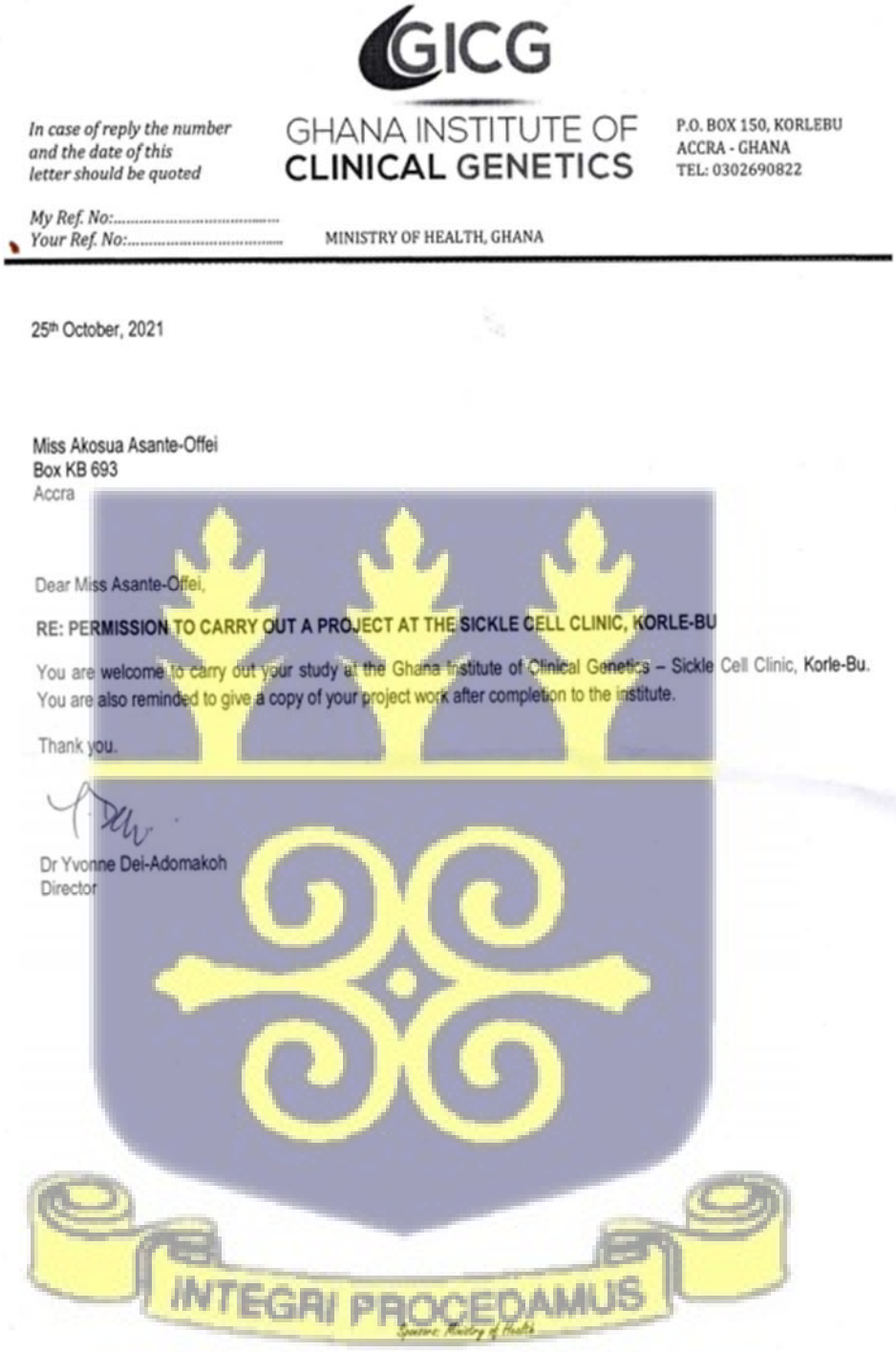
Please contact the Head of Department to discuss the commencement date of the study.

Please note that, this institutional approval is rendered invalid if the terms of the Institutional Reviewed Board/Scientific and Technical Committee approval are violated.

Sincere regards,

Dr. Ali Samba
Director of Medical Affairs
For: Chief Executive

APPENDIX 3



GICG

GHANA INSTITUTE OF CLINICAL GENETICS

In case of reply the number and the date of this letter should be quoted

My Ref. No:.....
Your Ref. No:.....

P.O. BOX 150, KORLEBU
ACCRA - GHANA
TEL: 0302690822

MINISTRY OF HEALTH, GHANA

25th October, 2021

Miss Akosua Asante-Offei
Box KB 693
Accra

Dear Miss Asante-Offei,

RE: PERMISSION TO CARRY OUT A PROJECT AT THE SICKLE CELL CLINIC, KORLE-BU

You are welcome to carry out your study at the Ghana Institute of Clinical Genetics – Sickle Cell Clinic, Korle-Bu.
You are also reminded to give a copy of your project work after completion to the institute.

Thank you.

Dr Yvonne Dei-Adomakoh
Director

INTEGRI PROCEDAMUS