STORAGE RELATED HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN SICKLE CELL TRAIT DONOR BLOOD AT KISUMU REGIONAL BLOOD TRANSFUSION CENTRE, KENYA

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Storage Related Hematological and Biochemical Changes in Sickle Cell Trait Donor Blood at Kisumu Regional Blood Transfusion Centre, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This work is dedicated to my beloved family. Thank you for the support shown during the designing and drafting of this thesis.

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ABBREVIATIONS AND ACRONYMS

2,3 BPG	2,3-Biphosphoglycerate
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
Bas	Basophils
BRMs	Biological Response Modifiers
CPDA	Citrate Phosphate Dextrose Adenine
CV	Cardiovascular Disease
Enos	Endothelial Nitric Oxide Synthase
Eos	Eosinophils
Hb	Hemoglobin
HCT	Hematocrit
INOBA	Insufficient Nitric Oxide Bioavailability
KNBTS	Kenya National Blood Transfusion Services
LBR	Lymphocyte-to-Basophil Ratio
LER	Lymphocyte-to-Eosinophil Ratio
LNR	Lymphocyte-to-Neutrophil Ratio

Lymph	Lymphocytes
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MOH	Ministry of Health
Mon	Monocytes
MPS	Microparticles
NACOSTI	National Commission for Science, Technology and Innovation
Neut	Neutrophils
NTBI	Non-Transferrin-Bound Iron
PCV	Packed Cell Volume
PE	Phosphatidylethanolamine
Plt	Platelets
PS	Phosphatidylserine
RBCs	Red Blood Cells
RBTCs	Regional Blood Transfusion Centers
RDT	Rapid Diagnostic Test

ROS	Reactive Oxygen Species
SCD	Sickle Cell Disease
SCT	Sickle Cell Trait
SPSS	Statistical Package for the Social Sciences
TTIs	Transfusion-Transmissible Infections
WBCs	White Blood Cells
WHO	World Health Organization

ABSTRACT

Sickle cell trait (HbAS) donor blood is widely used for transfusion in malaria-endemic regions, yet evidence comparing its storage-related deterioration with normal hemoglobin (HbAA) blood remains limited. This study evaluated hematological and biochemical changes occurring during storage of HbAS and HbAA donor blood at the Kisumu Regional Blood Transfusion Centre, Kenya. A prospective laboratory-based experimental design was conducted using 60 donor blood units comprising 30 HbAS and 30 HbAA units selected from 336 voluntary blood donors screened for sickle cell trait. Hematological and biochemical parameters were measured at baseline and weekly for 28 days using standardized laboratory methods, while data were analyzed using one-way ANOVA, R statistical software, and SPSS version 27 at a significance level of $p<0.05$. Both donor groups demonstrated progressive storage lesions, although changes were consistently more pronounced in HbAS blood. Hemoglobin and hematocrit increased, whereas platelet and total white blood cell counts declined markedly. Percentage hemolysis reached 0.78% in HbAS compared with 0.54% in HbAA by day 28. Lactate dehydrogenase increased steadily from 210.87 to 635.80 U/L in HbAS and from 196.09 to 530.69 U/L in HbAA, indicating greater cellular degradation in HbAS blood. Plasma potassium increased substantially from 4.98 to 19.28 mmol/L in HbAS versus 4.53 to 14.92 mmol/L in HbAA, while sodium declined progressively in both groups. Plasma bilirubin also increased throughout storage, reaching 19.98 ng/dL in HbAS and 13.48 ng/dL in HbAA, reflecting enhanced erythrocyte breakdown. The findings demonstrate that HbAS donor blood undergoes greater storage-related hematological and biochemical deterioration than HbAA blood, potentially compromising transfusion quality and safety. Routine screening for sickle cell trait among blood donors, differential storage protocols with shorter storage durations for HbAS units, and regular monitoring of biomarkers including lactate dehydrogenase, potassium, bilirubin, and hemolysis are recommended to improve transfusion safety and optimize blood quality in endemic settings.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Sickle Cell Trait (SCT) is a genetic disorder characterized by the presence of one hemoglobin beta sickle allele (rs334-T*)* and one normal beta allele (Verma et al., 2022). It is prevalent in many regions worldwide, particularly among Africans and African descendants (Verma et al., 2022). Globally, approximately 20 million individuals are SCT carriers, with an estimated 300,000 newborns with sickle cell disease (SCD) yearly (Rees et al., 2022). On the other hand, in Kenya, approximately 18% of children are born with SCT with 4% of the trait prevalent among voluntary blood donors (Mutua et al., 2022). In hypothermic storage (2–8 °C), SCT-negative donor blood RBCs exhibit significant changes in deformability, undergoing hematological and biochemical changes resulting in a limited shelf life that results from the gradual degradation of certain RBC components, quality, and safety (Yurkovich et al., 2017). The most common types of hemoglobin include HbAS, HbA2, HbS, HbAS, and HbC with healthy adults possessing a significant amount of HbA and HbA2.

Sickle Cell Trait donor blood, like normal hemoglobin blood, when stored, it undergoes storage-related hematological and biochemical changes (RBC's storage lesions). Storage-related biochemical changes in donor blood include but are not limited to pH variation due to glycolysis, variation in ATP production, and adenosine deaminase breakdown (Hess et al., 2014), 2, 3-Diphosphoglycerate increases due to a decrease in pH resulting in increased oxygen affinity of Hb shifting the oxygen dissociation curve to the left (Zubair et al., 2019). Moreover, there are variations in electrolytes, plasma hemolysis, LDH, Ferritin level and bilirubin level during donor blood storage period (Kotla et al., 2022 & Lin et al., 2019). On other hand, at donation time, donor blood hematological baseline parameters are normally within normal range that is Hb 12.5- 18.0g/dl, and plasma

hemoglobin- 0g/dl. Stored RBCs exhibit significant changes in deformability during storage. Oxidative damage manifests itself in RBCs, which are particularly vulnerable to stress evidenced by increased osmotic fragility during storage, resulting in the release of intracellular enzymes. Platelets that lose viability rapidly, WBC and its types, RBC counts, Lymphocytes Eosionphils ratio (LER) or in Lymphs: Neut ratio, Packed cell volume (PCV), and RBCs indices (MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration) are significantly altered in stored blood (Maruti et al., 2021, Klein et al., 2008 & Ravel et al., 2004). These changes reduce RBC's lifespan, quality, and recipients' safety.

These storage-related changes result in the gradual degradation of RBC components, leading to a loss of potency of about 17.6 % (Oyet et al., 2018). The depletion of important nutrients and energy sources, accumulation of harmful substances, and altered RBC deformability could be more drastic in SCT blood due to the lack of normal membrane deformability, fluidity, and flexibility (Pinto et al., 2022 & Yurkovich et al., 2017). During refrigerated storage, red blood cells (RBCs) are separated from plasma and preserved in an acidic, glucose-containing additive solution, where they are exposed to plasticizers, oxygen, and progressive acidification throughout the storage period (Bardyn et al., 2017). Although RBCs possess natural mechanisms to withstand oxidative and mechanical stress under physiological conditions, they lack effective adaptive responses to the unique stresses encountered during storage, making them susceptible to storage-related damage. The RBC memory impairment can be attributed to RBCs separation, plasma dilution with additive solutions, and long-term cryopreservation in closed bags. Oxidative stress and loss of biochemical countermeasures are the main causes of storage damage in stored erythrocytes. Metabolic impairment of stored RBCs occurs due to the removal of RBCs from the donor's circulation, separation from plasma, and storage in hypothermia and volume-limited acidic solutions.

This leads to the degradation of important substrates, accumulating metabolic waste products, and impaired activity of key enzymes that provide energy and antioxidant

defenses (Hess et al., 2014). Therefore, this study evaluated storage-related hematological and biochemical changes in SCT donor blood at Kisumu Regional Blood Transfusion Centre, Kenya. The findings from this study would provide critical insights into the safety and quality ramifications of SCT-positive blood transfusion and informed policies on SCT screening during blood donation locally and globally.

Although individuals with sickle cell traits are generally asymptomatic, the prevalence of SCT among blood donors is an important determinant of blood safety in regions where the trait is common (Piel et al., 2017). Understanding the burden of SCT among voluntary blood donors provides essential evidence for estimating the number of potentially affected blood units entering the blood supply (WHO, 2021). Furthermore, assessment of demographic characteristics associated with SCT distribution enables identification of high-risk donor populations and informs targeted donor education, recruitment strategies, and policy decisions regarding routine SCT screening during blood donation (Kato et al., 2018). Therefore, determination of SCT prevalence and its demographic distribution formed an important preliminary component of the present study before evaluating storage-related hematological and biochemical changes.

1.2 Statement of the Problem

In Africa, nearly 7 million blood donations are required annually to meet transfusion needs, but only half are being collected, resulting in severe blood shortages according to WHO. (2021). The scarcity of blood and blood products is alarming, with about seven people in Kenya requiring urgent transfusions every ten minutes (World Bank., 2022). Furthermore, this shortage may lead to increased mortality rates, particularly among individuals with SCD, iron deficiency anemia (IDA), and maternal complications resulting from severe bleeding (World Bank., 2022). This situation accentuates overreliance on family and friends as blood donors which may lead to unintended consequences (Mutua et al., 2022). For instance, it increases the likelihood of transfusing blood from individuals who are carriers of SCT (Mutua et al., 2022). Although the general

effects of storage on blood have been established, the specific hematological and biochemical changes that occur in stored SCT donor blood remained largely unexplored (Aninagyei et al., 2018). Africa has a prevalence (10-20%) and the potential safety risks associated with transfusing SCT-positive blood, screening for SCT in donor blood is critical for ensuring the safety of both donors and recipients, improving transfusion outcomes, and reducing the risk of allo-immunization (Oyet et al., 2018). However, SCT is not universally screened during blood donation, posing a significant safety and quality concern, particularly in SCT/SCD endemic areas (Oyet et al., 2018). Therefore, there was an urgent need to conduct a comprehensive analysis of the storage-related hematological and biochemical changes in SCT donor blood at Kisumu RBTC, in order to identify potential strategies for improving the SCT donor blood safety, quality, and efficacy. Despite increasing evidence that sickle cell trait red blood cells exhibit altered membrane physiology and reduced deformability under hypothermic storage, most blood transfusion services in sub-Saharan Africa, including Kenya, do not routinely screen donor blood for SCT before storage or transfusion (Bardyn et al., 2017; Nestheide et al., 2023). Consequently, blood units with potentially accelerated storage lesions are transfused without adequate evidence regarding their quality and safety (WHO, 2021). The absence of locally generated evidence limits formulation of evidence-based transfusion policies regarding SCT-positive donor blood

The results of this study would not only benefit Kenya but also provided valuable insights for developing effective and safe transfusion practices and guidelines globally.

1.3 Justification

The findings of this study would not only benefit Kenya and its neighbors but also provide valuable insights for developing effective, healthy lives, safe transfusion practices, and guidelines globally but also promoting well-being for all, especially in SCD/SCT endemic regions. This would go hand in hand with Sustainable development goal number Three (3), “Ensuring healthy lives and promote well- being for all at all ages.” Furthermore, the

findings from this study would be an impetus for the initiation of sustainable policies and guidelines on the safety of blood donation and blood products. For instance, developing a policy on mandatory screening for SCT during blood donors' recruitment and selection. The policy could be adopted locally by the Ministry of Health (MOH)-Kenya and Kenya National Blood Transfusion Services (KNBTs) or globally by Food and Drug Administration (FDA) and WHO Organization (WHO). This could ensure quality and safe blood products that are in line with WHO 2021 goals, "Supporting the quality and safety of blood products as well ensuring universal access to safe, effective and quality blood and blood products."

In addition, the findings from this study would go hand in hand with Kenya Health Policy 2014-2030 which guarantees its citizens the provision of essential healthcare. This would also be in line with the Kenya Health Strategic Plan 2018-2023 which advocates for, "Attaining the highest possible standard of health in a responsive manner." by ensuring a safe blood donation process and blood products. This study would be an arsenal in educating voluntary blood donors on their hemoglobin S variant status thus improving donors' blood quality, potency, and safety. Moreover, this study sought to update the database on the prevalence of SCT among voluntary blood donors attending Kisumu RBTC since the available literature dates to 2022 (Mutua et al.,2022). According to available literature then, most studies had focused on storage-related hematological and biochemical changes in normal hemoglobin (Hb AA) donor blood with limited research in Kenya, Eastern Africa, and globally (Maruti et al., 2021). It's against this backdrop that this study sought to assess the storage-related hematological and biochemical changes in SCT donors' blood at Kisumu RBTC, Kenya.

1.4 Study Objectives

1.4.1 General Objective

To evaluate storage-related hematological and biochemical changes in Sickle cell trait donor blood at Kisumu RBTC, Kenya.

1.4.2 Specific Objectives

i. To determine the prevalence of sickle cell trait among voluntary blood donors at Kisumu Regional blood transfusion centre, Kenya.
ii. To determine and compare the storage-related hematological and biochemical changes in progressing storage period of sickle cell trait and sickle cell trait Negative donor blood at Kisumu Regional Blood Transfusion Centre.
iii. To assess the association between demographic factors and the sickle cell trait status among voluntary blood donors at Kisumu Regional Blood Transfusion Centre, Kenya.

1.5 Research Questions

i. What is the prevalence of sickle cell trait among voluntary blood donors at Kisumu Regional Blood Transfusion Centre?
ii. What are the storage-related hematological and biochemical changes in progressing storage period of sickle cell trait and sickle cell trait Negative donor's blood at Kisumu Regional Blood Transfusion Centre?
iii. Is there any association between demographic factors and the sickle cell trait status among voluntary blood donors at Kisumu Regional Blood Transfusion Centre, Kenya?

1.6 Significance of the Study

The results obtained from this study would ensure there are:

i. Improved understanding of the prevalence and distribution of sickle cell trait among voluntary blood donors at Kisumu RBTC: The study would provide comprehensive insights into the epidemiology of SCT in the region, including the prevalence rates among different age groups and genders. This information would enable policymakers and health practitioners to develop evidence-based interventions to prevent and manage SCT in the county and beyond.
ii. Identification of hematological and biochemical changes in sickle cell trait donor blood: Understanding these changes could inform the development of best practices for blood transfusions and ensure the safety and efficacy of transfused blood in individuals with SCT/SCD. This information could be used to optimize blood donor screening and selection criteria, improving the quality of blood products available for transfusion in Kisumu County and beyond.
iii. The results of this project would be submitted to a peer-reviewed journal for publication and the abstract submitted to several conferences by way of application for presentations to provide valuable insights for developing effective and safe transfusion practices, policies, and guidelines globally.

1.7 Limitations of the Study

The study did not quantify the proportion of haemoglobin S (HbS) in sickle cell trait donor blood units because of limitations inherent in the confirmatory method used. Additionally, the analysis did not evaluate whether ABO blood groups and Rhesus phenotypes influenced the observed storage-related haematological and biochemical changes. These limitations should be considered when interpreting and generalizing the findings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Sickle Cell Trait (SCT)

Sickle cell trait (SCT) is an inherited hemoglobin condition that occurs when an individual inherits one normal hemoglobin (HbA) gene and one sickle hemoglobin (HbS) gene from their parents (Verma et al., 2022). Unlike individuals with sickle cell disease, those with SCT are generally asymptomatic and lead normal, healthy lives, although they may experience complications under extreme physiological stress. SCT is clinically significant because carriers can transmit the HbS gene to their offspring. When both parents have SCT, each pregnancy carries a 25% chance of producing a child with sickle cell disease, a 50% chance of producing a child with SCT, and a 25% chance of producing a child with normal hemoglobin (Rees et al., 2022).

2.2 Storage-Related Biochemical Changes in Donor Blood At donation time, donor blood biochemical baseline parameters are within normal ranges i.e. Plasma sodium-135-145mmol/l, plasma potassium- 3.0-5.2mol/l, chloride 95-108mmol/l, LDH- 100-250U/l with donor blood with % hemolysis of 0.0%. These biochemical parameters of the RBCs change during the donor blood storage period. This is mainly due to anaerobic glycolysis (Hess et al., 2014). Storage-related biochemical changes in donor blood include:

pH; when the donor blood is stored in plastic bags, glycolysis kicks off (Hess et al., 2014). Adenosine deaminase breaks down adenosine into inosine and ammonia. An increase in protons leads to a decrease in donor blood pH, resulting in altered glycolytic metabolism (Hess et al., 2014). A decrease in pH decreases the level of 2,3-diphosphoglycerate and simultaneously increases the production of ATP. Glycolysis slows down, ATP levels drop as acid accumulates, and the shape of RBCS gradually changes from discoid to echinocyte formation. This change in erythropoiesis is

attenuated when the stored donor blood is rejuvenated when warmed (Hess et al., 2014).

The 2, 3-Diphosphoglycerate (2, 3 DPG); is an enzymatic regulator of hemoglobin and aids in the transport of oxygen to the body's tissues (Yurkovich et al., 2017). A decrease in pH results in an increase in 2, 3-DPG degradation. This increases the oxygen affinity of Hb, shifts the oxygen dissociation curve to the left, and reduces oxygen in peripheral tissues. In hypoxia, the oxygen dissociation curve shifts delivery to the right, increasing oxygen transport to tissues. After the donor blood 35-42-day storage period, an RBC unit may lose more than 90% of its 2, 3 DPG concentration (Yoshida et al., 2019). 2, 3 DPG levels may become undetectable within 2 weeks of storage, and levels normalize within 72 hours after transfusion without any irreversible outcome being observed (Reisy et al., 2016). Adenosine triphosphate: there is a progressive loss of ATP as per the literature regarding morphological changes and RBCs' deformability during the storage period (Yurkovich et al., 2017). ATP is an intracellular energy source that stimulates the production of nitric oxide leading to vasodilation during hypoxic conditions. The decrease of ATP concentration during donor blood storage causes the cellular reactions requiring energy i.e. phospholipids membrane distribution, active transport, and antioxidant reactions, to also decrease (Yoshida et al., 2019). From the available literature, there is an approximate 60% decrease in ATP levels after more than 5 weeks of donor blood storage (Yoshida et al., 2019). The continuous reduction in ATP concentrations and acidification results in irreversible shape alteration of the RBC as echinocytic surface protrusions appear, phospholipids bilayer loses its asymmetry and the shedding of micro-vesicles occur (Yurkovich et al., 2017).

Potassium and Sodium ions: donor blood stored at 2-8°C decreases the rate of cellular metabolism and energy demand which allows blood to be stored for 35-42 days. This makes the sodium-potassium pump inoperative and consequently allows potassium ions to exit the cell and sodium ions to enter via the semi-permeable membrane (Yurkovich et al., 2017). The extracellular potassium levels of stored blood increase daily at approximately 1 mEq/L with higher concentrations observed during the early days of

storage (Yoshida et al., 2019).

Increased potassium levels in RBCs may lead to arrhythmia when neonates or infants are transfused with large volumes of stored blood (Yoshida et al., 2019).

Plasma hemolysis due to extended storage period, the RBCs membrane undergoes both biochemical and morphological changes indicated by plasma % hemolysis. Hemolysis of RBCs may occur during blood donation due to bacterial-contamination, transportation, storage, donor RBCs' membranes' deficiencies, and presence of leucocytes in unfiltered RBC, mechanical penicillin in the donor (Han et al., 2010). The interaction of plasma Hb with nitric oxide causes endothelial dysfunction which is a risk factor for vasoconstriction, leucocyte adhesion, and intravascular thrombosis (Yoshida et al., 2019). The hydrogen peroxidase and proteases released by the leucocytes present in unfiltered blood may cause the lysis/breakdown of RBCs during the storage period. Hemolysis signs in the plasma or suspending fluid suggest that the RBCs have either ruptured or it may be due to the loss of membrane-bound hemoglobin in the micro vesicles found on the cell surface of intact RBCs. The addition of membrane stabilizers (mannitol and citrate) decreases hemolysis (Khisa et al., 2021). The easiest approach to assess the presence of hemolysis in an RBC unit is by observation, but this visual inspection is often deceptive as it leads to an overestimation of hemolysis levels (Mutua et al.,2022). It has also been reported that pink/red discoloration due to hemolysis observed in either plasma or suspending fluid may often be due to plasma hemolysis as low as 25 g/dl and under normal storage conditions, these blood units are discarded unnecessarily. The clinical-implication of RBCs' hemolysis for the transfused patients is very serious and may lead to redox injury of the body tissues, endothelium, or the proximal-tubules of the kidney (Relevy et al., 2007).

Oxidative injury manifests RBCs as extra vulnerable to stress as indicated by increased osmotic fragility in the course of the storage period and resultant discharge of hemoglobin and intracellular enzymes such as LDH into the donor blood floating plasma

(Yoshida et al., 2019). Ferritin level; ferritin plays a key role in iron metabolism, with the inherent ability to store and release iron when the body needs it (Kotla et al., 2022). Body ferritin levels can be used as an indicator to measure the amount of iron stored in the body.

Ferritin can reduce the accumulation of Reactive oxygen species (ROS) in the body and it's known for its significant function in iron homeostasis both at the cellular and organismal level. It also has anti-oxidative attributes (Mahomoodally et al., 2022). Intracellular ferritin synthesis is controlled at translational and transcriptional levels in both an iron-dependent and an iron-independent manner. Iron is an essential/vital trace element for living organisms and plays a crucial function in a number of cellular functions in metabolism, growth, and cell-differentiation, such as energy production, oxygen transport, DNA synthesis and storage, and cell cycle. Bilirubin functions as antioxidant in vitro and its redox movement is especially critical within the brain, where it anticipates excitotoxicity and neuronal passing by rummaging O2·- amid NMDA neurotransmission. Bilirubin's interesting redox action toward O2·- may underlie a critical physiologic part despite being altogether less inexhaustible than other endogenous and exogenous antioxidants (Vasavda et al., 2019). It plays a role as a signaling molecule (Vítek et al., 2020), and as a metabolic hormone (Creeden et al., 2021), and it, is also associated with a decreased risk of developing cardiovascular disease (CV) and all-cause death in diabetic patients at its higher concentration (McQuilten et al.,2018 & Lin et al., 2019).

2.2.1 Causes of Storage-Related Biochemical Changes in Donor Blood

During hypothermic storage, red blood cells (RBCs) are separated from plasma and suspended in a preservative solution rich in glucose to maintain cellular metabolism. Throughout storage, the cells are exposed to plasticizers leaching from blood storage bags, oxygen that diffuses through the bag from the surrounding environment, progressive acidification, and the accumulation of metabolic waste products, all of which contribute

to cellular stress (Wither et al., 2016). Under physiological conditions, RBCs possess adaptive mechanisms that enable them to withstand oxidative and mechanical stress while transporting oxygen. However, during ex vivo storage, these protective physiological processes are absent, rendering RBCs more susceptible to oxidative injury, membrane damage, metabolic deterioration, and other biochemical and structural alterations collectively referred to as the storage lesion (Wither et al., 2016). These causes of storage-related hematological and biochemical changes include:

The chemical oxidation of iron in hemoglobin causes oxidative stress and is a major factor in the development of storage damage in stored erythrocytes. RBCs contain high concentrations of dissolved oxygen and high concentrations of reactive iron (II) in the hematopoietic group of Hb. The four iron units of hemoglobin chemically react with oxygen to form met-hemoglobin (Wither et al., 2016). The superoxide anion is produced as a by-product and converted by superoxide dismutase to H_2O_2, an important reactive oxygen species (ROS) and substrate for hydroxyl radical (OH) production. Reductase enzymes convert methemoglobin back to hemoglobin in vivo; however, cryopreservation conditions cause their enzymatic activity to decline. Superoxide reduces iron (III) in heme by forming met-hemoglobin. Through the Fenton reaction, the resultant ferrous ion combines with H2O2 to generate net (•OH) through the Haber-Weiss reaction (Reisz et al., 2016). Ferryl (+IV) hemoglobin is also created when H2O2 combines with oxyhemoglobin. Ferryl hemoglobin and (•OH) are both extremely reactive substances that oxidize nearby lipids and enzymes. The occurrence of this cascade of events increases exponentially after 2-3 weeks of storage in the SAGM and other additives causing reversible and irreversible oxidation, thereby impairing metabolism (Wither et al., 2016). There is exposure of phosphatidylserine on RBCs' surfaces and imbalances in K^+ and Ca^{2+}ions (Liang et al., 2014). Oxidized proteins bind to the cytoskeleton and disrupt network structure causing morphological changes and reduced deformability (Yoshida et al., 2019). Oxidized and denatured proteins aggregate and precipitate in/on RBCs membranes destroying their oxygen-

binding capacity (Wither et al., 2016). Ferryl-hemoglobin and OH are ROS that initiates the lipid peroxidation cycle (Wither et al., 2016).

Metabolic impairments: occur as a result of RBCs being removed from the donor's circulation, separated from the plasma, and stored in hypothermia and volume-limited acidic solutions. Important substrates such as glucose and purine derivatives such as uric acid (Bardyn et al., 2017) are degraded, accumulating metabolic waste products, dominated by lactic occur in blood component processing and storage. Glycolysis results in a drop in pH and impaired activity of key enzymes and antioxidant defenses (Hess et al., 2014).

Low pH additive solutions reduce the activities of the rate-limiting enzymes of glycolysis, the pentose phosphate pathway (PPP), and the Rapoport-Luebbering shunts, and contribute to the rapid depletion of 2,3-diphosphoglycerate and a gradual reduction of ATP during storage. Glycolytic enzymes are reversibly and irreversibly oxidized progressively during storage (Reisz et al., 2016). 2,3-DPG breakdown fuels ATP generation at the expense of hemoglobin capacity to off-load oxygen, due to the ensuing low 2,3-DPG levels. As 2,3-DPG is consumed and hemoglobin oxygen saturation is increased by 2-3 storage weeks, ROS accumulation reaches its plateau in classic additives (Wither et al., 2016).

2.3 Storage-Related Hematological Changes in Donor Blood

At donation time, donor blood hematological baseline parameters are normally within normal range i.e. Hb 12.5- 18.0g/dl, plasma hemoglobin- 0g/dl. In hypothermic storage (2–8°C), chemical reactions continue, albeit more slowly, resulting in a limited shelf life of up to six weeks results from the gradual degradation of certain RBC components, collectively termed as the "storage lesion"(Yurkovich et al., 2017. Stored RBCs exhibit significant changes in deformability during storage. Oxidative stress induces progressive damage to red blood cells (RBCs), which are particularly susceptible to oxidative injury

during storage. This damage is characterized by increased osmotic fragility, membrane instability, and subsequent hemolysis, leading to the leakage of intracellular enzymes into the storage medium. In addition, prolonged storage results in significant alterations in hematological parameters, including platelet and white blood cell counts, red blood cell counts, and red blood cell indices, reflecting the cumulative effects of storage-related cellular deterioration (Maruti et al., 2021). These hematological changes in donor blood may manifest itself in:

Hemoglobin, RBCs count, and PCV; are significantly affected during storage with PCV being more affected than Hb. This could be due to increased (MCV) or the real effect of age on blood, meaning new blood is better than old blood in raising (PCV) (Klein et al., 2008). With prolonged storage, there is a shortage of ATP and the pumps may not be able to maintain the ionic homeostasis of the RBCs, leading to changes in shape and mean cell volume (MCV), hematocrit (HCT), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) as well as RDW-CV% (Klein et al., 2008).

The water influx to the cytosol gives rise to the swelling of erythrocytes during storage and the non-existence of selective channels of performance as done in the spleen is associated with the change in shape and volume of the RBCs during storage (Maruti et al., 2021). During storage, the total WBC count decrease is associated with the granulocytes' degeneration (Nuaimy et al., 2008).

White blood cells have an abbreviated life span in stored blood (a few hours) and transfusion proved ineffective in elevating the leukocyte count, From the literature, many studies showed that during storage the total leukocyte count decreases, they attribute this decrease to degeneration of the granulocytes (Ravel *et a*l., 2004). WBC changes could also be in; Neutrophil, Basophil, Lymphocyte, Monocytes, Eosionphils counts or in Lymphocyte: Eosinophil ratio (LER) or in Lymphs: Neutrophil ratio or in Lymphs: Baso ratio (LBR). WBCs have an adverse effect on platelets function and post-transfusion recovery (Ravel et al., 2004). Platelets lose viability rapidly in storage in refrigerators.

Platelets transfusion to be physiologically effective must be completed within 4-6 hours after blood donation. Changes take place in both platelet and storage device, which may lead to the activation of platelets and malfunction (Aubron et al., 2018). The platelets' viability and functions are affected by WBCs present in stored platelet concentrate. In fresh whole blood, platelets are about 60 % effective at 24 hours and almost completely ineffective after 48 hours (Ravel et al., 2004). This rapid disintegration of platelets during storage may be due to a rapid disappearance of the WBCs; their hydrolytic enzyme affects the platelet membrane and causes platelet degeneration (Ravel et al., 2004).

2.3.1 Causes of Storage-Related Hematological Changes in Donor Blood

Amid the hypothermic capacity of donor blood, RBCs are isolated from plasma and re-suspended in an arrangement containing high concentrations of glucose. Red blood cells are uncovered to plasticizers within the capacity sack, uncovered to oxygen diffusing from the encompassing discuss amid the collection of metabolic squanders, and advanced fermented over the capacity period (Wither et al., 2016).

Red blood cells have advanced to manage the oxidative and mechanical stresses they experience whereas satisfying their critical work as oxygen carriers in vivo but in their disconnected state beneath ex-vivo cryopreservation, RBCs are uncovered to diverse chemical and mechanical stresses without physiological countermeasures in put driving to capacity harm (Wither et al., 2016). These causes of storage-related hematological and biochemical changes incorporate Oxidative harm; the chemical oxidation of iron in hemoglobin causes oxidative stress and may be a major calculate within the improvement of storage damage in put-away erythrocytes. RBCs contain tall concentrations of broken-down oxygen and tall concentrations of reactive iron (II) within the hematopoietic gather of Hb. The four press units of hemoglobin chemically respond with oxygen to create methemoglobin (Wither et al., 2016). The superoxide anion is created as a by-product and changed over by superoxide dismutase to H_2O_2, a vital responsive oxygen species (ROS) and substrate for hydroxyl radical ($^{\bullet}OH$) generation. In vivo, methemoglobin is diminished

back to hemoglobin by reductase chemicals, but these enzymatic exercises diminish beneath cryopreservation conditions. Iron (III) in heme is decreased by superoxide through met-hemoglobin arrangement.

The coming about ferrous particles responds with H_2O_2 through the Fenton response to deliver net (OH) by means of the Haber-Weiss response (Reisz et al., 2016). Hydrogen peroxide H_2O_2 moreover responds with oxyhemoglobin to create ferryl (+IV) hemoglobin. Both (OH) and ferryl hemoglobin are exceedingly responsive and oxidize adjoining proteins and lipids. The event of this cascade of occasion's increments exponentially after 2-3 weeks of capacity within the SAGM and other added substances causing reversible and irreversible oxidation, in this manner disabling the digestion system (Wither et al., 2016). There's the presentation of phosphatidylserine on RBCs' surfaces and lopsided characteristics in K^+ and Ca^{2+}ions (Liang et al., 2014). Oxidized proteins tie to the cytoskeleton and disturb arrange structure causing morphological changes and diminished deformability (Yoshida et al., 2019). Oxidized and denatured proteins total and accelerate in/on RBC layers wrecking their oxygen authoritative capacity (Wither et al., 2016). Ferryl-hemoglobin and globin free radicals are ROS that start the lipid peroxidation cycle (Wither et al., 2016).

Metabolic impairments occur when red blood cells (RBCs) are removed from the donor's circulation, separated from plasma, and stored under hypothermic conditions in acidic, volume-restricted preservative solutions. During storage, essential metabolic substrates, particularly glucose, and antioxidant compounds such as purine derivatives (e.g., uric acid), are progressively depleted, while metabolic waste products accumulate, with lactate being the predominant by-product of glycolysis (Bardyn et al., 2017). Continuous anaerobic glycolysis results in a gradual decline in pH, creating an acidic environment that inhibits the activity of key metabolic enzymes and weakens the antioxidant defense system. These metabolic disturbances compromise RBC energy production, membrane integrity, and cellular function, thereby contributing to the development of storage lesions and reduced post-transfusion viability (Hess et al., 2014).

More pH added substance arrangements diminish the exercises of the rate-limiting proteins of glycolysis, the pentose phosphate pathway (PPP), and the Rapoport-Luebbering shunts, and contribute to the quick consumption of 2,3-diphosphoglycerate and a continuous lessening of ATP amid capacity. Glycolytic chemicals are reversibly and irreversibly oxidized continuously amid capacity (Reisz et al., 2016). 2,3-DPG breakdown powers ATP era at the cost of hemoglobin capacity to off-load oxygen, due to the following moo 2,3-DPG levels. As 2,3-DPG is expended and hemoglobin oxygen immersion is increased by 2-3 capacity weeks, ROS aggregation comes to its level in classic added substances (Yoshida et al., 2019)

2.4 Effects of Stored Donor Blood Transfusion on Hematological and Biochemical Changes

ATP and 2,3-DPG, and reduced equivalent glutathione (GSH) and (NAD(P)H) decrease after 2-3 weeks of hypothermic storage (Nemkov et al., 2016). Depleted ATP affects several enzymatic functions and ion pumps such as Ca^{2+}. ATP depletion deregulates cation homeostasis, disrupts membrane asymmetry, and leads to the exposure of phosphatidylserine (PS) and phosphatidyl ethanolamine (PE), which are normally trapped in the inner bilayer leading to the formation of fine particles. ATP depletion also alters the ability of kinases to phosphorylate proteins evidenced by the restoration of the ability to phosphorylate membrane proteins after the rejuvenation of long-term stored erythrocytes (Prudent et al., 2018). Depleted ATP also causes cytoskeletal rearrangement, leading to echinocytosis (Park et al., 2010). Depletion of reducing equivalents leads to reduced antioxidant capacity, further exacerbating oxidative stress-induced damage in erythrocyte recipients during storage and post-transfusion. Rapid loss of hemoglobin S-nitrosylation (SNO) in stored RBCs is thought to interfere with vasodilation in transfused patients by causing insufficient nitric oxide bioavailability (INOBA) (Reynolds et al., 2007).

The RBCs unit contains a continuum of cell ages, from those just released into circulation to those at the end of their circulatory lifespan in senescence. Thus, at any given time

during storage, a unit of stored RBCs contains several senescent cells that have lost excessive membrane area through vesicle formation and reduced antioxidant capacity (Rodgers et al., 1983). Morphological analysis reports 6-9% of erythrocytes with irreversible changes (Bardyn et al., 2017). To accommodate long-term ex vivo storage (Tuo et al., 2014 & Aung et al., 2017), an average potency loss of approximately 17.6% occurs after the storage of packaged RBCs is terminated. Accumulation of denatured methemoglobin and damage by ROS change the morphology of RBCs from discoid cells to spinous cells and then irreversibly to spherical cells by releasing reversible micro-particles (MPs) after transfusion. Oxidation of membrane lipids and proteins exposes inner membrane phospholipids (PS & PE) (Larson et al., 2017). Large loss of membrane area compared to volume occurs during MP formation, resulting in a loss of the extra surface area required for RBC passage through thin splenic capillaries (Pecker et al., 2018). Minimizing the surface area to volume ratio also increases the osmotic fragility of RBCs. Cross-linking of cytoskeletal and membrane proteins (Yoshida et al., 2019 &Hebbel et al., 1990), dysregulation of cytoskeletal protein phosphorylation (Yurkovich et al., 2017) and dehydration caused by Ca^{2+} influx and K^{+} efflux (Yoshida et al., 2019).

Oxidation of the membrane cytoskeleton disrupts the anchorage between membrane proteins and the cytoskeleton, leading to the formation of the erythropoiesis signal by band 3 clustering (Yoshida et al., 2019). Oxidation of CD47 leads to the formation of erythropoietic signals (Burger et al, 2012), whereas storage-dependent depletion of her CD47 in the membrane (Stewart et al., 2005). Erythrocytes are susceptible to removal mediated by the recipient's reticuloendothelial system. Red blood cells that are nearly senescent at the time of collection may represent the majority of cells that are haemolyzed during storage, but the incidence of hemolysis is very low, typically less than 0.8%.

Biological response modifiers (BRMs) such as cytokines, chemokine, bioactive lipids, and metabolites accumulate during storage (Casali et al., 2016). BRMs act as pro-inflammatory agents in transfusion recipients. Transfusion of the freshest available blood does not decrease the risk of mortality in several categories of recipients

when compared to the standard of care (McQuilten et al., 2018 & Cushing et al., 2017) Ionic homeostasis imbalance adversely affects proton pumps, and deregulations of ion homeostasis (such as calcium) affects kinase activity and metabolic signaling (Bawazir et al., 2014).

Moreover, when stored RBCs are transfused, approximately 17.6% of RBCs are cleared from circulation within 24-hr (Mays et al., 2017). A small fraction of mechanically damaged RBCs may hemolyse intravascularly after transfusion with majority being phagocytosed extravascular by macrophages in the recipient's reticuloendothelial system (Wojczyk et al., 2014). Mechanisms of programmed cell death may activate during storage in parallel, resulting in eryptosis, which is induced by calcium influx and K^+ efflux (Lion et al.,2010 & Liang et al.,2014), cell shrinkage, exposure of PS (Burger et al., 2012) and PE (Larson et al.,2017) from inner membrane bilayer, vesiculation of MPs with loss of excess surface area (Roussel et al.,2017), activation of calpains and caspases (Reisz et al., 2016), and reduced deformability (Daly et al., 2014).

In vivo, RBCs haemolyzed in the circulation release iron from heme but are sequestered quickly by transferrin (Rapido et al., 2017). In chronically transfused patients, the recipient's ability to process the excess iron from the non-viable RBCs contained in each transfused unit is overwhelmed, leading to tissue iron overload and organ dysfunction (Wood et al., 2015). Ferryl-l hemoglobin, the oxidation product of methemoglobin by ROS, is also a pro-inflammatory agonist and can cause endothelial damage (Silva et al., 2009).NO is a vasodilatory signal produced by endothelial nitric oxide synthase (eNOS) near precapillary arterioles. Blood flow deregulation by interfering with NO-mediated vascular regulation is another important physiological effect of preserved RBC transfusion. RBCs, which are difficult to deform, also participate in the formation of INOBA (Yalcin et al., 2014).

Red blood cells play a direct role in regulating NO flow in capillaries. Red blood cells Hb reduces plasma nitrite to produce NO (Chen et al., 2008), and endothelial eNOS is

stimulated by ATP released from RBCs (Bogle et al., 1991). Storage under conventional conditions alters the latter mechanism, with lower glucose concentration in the additive solution allowing better NO production from endothelial cells stimulated by ATP release (Wang et al., 2013). Transfused stored RBCs can induce pro-inflammatory responses over time through cytokines, eicosanoids, and free hems (Zecher et al., 2014).

2.5 Sickle Cell Trait among Blood Donors

Sickle cell trait (SCT) is the heterozygous inheritance of one normal hemoglobin A (HbA) gene and one sickle hemoglobin (HbS) gene (HbAS). Although individuals with SCT are generally asymptomatic, they can donate blood, and their red blood cells may exhibit altered physiological characteristics that influence storage quality and post-transfusion performance under certain conditions (Piel et al., 2017). Globally, it is estimated that over 300 million people carry the HbS gene, with the highest burden occurring in sub-Saharan Africa, the Middle East, India, and parts of the Mediterranean, where SCT prevalence ranges from 10% to 40% in malaria-endemic populations due to the selective advantage conferred against severe malaria (Kato et al., 2018; WHO, 2021).

Kenya is among the countries with a significant burden of SCT, although prevalence varies geographically according to malaria transmission intensity and ethnic distribution. Western Kenya, particularly the Lake Victoria region, reports some of the highest SCT frequencies in the country, with prevalence estimates ranging between 10% and 20%, compared with much lower frequencies in non-endemic regions (MOH Kenya, 2023; Ambrose et al., 2018). Among blood donors, studies conducted in several African countries have reported SCT prevalence ranging from 5% to 20%, indicating that a considerable proportion of donated blood units may originate from SCT-positive donors (Jeremiah & Koate, 2010; Nnodu et al., 2021).

Several demographic factors have been associated with SCT distribution, including ethnicity, geographical location, age, and family genetic background. Ethnic communities

residing in malaria-endemic regions generally exhibit higher SCT prevalence because of the long-standing evolutionary selection pressure exerted by *Plasmodium falciparum* malaria (Piel et al., 2017). While sex has not consistently been associated with SCT occurrence, regional and ethnic variations remain important predictors of carrier frequency (Kato et al., 2018). Understanding these demographic characteristics among blood donors is important for identifying populations contributing disproportionately to the blood supply and informing targeted donor education and recruitment strategies.

The presence of SCT among blood donors has important implications for transfusion services. Although SCT blood is generally considered suitable for transfusion in many settings, increasing evidence suggests that HbAS red blood cells may develop storage lesions more rapidly than normal red blood cells, resulting in increased hemolysis, potassium leakage, oxidative stress, and reduced deformability during prolonged storage (Bardyn et al., 2017; Nestheide et al., 2023). These changes may compromise blood quality, particularly for vulnerable recipients such as neonates, patients with sickle cell disease, and individuals requiring chronic transfusion therapy. Consequently, several authors have recommended consideration of routine SCT screening and appropriate labeling of donor units in regions with high SCT prevalence to enhance transfusion safety (WHO, 2021).

Despite these studies, little information exists regarding the prevalence of SCT among voluntary blood donors in western Kenya and whether demographic characteristics influence SCT occurrence within this population

2.6 Conceptual Framework

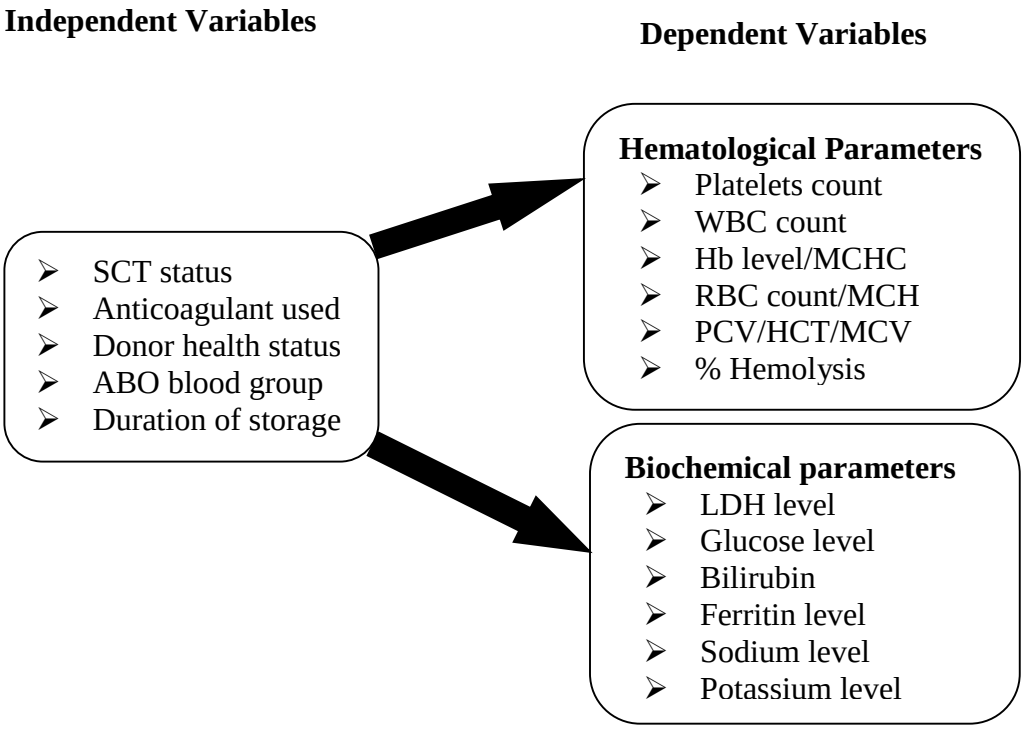

Figure 2.1: Conceptual Framework

2.7 Knowledge Gap

Although numerous studies have characterized storage lesions in conventional donor blood and others have documented the prevalence and distribution of sickle cell trait (SCT) in different populations, limited research has comprehensively linked these areas by evaluating storage-induced hematological and biochemical changes in SCT-positive donor blood under routine blood bank conditions (Bardyn et al., 2017; Piel et al., 2017). Existing studies have largely focused either on the epidemiology of SCT or on red blood cell storage lesions in the general donor population, with few investigations examining how the presence of HbAS influences blood quality throughout the storage period (Nestheide et al., 2023).

In Kenya, published evidence on SCT among blood donors remains scarce, particularly in western Kenya where the burden of SCT is relatively high because of the endemicity of malaria (Ministry of Health Kenya, 2023). To date, no published Kenyan study has simultaneously determined the prevalence of SCT among voluntary blood donors, evaluated the demographic characteristics associated with SCT occurrence, and prospectively compared storage-related hematological and biochemical changes between HbAS and HbAA donor blood throughout the recommended storage period under routine blood bank conditions. Consequently, the potential implications of SCT-positive donor blood for blood quality, storage stability, and transfusion practice remain inadequately understood. Addressing this evidence gap is essential for informing donor screening policies, improving blood inventory management, and generating locally relevant evidence to guide transfusion practices in Kenya and other malaria-endemic settings. Therefore, this study was undertaken to bridge these critical knowledge gaps and provide evidence to support policy and clinical decision-making

CHAPTER THREE

METHODOLOGY

3.1 Study Site

The study was carried out at the Kisumu RBTC, strategically positioned on geographical coordinates -0.0868146°S and 34.7716936°E, within Kisumu County, Kenya. Kisumu County is renowned for being a significant area of SCT endemicity, with a prevalence rate of 4% among voluntary blood donors (Mutua et al., 2022). This made it an ideal location for the study, which sought to explore and understand the epidemiology of SCT in the region.

3.2 Study Design

A prospective laboratory-based experimental study design was employed in this study (Rebecca et at., 2022). Blood samples (2X EDTA) drawn on days 0(baseline), day 7(week 1), day 14(week 2), Day 21(week 3) and day 28 (week 4), and hematological and biochemical changes accessed as per the laid down standard operating procedures. The study consisted of two complementary phases. Phase I was a descriptive cross-sectional component involving screening of 336 voluntary blood donors to determine the prevalence of sickle cell trait and its association with selected demographic characteristics. Phase II was a prospective laboratory-based experimental study involving 60 donor blood units (30 HbAS and 30 HbAA) that were followed longitudinally during 28 days of storage to evaluate changes in hematological and biochemical parameters.

3.3 Study Population

The study population comprised volunteer blood donors who visited Kisumu RBTC during the data collection period (2nd January, 2024 to 3rd April 2024). The target population for the prevalence component comprised all eligible voluntary blood donors

presenting to Kisumu Regional Blood Transfusion Centre during the study period. The experimental component comprised donor blood units confirmed as HbAS or HbAA following SCT screening.

3.3.1 Inclusion Criteria

Inclusion criteria for the prevalence component included all eligible voluntary blood donors who met Kenya National Blood Transfusion Service donor selection criteria and consented to participate. For the experimental component, donor blood units were eligible if they were confirmed as HbAS or HbAA, negative for transfusion-transmissible infections and fulfilled all predefined hematological and biochemical quality criteria. The study included donor blood units that met the following criteria:

- Thirty (30) sickle cell trait-positive (HbAS) donor blood units.
- Thirty (30) sickle cell trait-negative (HbAA) donor blood units.
- Blood units collected from voluntary blood donors who provided written informed consent to participate in the study.
- Donors who met the Kenya National Blood Transfusion Service donor selection and recruitment guidelines.
- Blood units that tested negative for all transfusion-transmissible infections (TTIs), including:
    - Human Immunodeficiency Virus (HIV)
    - Hepatitis B virus (HBV)
    - Hepatitis C virus (HCV)
    - Syphilis
    - Malaria
- Blood units with baseline hemoglobin concentration between 12.5 and 18.0 g/dL.
- Blood units with 0 g/dL plasma hemoglobin at baseline.
- Blood units with 0.0% percentage hemolysis at baseline.

- Blood units with baseline biochemical parameters within the following reference ranges:
    - Plasma sodium: 135-145 mmol/L
    - Plasma potassium: 3.0-5.2 mmol/L
    - Lactate dehydrogenase (LDH): 100-250 U/L.

3.3.2 Exclusion Criteria

Donors who failed routine blood donor eligibility screening or declined consent were excluded from the prevalence component. Blood units showing evidence of hemolysis, positive transfusion-transmissible infections or abnormal baseline laboratory parameters were excluded from the experimental component. The following donor blood units were excluded from the study:

- Blood units with a baseline hemoglobin concentration of <12.5 g/dL.
- Blood units that tested positive for one or more transfusion-transmissible infections (TTIs), including:
    - Human Immunodeficiency Virus (HIV)
    - Hepatitis B virus (HBV)
    - Hepatitis C virus (HCV)
    - Syphilis
    - Malaria
- Blood units showing evidence of hemolysis at baseline (plasma hemoglobin >0 g/dL).
- Blood units with a baseline percentage hemolysis >0.0%.
- Blood units with baseline hematological or biochemical parameters outside the established normal reference ranges.
- Blood units collected from voluntary blood donors who did not provide informed consent to participate in the study.

3.4 Sampling Technique

A convenient purposive sampling technique was employed (Wiliyanarti et al., 2022) that entailed strict recruitment of voluntary blood donors who satisfied the defined inclusion criteria.

3.5 Sample Size Determination

Sample size was determined using Cochran's formulae at a prevalence rate of 4% (Mutua et al., 2022) as shown below:

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where;

Z=1.96 P=4% q=1-p e=0.05

$$\frac{1.96^2 \times 0.04 \times (1 - 0.04)}{0.05^2}$$

$$\frac{= 1.96^2 \times 0.04 \times 0.96}{0.0025}$$

$$\frac{= 3.8416 \times 0.04 \times 0.96}{0.0025} = \frac{0.14751744}{0.0025}$$

=59.006976=**60**

The sample size of 336 voluntary blood donors was calculated to estimate the prevalence of SCT among blood donors. Following SCT screening, all HbAS-positive donor blood units identified during the study period were included in the experimental phase. Thirty HbAS blood units were obtained and matched with thirty HbAA blood units of similar storage age to provide equal comparison groups for longitudinal laboratory analysis.

3.6 Data Collection Instruments/Equipment

The data collection instruments included:

3.6.1 Kenya National Blood Transfusion Services Questionnaire (KNBTSQ)

The Kenya National Blood Transfusion Service Questionnaire (KNBTSQ) was used to collect data on age, body weight, hemoglobin level, history of donation, health status, blood pressure, and pulse rate. This tool is routinely used by Kenya National Blood Transfusion Centers and shall be adopted for use in this study with permission from Kisumu Regional Blood Transfusion Center.

3.6.2 Tools for Collection of Laboratory Data

3.6.2.1 Sickle SCAN Test Kit

Sickle SCAN test kit (BioMedomics, USA) is a rapid, qualitative lateral flow immunoassay test for the identification and confirmation of sickle cell disorder of hemoglobin A, S, and C. It has three indicators which detect the presence of defective hemoglobin, allowing rapid distinguishing between normal, carrier, and sickle cell samples using five microliters of blood (Katanga et al., 2020). In summary, blood was placed into the buffered-loaded pretreatment module to release hemoglobin by lysing erythrocytes. Three drops of the treated samples dropped from the pretreatment module and added to the sample inlet where it interacted with antibody-conjugated colorimetric detector nanoparticles and travels to the capture zone. Results were read within five minutes where the presence of hemoglobin variants A, S, and C was indicated by blue lines as illustrated in appendix VI (Katanga et al., 2020).

3.6.2.2 Automated Hematology Analyzer

Automated Hematology Analyzer (Mindray BC-5000, Mindray Global, China) was used to evaluate hematological parameters (Hb level, RBC Count, RBC indices (MCV, MCH, MCHC, HCT), WBC count (Mon, Neut, Eos, Lymph, and Bas) and PLT) on day 0 (baseline), day 7(week 1), day14(week 2), day 21(week 3) and day 28(week 4) in reference to the standard operating procedure (Appendix 4).

3.6.2.3 Biochemistry Analyzer (Cobas Integra 400 Plus)

The fully Automated Biochemistry Analyzer (Cobas Integra 400 plus, Switzerland) was used to evaluate biochemical changes (Ferritin level, Sodium, potassium, Bilirubin level, LDH, and Glucose) on day 0(baseline), day 7(week 1), day14(week 2), Day 21(week3) and day 28(week 4) as per the standard operating procedure. Whole blood plasma was analyzed after being drawn from donor blood units into vacutainers aseptically on day 0 (baseline), day 7(week 1), day 14(week 2), day 21(week 3) and day 28(week 4) and used during the analysis of these biochemical parameters according to the standard operating procedure in appendix 7. These biochemical parameters are very key in determining the potency and quality of SCT-donated blood. The detailed standard operating procedure is illustrated in Appendix 7 for reference.

3.7 Validation and Reliability of Data

Before analysing the study samples, internal quality-control materials at normal and abnormal concentration levels were processed on the Mindray BC-5000 Haematology analyzer and Cobas Integra 400 Plus Biochemistry Analyse. Study samples were analyzed only when the quality-control results fell within the manufacturer's acceptable range. The analyzers were calibrated and maintained according to the manufacturers' instructions and laboratory standard operating procedures. Internal quality-control records were documented and retained as evidence of analytical validity and reliability.

A pretest was conducted among ten voluntary blood donors who attended Kisumu Regional Blood Transfusion Center (KRBTC). Furthermore, Internal Quality Control (IQC) was done before every test run.

The Kenya National Blood Transfusion Service Questionnaire had been rated by different experts and found to be reliable. A total of four samples were randomly selected and sent to referral laboratory to be evaluated for the reproducibility of the results.

3.8 Laboratory Procedures and Methods

Laboratory methods and procedures provided a sequential flow of events during the data collection period. This entailed the following laboratory methods and procedures:

3.8.1 Recruitment of Study Subjects

After the acquisition of study approvals (Ethical approval from JKUAT ISEREC, data collection permission from NACOSTI, and site permission from KNBTs), the study subjects were identified and recruited based on their demographic factors as outlined in the KNBTS questionnaire. Thereafter, subjects were given an explanation of the study's details and selected according to the guidelines in the KNBTs Questionnaire. Eligible voluntary blood donors were recruited consecutively during routine blood donation sessions after satisfying Kenya National Blood Transfusion Service donor selection criteria. Written informed consent was obtained before SCT screening. Following laboratory confirmation, donor blood units meeting eligibility criteria were enrolled into the experimental storage study.

3.8.2 Collection of Blood Samples

Whole-blood units were collected into sterile blood bags containing citrate-phosphate-dextrose-adenine (CPDA-1), which served as the anticoagulant and preservative. The units were stored at 2–8 °C and analyzed at baseline and on days 7, 14, 21 and 28. At each

time point, the blood bag was gently mixed, and a small aliquot was aseptically withdrawn through the sampling port using a sterile syringe. This procedure maintained the integrity of the blood units and prevented unnecessary wastage. EDTA tubes were used exclusively for haematological analysis and not for biochemical testing.

3.8.3 Performing Sickling Test

The sickling test was performed according to the standard operating procedure (Khan et al., 2023) and was used to determine the Sickle cell disease/carrier status of donor blood unit. This was performed by mixing 10µL of fresh whole blood with 50µL (2%) sodium metabisulphite ($Na_2S_2O_5$), cover slipped, and airtight-sealed it with wax mold at incubating at 37C for 2-3 hours. The slide was then observed microscopically under low power objectives for the detection of sickle-shaped RBCs (Khan et al., 2023). In this test, the deoxy-hemoglobin formed polymers, causing change in the morphology of the RBCs, acquiring the classical sickle shape. The Sickling test positive results were confirmed with Sickle SCAN Test without any discrepancies noted.

3.8.4 Sickle SCAN Test

The Sickle SCAN test (qualitative lateral flow immunoassay test) was carried out according to (Katenga et al., 2020). This test played a crucial role in the identification and confirmation of sickle cell disorder of hemoglobin A, S, and C using three indicators that detected the presence of defective hemoglobin, allowing rapid distinguishing between normal, carrier, and sickle cell samples using five microliters of blood (Katanga et al., 2020). Blood was placed into the buffered-loaded pretreatment module to release hemoglobin by lysing erythrocytes. Three drops of the treated samples were dropped from the pretreatment module and added to the sample inlet where it interacted with antibody-conjugated colorimetric detector nanoparticles and traveled to the capture zone. Results were read within five minutes where the presence of hemoglobin variants A, S, and C was indicated by blue lines (Katanga et al., 2020).

3.8.5 Running Haematological Tests

Haematological analyses were performed at [insert laboratory name] using the Mindray BC-5000 automated haematology analyzer. The analyzer determines haemoglobin concentration by the colorimetric method, while red blood cell, white blood cell and platelet counts are measured using electrical impedance. Haematocrit and red cell indices including mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration are calculated from directly measured parameters. EDTA-anticoagulated aliquots were used for these analyses.

3.8.6 Running Biochemical Tests

Biochemical analyses were performed at [insert laboratory name] using the Cobas Integra 400 Plus automated chemistry analyzer. Plasma obtained directly from the CPDA-1-preserved donor-blood aliquots was used for biochemical testing; no additional EDTA was introduced. Lactate dehydrogenase was measured using an enzymatic kinetic method, while glucose, ferritin and bilirubin were determined using the analyzer's validated photometric and immunological methods. Sodium and potassium concentrations were measured using ion-selective electrodes. All procedures followed the manufacturer's instructions and laboratory standard operating procedures.

3.8.7 Determination of Percentage Haemolysis

Whole blood was drawn from donor blood units aseptically into EDTA vacutainers tubes and used to calculate and determine the percentage of hemolysis on day 0 (baseline), day 7(week 1), day 14(week 2), day 21(week 3) and day 28(week 4) Khisa et al. (2021). The % hemolysis in RBC was determined on days 0, 7, 14, 21 and lastly on day 28 using the formulae below:

(100- HCT) x plasma Hemoglobin (g dl-1) / Total Hb (g dl-1).

3.8.8 Data Processing and Statistical Analysis

Baseline and weekly data were entered Microsoft Excel 2023. The statistical analysis was done by SPSS version 27 and R statistical models. Based on SCT status, participants were grouped into two: SCT blood donors (HbAS) and SCT negative (HbAA) blood donors for subsequent analysis. The differences between baseline and the 4 weeks' hematological parameters and biochemical data set were determined by one-way analysis of variance (ANOVA) and R statistical models. The mean of baseline and 4 weeks' data set were also compared. Data was presented in the form of tables, graphs and charts. Normality was assessed using the Shapiro-Wilk test. Between-group comparisons were performed using independent samples t-tests, while repeated measurements across storage time were analyzed using repeated-measures ANOVA followed by Tukey's post hoc test where appropriate. Associations between SCT status and demographic variables were assessed using Chi-square tests and binary logistic regression. Statistical significance was set at $p < 0.05$.

3.9 Ethics Approval and Consent to Participate

Ethical approval of this study was granted by JKUAT Institutional Scientific and Ethics Review committee (Approval number: *JKU/ISERC/02316/1091*) and data collection permit sought from NACOSTI (License No: *NACOSTI/P/23/31896*). Moreover, written informed consent was obtained from each voluntary blood donor attending Kisumu RBTC during the study period (2nd January, 2024 to 3rd April 2024). The study participants' data privacy and confidentiality were adhered to.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Sickle Cell Trait among Voluntary Blood Donors at Kisumu RBTC

Figure 4.1 presents the prevalence of sickle cell trait (SCT) among 336 eligible voluntary blood donors who were consecutively screened for hemoglobin phenotype at the Kisumu Regional Blood Transfusion Centre (RBTC) between January and April 2024**.** The sample of 336 donors constituted the study population used to estimate SCT prevalence before selecting eligible donor units for the experimental component of the study. Of the 336 donors screened, 303 (90.2%) had normal adult hemoglobin (HbAA), while 33 (9.8%) were identified as having sickle cell trait (HbAS), giving an overall SCT prevalence of 9.8% (approximately 10%) among voluntary blood donors during the study period. These findings indicate that approximately one in every ten blood donors at Kisumu RBTC carries the sickle cell trait, highlighting the likelihood of collecting HbAS donor blood units within the routine blood donation program.

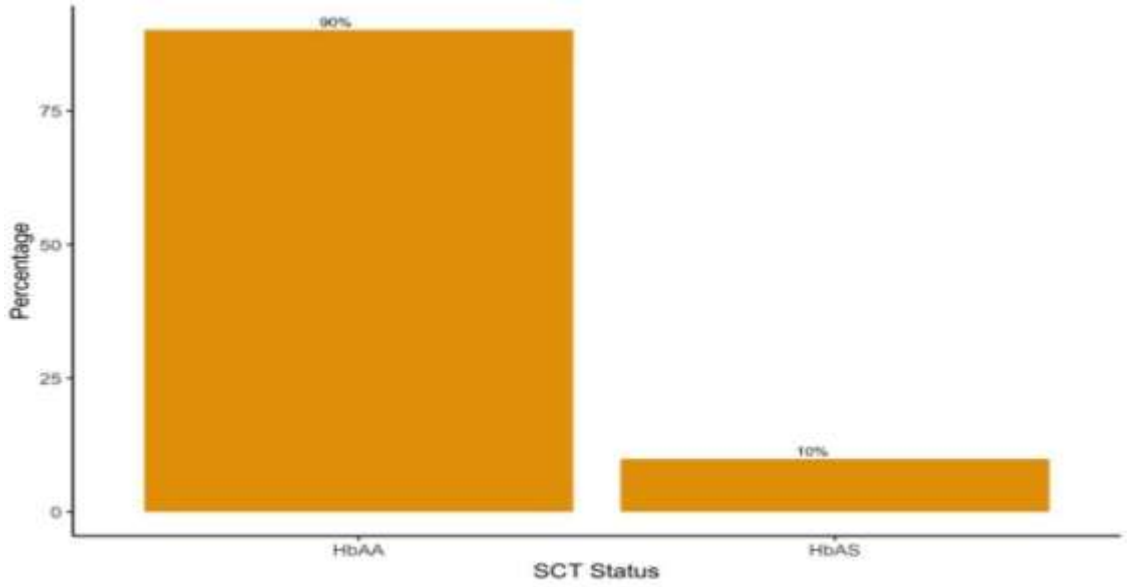

Figure 4.1: A Bar Graph Showing Prevalence of SCT among Voluntary Blood Donors

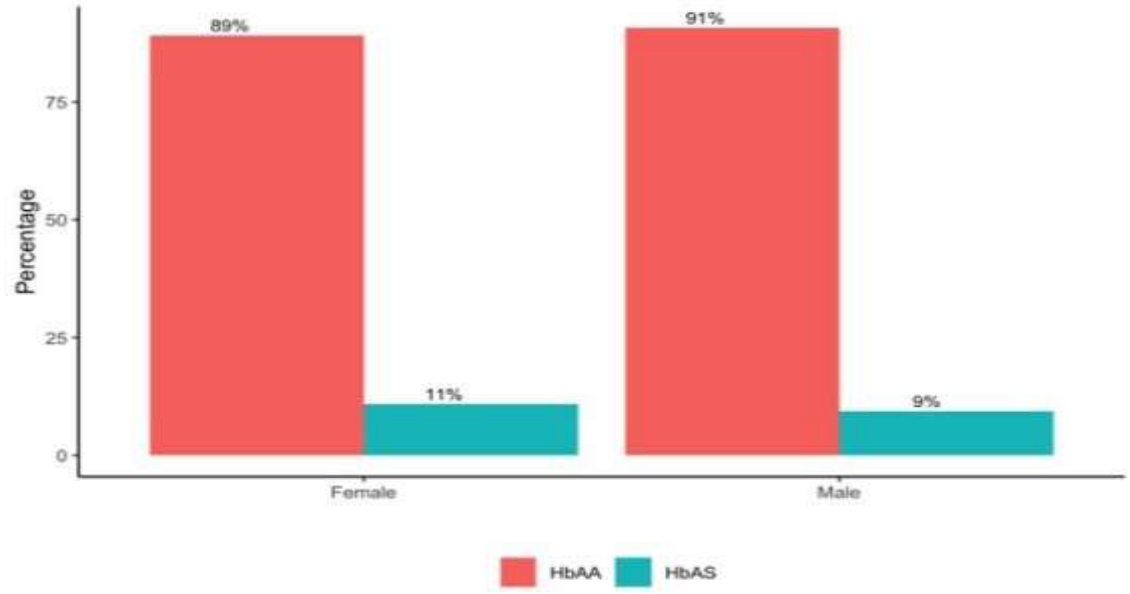

Figure 4.2: Sickle Cell Trait Status among Voluntary Blood Donors by Gender

The table 1 below presents the prevalence of sickle cell trait (SCT) among voluntary blood donors at Kisumu Regional Blood Transfusion Centre in Kenya, categorized by tribe. The table lists the tribe, SCT status (HbAA for normal hemoglobin, HbAS for sickle cell trait), total count of donors, and corresponding percentages. India: 1 donor with HbAA, representing 100% of the donors from this tribe, Kalenjin: 5 donors with HbAA, representing 100% of the donors from this tribe, Kamba: 1 donor with HbAA, representing 100% of the donors from this tribe, Kisii: 9 donors with HbAA, representing 100% of the donors from this tribe, Luhya: 43 donors with HbAA, representing 97.73% of the donors from this tribe.1 donor with HbAS, representing 2.27% of the donors from this tribe,Luo:243 donors with HbAA, representing 88.36% of the donors from this tribe. 32 donors with HbAS, representing 11.64% of the donors from this tribe and Meru:1 donor with HbAA, representing 100% of the donors from this tribe.

Table 4.1: Sickle Cell Trait Status among Voluntary Blood Donors by Tribe

Tribe	SCT_STATUS	Total count	Percentages
INDIA	HbAA	1	100
KALENJIN	HbAA	5	100
KAMBA	HbAA	1	100
KISII	HbAA	9	100
LUHYA	HbAA	43	97.73
LUHYA	HbAS	1	2.27
LUO	HbAA	243	88.36
LUO	HbAS	32	11.64
MERU	HbAA	1	100

Table 4.2 presents the distribution of sickle cell trait (SCT) among 336 voluntary blood donors according to their place of birth. Overall, most donors from all regions had normal hemoglobin (HbAA), while a smaller proportion were identified with sickle cell trait (HbAS). Kisumu County contributed the largest proportion of donors (250/336, 74.4%), of whom 223 (89.2%) had HbAA and 27 (10.8%) had HbAS, accounting for most SCT-

positive cases identified in the study. Siaya County recorded the second-highest proportion of SCT-positive donors, with 4 of 31 donors (12.9%) exhibiting HbAS, while Homabay had 1 of 8 donors (12.5%) with HbAS. Vihiga County reported 1 of 23 donors (4.4%) with HbAS. In contrast, all donors from Bungoma, Kakamega, Kericho, Kisii, Kitui, Migori, Nairobi, Nandi, Nyeri, and Trans Nzoia had normal hemoglobin (HbAA), with no SCT-positive cases detected. However, the absence of HbAS in these counties should be interpreted cautiously because the number of donors from most of these regions was very small. These findings suggest that SCT among voluntary blood donors was predominantly observed among individuals born in the western Kenya counties of Kisumu, Siaya, Homabay, and Vihiga, reflecting the known geographical distribution of the sickle cell gene in malaria-endemic regions.

Table 4.2: Sickle Cell Trait Status among Voluntary Blood Donors by Place of Birth

Place_of_Birth	SCT_STATUS	Total count	Percentages
BUNGOMA	HbAA	3	100
HOMABAY	HbAA	7	87.5
HOMABAY	HbAS	1	12.5
KAKAMEGA	HbAA	2	100
KERICHO	HbAA	1	100
KISII	HbAA	3	100
KISUMU	HbAA	223	89.2
KISUMU	HbAS	27	10.8
KITUI	HbAA	1	100
MIGORI	HbAA	7	100
NAIROBI	HbAA	2	100
NANDI	HbAA	3	100
NYERI	HbAA	1	100
SIAYA	HbAA	27	87.1
SIAYA	HbAS	4	12.9
TRANZOIA	HbAA	1	100
VIHIGA	HbAA	22	95.65
VIHIGA	HbAS	1	4.35

4.2 Storage-Related Haematological and Biochemical Changes

4.2.1 Storage-Related Hematological Changes in SCT Positive and SCT Negative Donor Blood

The table 3 below shows Sickle cell trait negative (SCT negative) and sickle cell trait positive (HbAS) donor blood storage-related haematological changes over a period of four weeks. For the SCT negative donor blood shows a gradual increase in Hb levels from 15.13 on day 0 to 15.67 by Week 4, the percentage of hemolysis starts at 0.00% on day 0 and gradually increases to 0.54% by day 28 which indicates a steady, albeit minimal, increase in cell breakdown over the storage period. HCT values showed a consistent increase from 37.94% on day 0 to 41.82% by day 28 that suggested a concentration effect likely due to the reduction in plasma volume over storage time. MCHC decreased from 30.9 g/dL on Day 0 to 27.5 g/dL by Day 28. This decline indicates a decrease in hemoglobin concentration within the red blood cells as storage time increases. MCV values increased from 76.96 fL on day 0 to 88.06 fL on day 28, indicating that the average volume of RBCs increased over the storage period. Moreover, PCT showed a decline from 0.24% on day 0 to 0.11% by day 28, reflecting a decrease in the overall platelet volume in the stored donor blood. Platelet count dropped significantly from 223.45 x 10^9/L on Day 0 to 127.13 x 10^9/L by day 28. This substantial reduction points to platelet degradation or aggregation over time. RBC count decreases from 5.03 x 10^12/L on Day 0 to 4.23 x 10^12/L by Day 28, indicating a reduction in the number of red blood cells during storage. TWBC count also decreases markedly from 5.64 x 10^9/L on Day 0 to 2.36 x 10^9/L by Day 28, suggesting a significant reduction in white blood cells over the storage period.

In HbAS donor blood, the percentage of hemolysis starts at 0.00% on day 0 and increases to 0.78% by Day 28. The rate of hemolysis was higher in SCT positive compared to SCT negative donor blood, indicating greater cell breakdown in HbAS donor blood. HCT values rose from 40.98% on day 0 to 45.07% by day 28, similar to the trend observed in

SCT negative blood but starting from a higher baseline average value. MCHC decreased from 33.68 g/dL on day 0 to 30.6 g/dL by day 28, mirroring the trend in SCT negative blood but at a consistently higher level. MCV increased from 79.54 fL on day 0 to 87.78 fL by day 28, similar to the pattern seen in SCT negative blood. Moreover, PCT declined from 0.21% on day 0 to 0.09% by day 28, indicating a significant reduction in platelet volume, more pronounced than in SCT negative blood. PLT count dropped from 223.65 x 10^9/L on day 0 to 99.07 x 10^9/L by day 28, a sharper decline compared to SCT negative donor blood, suggesting greater platelet degradation. RBC count decreases from 4.98 x 10^12/L on Day 0 to 3.78 x 10^12/L by Day 28, a more pronounced reduction compared to SCT negative blood. TWBC count declines from 5.99 x 10^9/L on Day 0 to 3.36 x 10^9/L by Day 28, indicating a significant reduction, though less steep compared to SCT negative blood.

Table 4.3: Average Hematological Storage- Related Changes Values at Different Points in Time

SCT STATUS Variables	Day 0	Day 7 (Week 1)	Day 14 (Week 2)	Day 21 (Week 3)	Day 28 (Week 4)
Sickle Cell trait negative					
% Haemolysis	0.00	0.16	0.35	0.44	0.54
HCT	37.94	40.07	41.02	41.26	41.82
MCHC	30.9	29.49	28.26	28.04	27.5
MCV	76.96	83.95	86.07	86.68	88.06
PCT	0.24	0.19	0.16	0.14	0.11
PLT	223.45	198.34	168.43	153.5	127.13
RBC	5.03	4.53	4.32	4.28	4.23
TWBC	5.64	3.94	2.99	2.58	2.36
Hb	15.13	15.24	15.35	15.49	15.67
Sickle cell trait positive (HbAS) donor blood					
% Haemolysis	0.00	0.44	0.58	0.67	0.78
HCT	40.98	43.18	44.17	44.48	45.07
MCHC	33.68	32.36	31.18	31.02	30.6
MCV	79.54	84.59	86.13	86.53	87.78
PCT	0.21	0.17	0.14	0.11	0.09
PLT	223.65	193.43	158.73	138.9	99.07
RBC	4.98	4.28	3.78	3.77	3.78
TWBC	5.99	4.74	4.07	3.88	3.36

SCT STATUS	Variables	Day 0	Day 7 (Week 1)	Day 14 (Week 2)	Day 21 (Week 3)	Day 28 (Week 4)
	Hb	13.78	14.03	14.32	14.71	15.12

Abbreviations: Hb=Haemoglobin, RBC=Red blood cells, TWBC=Total white blood cells, HCT=Hematocrit, MCHC=Mean cell haemoglobin concentration, MCV=Mean cell volume, PCT=Plateletcrit, PLT=Platelets.

From table 4 below, average weekly values (week 1-week 4) were calculated as baseline Percentage Differences for both donor groups. For the Sickle Cell Trait negative (HbAA) donor blood (Table 4 below), There was a gradual increase in hematocrit levels over time, with percentage differences of 6%, 8%, 9%, and 10% on days 7, 14, 21, and 28, respectively with a consistent rise in the proportion of Red blood cells in the SCT negative donor blood (*Figure 4.6*). The MCHC showed a decreasing trend, with percentage differences of -5%, -9%, -9%, and -11% over the SCT negative donor blood storage period (*Figure 4.4*). This indicates a reduction in the concentration of hemoglobin in the red blood cells with the MCV increasing by 9%, 12%, 13%, and 14% on days 7, 14, 21, and 28, respectively (*Figure 4.7*). This suggests an increase in the average size of the red blood cells over time. There was a significant decline in PCT, with percentage differences of -21%, -33%, -42%, and -54% over the storage period indicating a marked reduction in the volume percentage of platelets in the blood (*Figure 4.8*). Moreover, the platelet counts also decreased, with percentage differences of -11%, -25%, -31%, and -43% over the 28 days showing a substantial reduction in the number of platelets over storage period (*Figure 4.3*). In addition, the RBC count decreased by -10%, -14%, -15%, and -16% over the storage period, indicating a consistent decline in the number of Red blood cells. There was a significant reduction in the TWBC count, with percentage differences of -30%, -47%, -54%, and -58% over the 28 days indicating a notable decrease in the number of TWBC (*Figure 4.5*).

For the Sickle Cell Trait positive (HbAS) donor blood (*Table 4 below*)**,** The HCT levels increased by 5%, 8%, 9%, and 10% on days 7, 14, 21, and 28, respectively with a gradual rise in the proportion of Red blood cells in the stored SCT positive donor blood over time (*Figure 4.6*). The MCHC showed a decreasing trend, with percentage differences of -4%,

-7%, -8%, and -9% over the SCT positive donor blood storage period with the MCV increasing by 6%, 8%, 9%, and 10% on days 7, 14, 21, and 28, respectively (*Figure 4.4 & 4.7*), suggesting an increase in the average size of the red blood cells over time. There was a significant decline in PCT, with percentage differences of -19%, -33%, -48%, and -57% over the storage period which indicated a marked reduction in the volume percentage of platelets in the SCT positive donor blood (*Figure 4.8*) Moreover, the platelet count decreased, with percentage differences of -14%, -29%, -38%, and -56% over the 28 days and showed a substantial reduction in the number of platelets (*Figure 4.3*).

The RBC count decreased by -14%, -24%, -24%, and -24% respectively over the storage period, indicating a consistent decline in the number of RBCs. There was a significant reduction in the TWBC count, with percentage differences of -21%, -32%, -35%, and -44% respectively over the 28 days implying a notable decrease in the number of TWBC (*Figure 4.5*). Sickle cell trait positive donor blood exhibits an increase in Hb levels, starting at 13.78 on day 0 and reaching 15.12 by Week 4(*Figure 4.10*).

Table 4.4: Average Weekly Values (Week 1-Week 4) were Calculated as Baseline Percentage Differences

Variables	Day7_diff_perc	Day14_diff_perc	Day21_diff_perc	Day28_diff_%
Sickle cell trait negative (HbAA) donor blood				
% Hemolysis	Inf	Inf	Inf	Inf
HCT	6	8	9	10
MCHC	-5	-9	-9	-11
MCV	9	12	13	14
PCT	-21	-33	-42	-54
PLT	-11	-25	-31	-43
RBC	-10	-14	-15	-16
TWBC	-30	-47	-54	-58
Hb	0.73	1.45	2.38	3.57
Sickle cell trait positive (HbAS) donor blood				
%Haemolysis	Inf	Inf	Inf	Inf
HCT	5	8	9	10
MCHC	-4	-7	-8	-9
MCV	6	8	9	10
PCT	-19	-33	-48	-57

Variables	Day7_diff_perc	Day14_diff_perc	Day21_diff_perc	Day28_diff_%
PLT	-14	-29	-38	-56
RBC	-14	-24	-24	-24
TWBC	-21	-32	-35	-44
Hb	1.81	3.92	6.75	9.72

Abbreviations: Hb=Haemoglobin, RBC=Red blood cells, TWBC=Total white blood cells, HCT=Hematocrit, MCHC=Mean cell haemoglobin concentration, MCV=Mean cell volume, PCT=Plateletcrit, PLT=Platelets

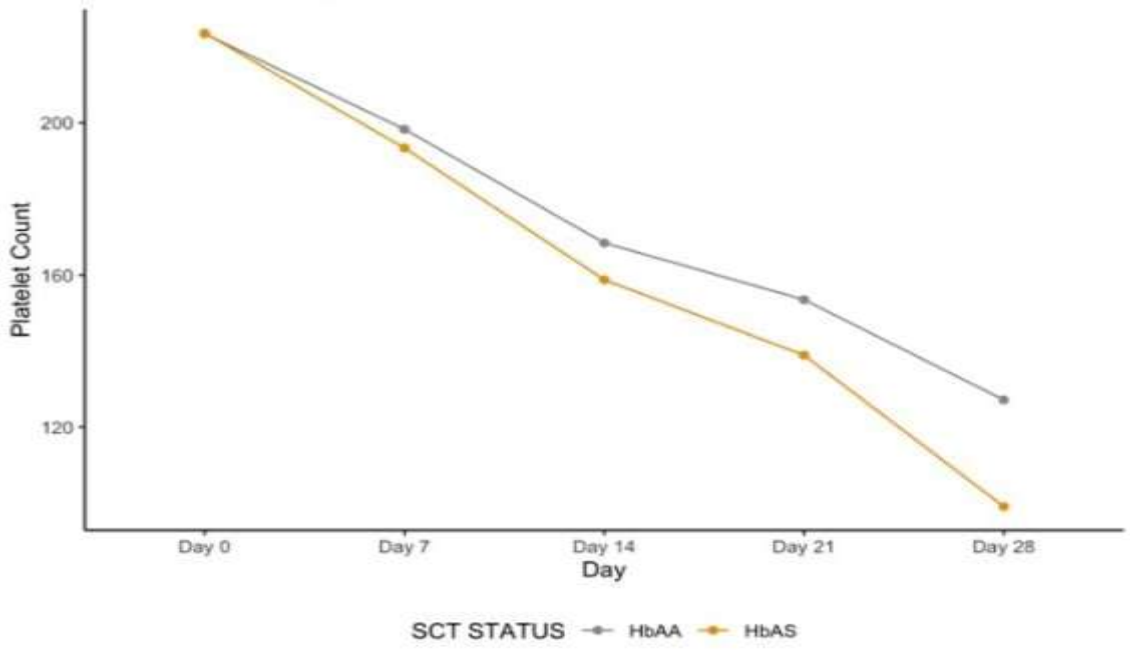

Figure 4.3: Changes in Average Platelet Count Over Time for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

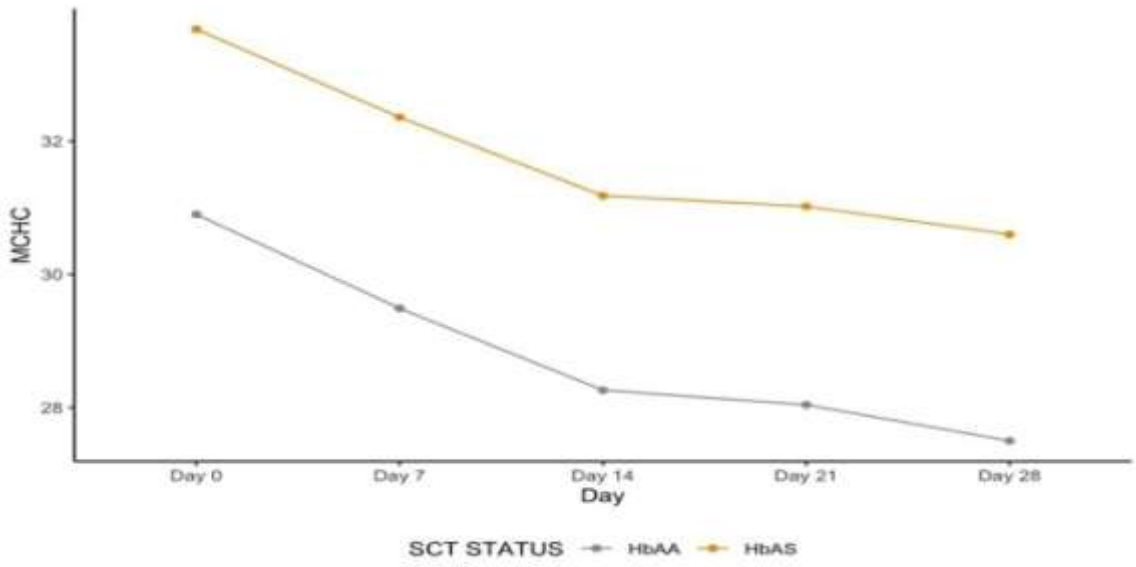

Figure 4.4: Changes in Average MCHC (g/dl) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

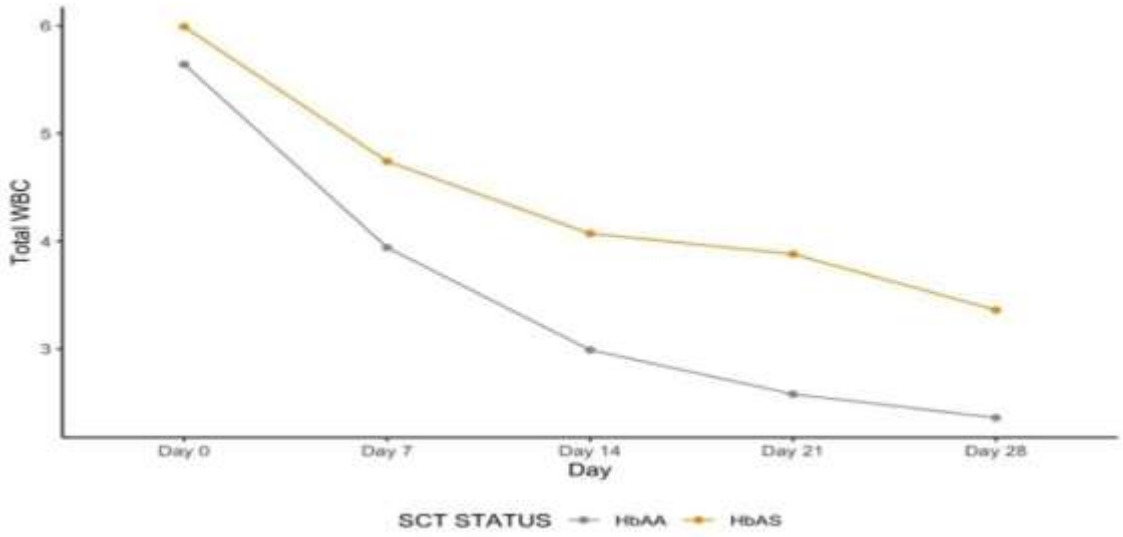

Figure 4.5: Changes in Average Total White Blood Cells (x 109/L) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

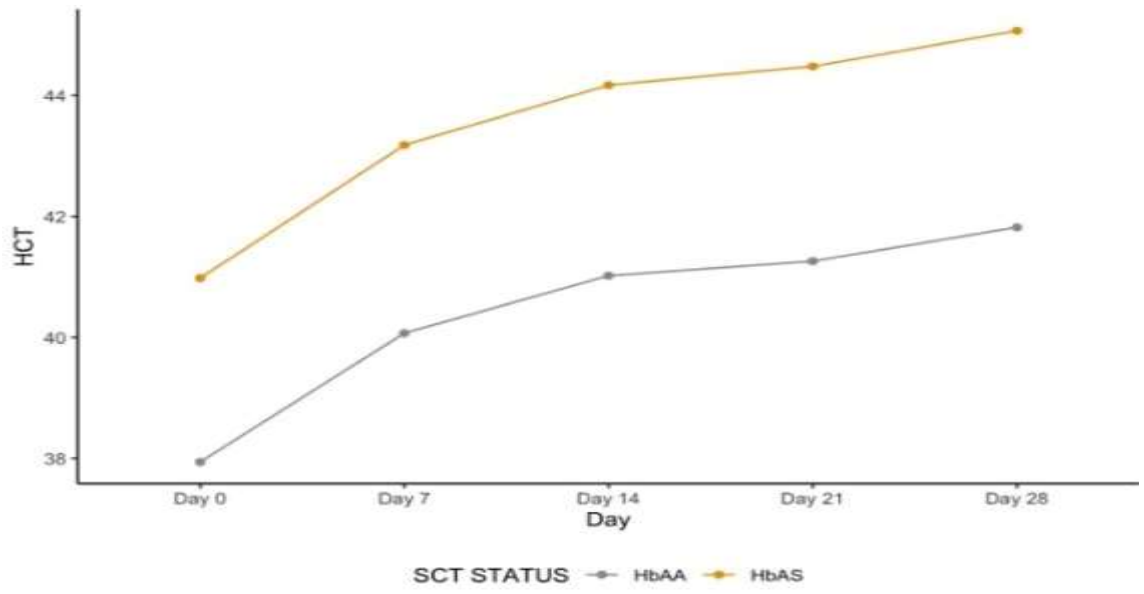

Figure 4.6: Changes in Average Hematocrit (%) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

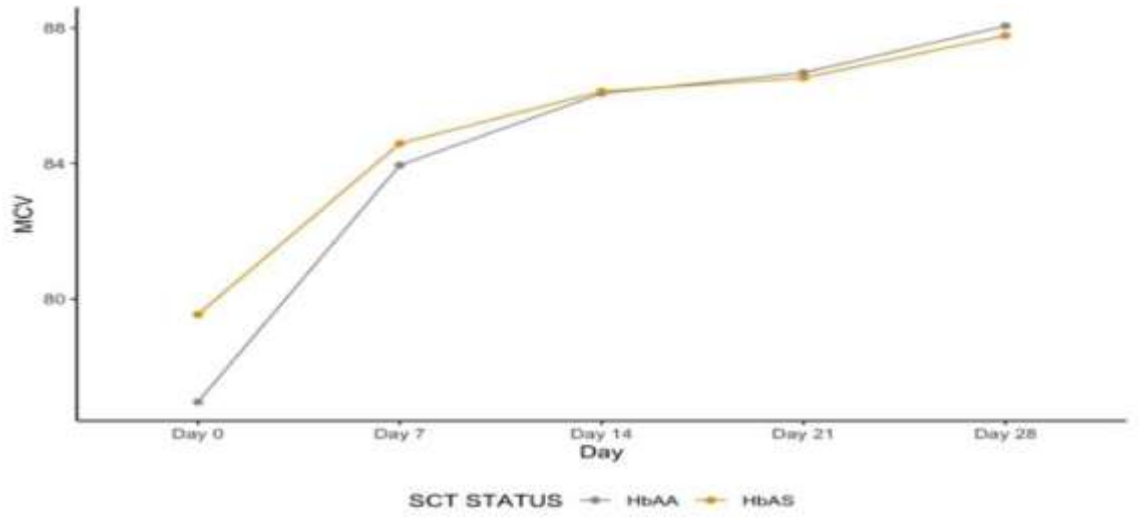

Figure 4.7: Changes in Average Mean Cell Volume (fL) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

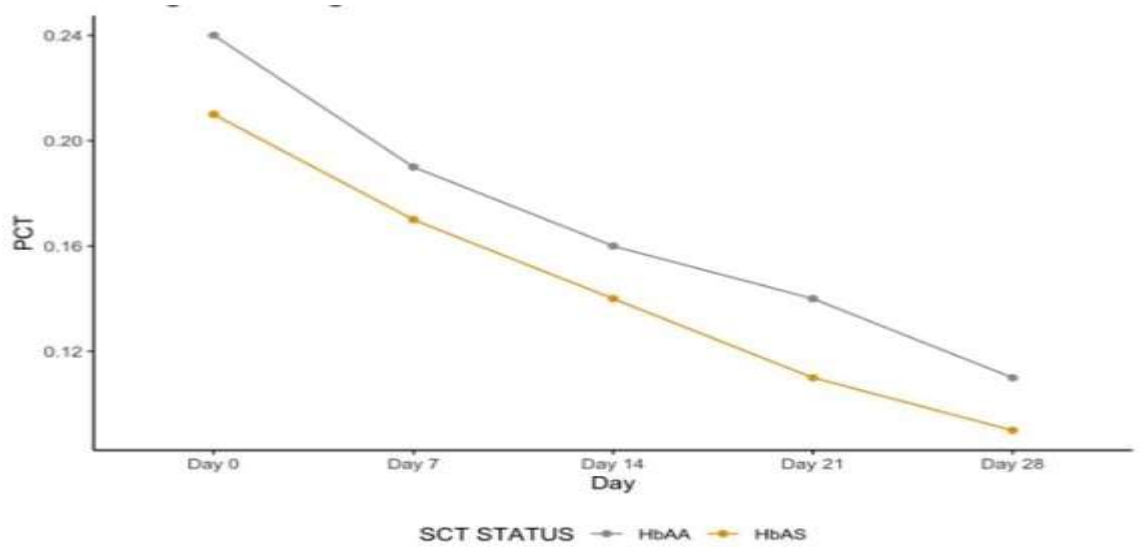

Figure 4.8: Changes in Average Plateletcrit (%) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

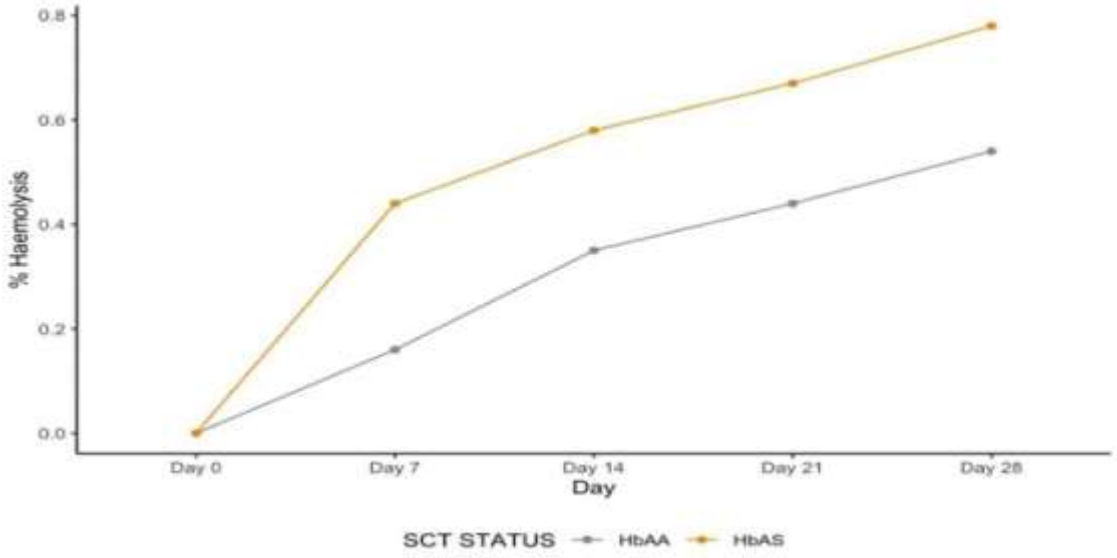

Figure 4.9: Changes in Average Percentage Haemolysis (%) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

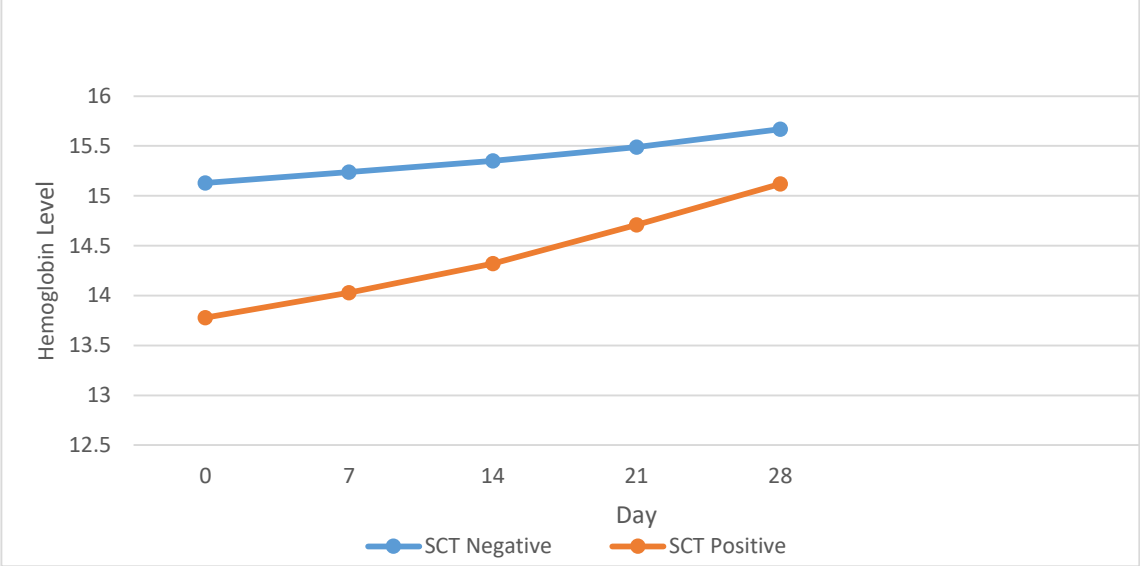

Figure 4.10: Changes in Average Haemoglobin Level Over Time for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

4.2.2 Storage-Related Biochemical Changes in SCT Positive and SCT Negative Blood Donor Units

Storage-related changes in average weekly LDH levels were observed in the two donor groups (*Table 5 below*). Weekly LDH levels increased consecutively and steadily in both donor groups (sickle cell trait positive and sickle cell trait negative) (*Table 5 &Figure 4.11*). The rates of increase were slightly higher in the sickle cell trait positive group, with the most significant increase of 202% occurring in week 4 compared to baseline, as depicted in Table 4. Although baseline plasma potassium levels were comparable between the groups, the potassium levels increased steadily from day 0 to week 3, followed by a sharp increase from week 3 to week 4 in both donor groups. Furthermore, average weekly plasm potassium levels were consistently higher in the SCT positive blood donor group compared to the SCT negative blood donor group (Table 5 & figure 4.15). The rates of decrease in plasma sodium levels were comparable in both the sickle cell trait positive and sickle cell trait negative blood donor groups (*Table 5 & Figure 4.14*).

The rates of increase in plasma ferritin levels remained consistent in both groups throughout the storage period (day 0 to day 28). However, the average weekly increase rates of plasma ferritin levels were slightly higher in the SCT-negative blood donor group compared to the SCT-positive blood donor group (*Table 5& Figure 4.13*). Although the baseline plasma glucose levels were similar in both groups, the levels increased from day 0 to day 7 (week 1) and then steadily declined from week 1 to week 4 in both blood donor groups (*Table 5 & figure 4.12*). The baseline levels and rates of increase in plasma bilirubin levels were comparable between the groups during the storage period (day 0 to day 28), but the average weekly increase rates of plasma bilirubin levels were slightly higher in the SCT-positive blood donor group compared to the SCT-negative blood donor group (*Table 5*).

Table 4.5: Analysis of Biochemical Parameters in the Blood Donor Groups Stored Over Time

Variables	Day 0	Day 7	Day 14	Day 21	Day 28
Sickle cell negative (HbAA) donor blood					
Ferritin Level(ng/ml)	65.34	70.55	76.57	83.71	91.81
Glucose Level	5.95	11.79	8.89	7.45	4.86
LDH(U/L)	196.09	247.36	309.45	403.76	530.69
Potassium level	4.53	6.04	8.11	10.42	14.92
Sodium level	137.16	133.74	130.23	129.21	126.82
Bilirubin level(ng/dl)	4.9	6.1	7.8	9.72	13.48
Sickle cell trait positive (HbAS) donor blood					
Ferritin level(ng/ml)	92.16	96.43	101.51	107.57	113.19
Glucose Level	6.98	14.21	10.18	6.69	4.26
LDH (U/L)	210.87	267.88	339.35	449.75	635.8
Potassium level	4.98	7.66	10.11	12.52	19.28
Sodium level	138.03	135.22	132.12	129.25	127.09
Bilirubin level (ng/dl)	6.78	8.35	10.86	14.07	19.98

Table 6 below presents an analysis of average weekly storage-related biochemical changes expressed as baseline percentage differences for two groups: sickle cell trait negative (HbAA) donor blood and sickle cell trait positive (HbAS) donor blood. For Sickle cell negative (HbAA) donor blood: plasma ferritin levels increased steadily over time, with an

8% rise by day 7, 17% by day 14, 28% by day 21, and 41% by day 28(Figure 4.13). Glucose levels decreased from 98% on day 7 to 49% at day 14, 25% on day 21, and showed a significant drop to -18% by day 28(Figure 4.12). Plasma LDH levels rose consistently, showing a 26% increase by day 7, 58% by day 14, 106% by day 21, and reaching 171% on day 28(Figure 4.11). Plasma potassium levels increased significantly, starting at 33% on day 7, then 79% on day 14, 130% on day 21, and a dramatic rise to 229% by day 28(Figure 4.15). Sodium levels decreased slightly, with a -2% change by day 7, -5% by day 14, -6% by day 21, and -8% by day 28(Figure 4.14). Bilirubin levels rose considerably, showing a 24% increase by day 7, 59% by day 14, 98% on day 21, and 173% by day 28.

For Sickle cell trait positive (HbAS) donor blood: plasma ferritin levels increased over time, with a 5% rise by day 7, 10% by day 14, 17% by day 21, and 23% by day 28(Figure 4.13). Glucose levels increased to 104% by day 7, decreased to 46% by day 14, then dropped to -4% by day 21 and further to -39% by day 28(Figure 4.12). LDH levels rose steadily, showing a 27% increase by day 7, 61% by day 14, 113% by day 21, and reaching 202% by day 28(Figure 4.11). Plasma potassium levels increased significantly, starting at 54% on day 7, 103% by day 14, 151% on day 21, and a dramatic rise to 287% by day 28(Figure 4.15). Plasma sodium levels decreased slightly, with a -2% change by day 7, -4% by day 14, -6% by day 21, and -8% by day 28(Figure 4.14). Plasma bilirubin level rose considerably, showing a 23% increase by day 7, 60% by day 14, 108% by day 21, and 195% by day 28.

Table 4.6: Analysis of Biochemical Changes Over Time (Day 0-Day 28) Expressed as Baseline Percentage Differences

Variables	Day7_diff_%	Day14_diff_%	Day21_diff_%	Day28_diff_%
Sickle cell negative (HbAA) donor blood				
Ferritin level(ng/ml)	8	17	28	41
Glucose Level(mmol/l)	98	49	25	-18
LDH (U/L)	26	58	106	171
Potassium level	33	79	130	229
Sodium level (mmol/l)	-2	-5	-6	-8
Bilirubin level(ng/dl)	24	59	98	173
Sickle cell trait positive (HbAS) donor blood				
Ferritin level(ng/ml)	5	10	17	23
Glucose Level	104	46	-4	-39
LDH (U/L)	27	61	113	202
Potassium level	54	103	151	287
Sodium-mmol/L	-2	-4	-6	-8
Bilirubin -ng/dl	23	60	108	195

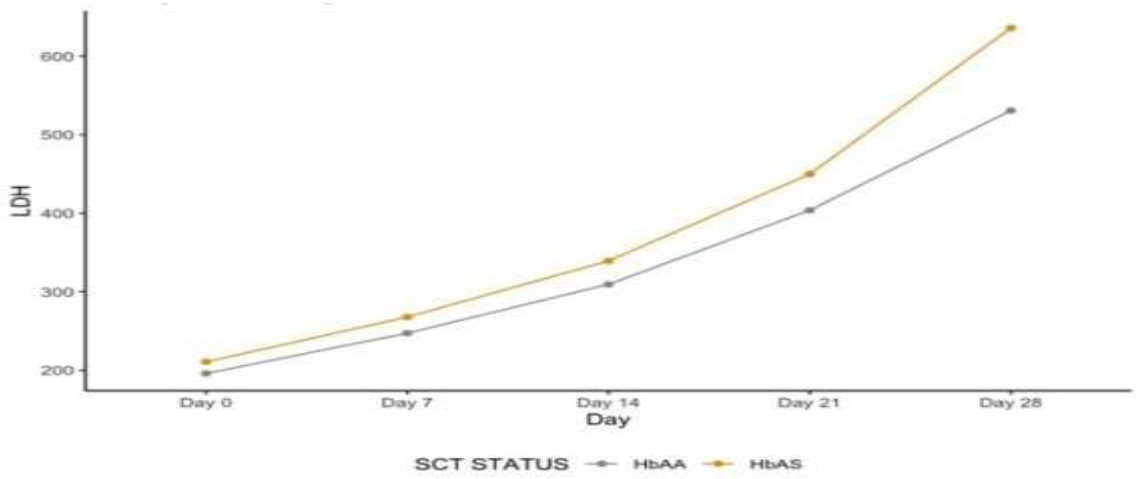

Figure 4.11: Changes in Average Lactate Dehydrogenase (U/L) Over Time (Day 0-day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

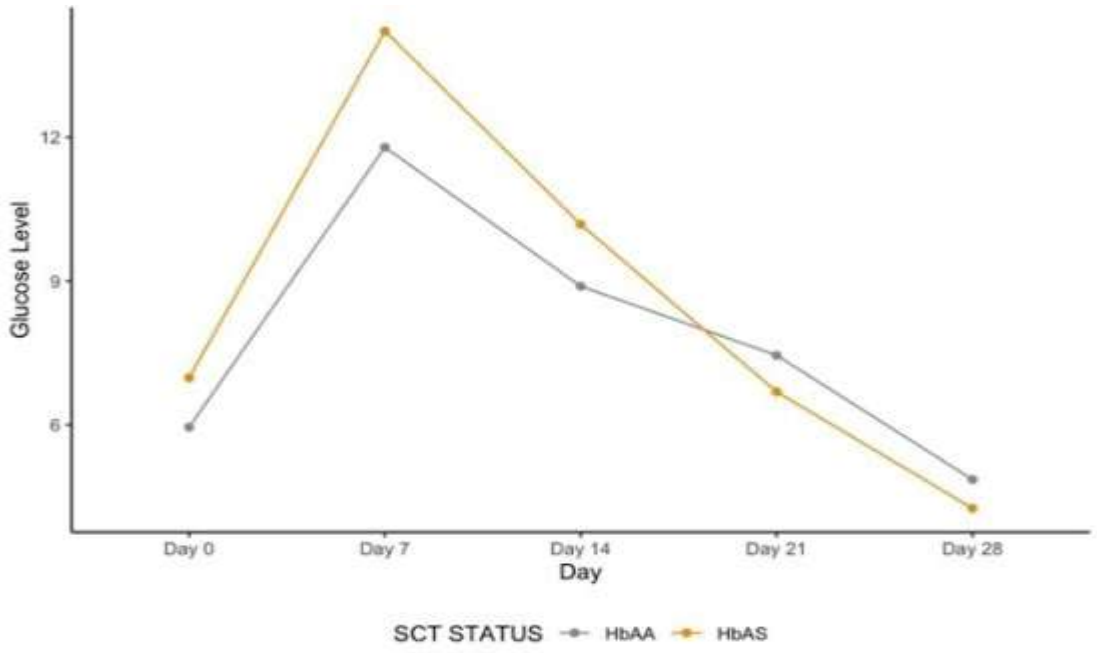

Figure 4.12: Changes in Average Glucose Level (mmol/L) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

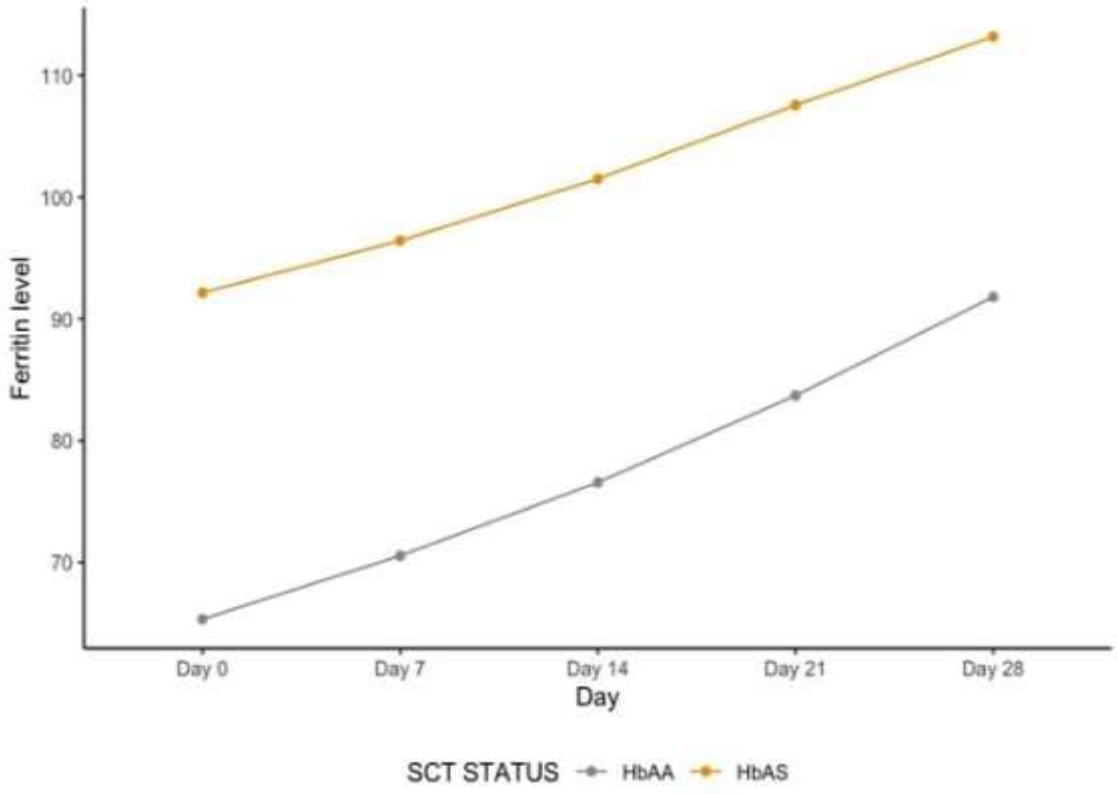

Figure 4.13: Changes in Average Ferritin Level (ng/ml) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

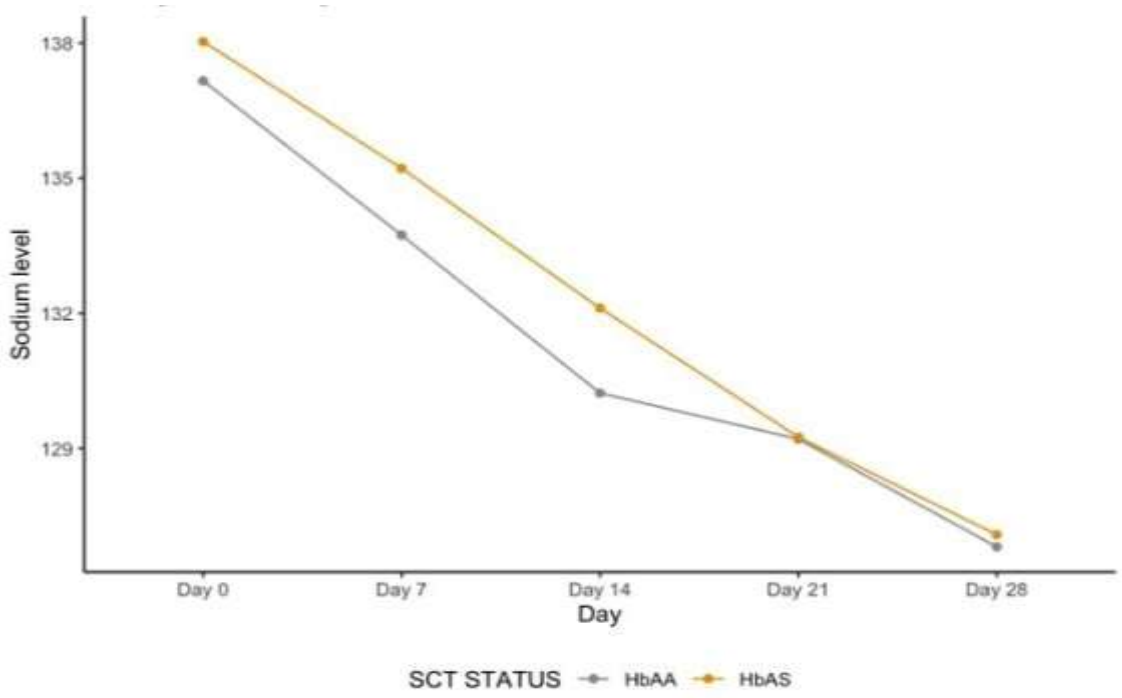

Figure 4.14: Changes in Average Sodium Level (mmol/L) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

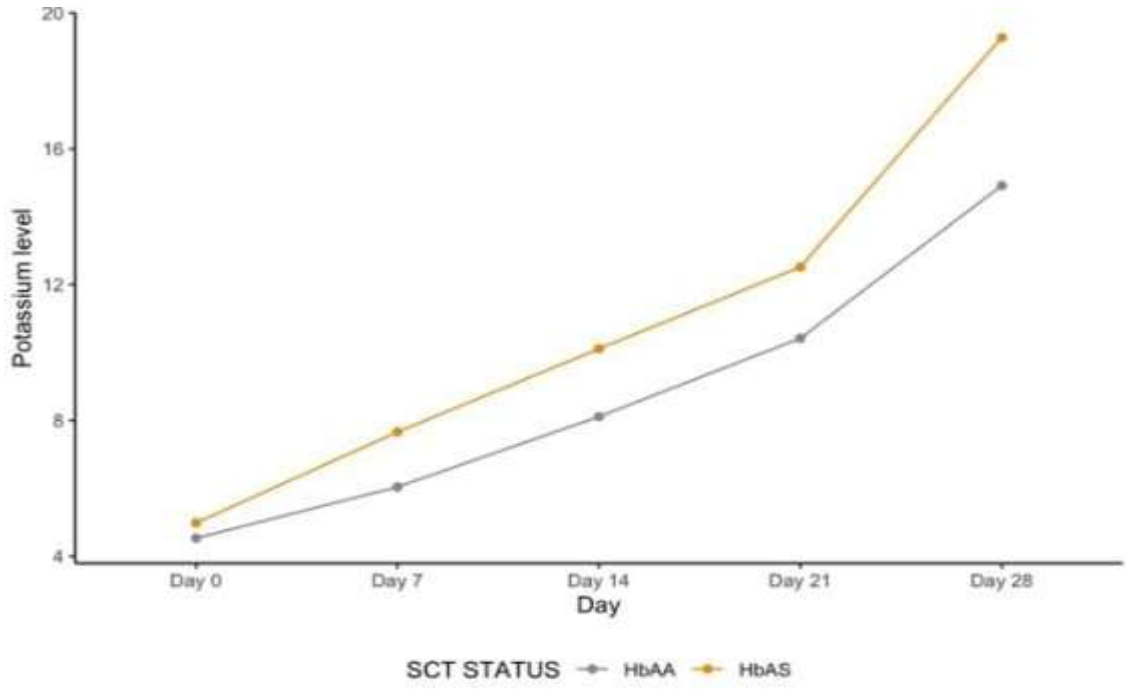

Figure 4.15: Changes in Average Potassium Level (mmol/L) Over Time (Day 0-Day 28) for Both Sickle Cell Trait Donor (HbAS) Blood and Sickle Cell Trait Negative (Hb AA) Donor Blood

CHAPTER FIVE

DISCUSSION

5.1 Prevalence and Factors Influencing SCT among Blood Donors at Kisumu RBTC

The present study established that the prevalence of sickle cell trait (SCT) among voluntary blood donors at Kisumu Regional Blood Transfusion Centre (RBTC) was 10%, with 33 of the 336 screened donors exhibiting the HbAS phenotype, while 303 (90%) had normal adult hemoglobin (HbAA). This prevalence is comparable to previous reports from western Kenya and other malaria-endemic regions of sub-Saharan Africa, where SCT prevalence ranges between 5% and 20% due to the selective survival advantage conferred by the sickle cell gene against severe malaria (Piel et al., 2017; Nnodu et al., 2021; Kato et al.,2018). The observed prevalence also concurs with studies conducted among Kenyan blood donors and the general population, indicating that a substantial proportion of donated blood units may originate from donors with SCT (Khisa et al., 2021; Mutua et al.,2022). These findings underscore the importance of considering SCT status in blood donor screening, particularly because HbAS red blood cells may exhibit altered storage characteristics that could influence transfusion quality.

5.1.1 Gender and SCT Prevalence

The study found no statistically significant association between gender and the prevalence of SCT among voluntary blood donors, with both male and female donors demonstrating comparable proportions of the HbAS phenotype. This finding is expected because sickle cell trait is an autosomal recessive genetic condition inherited independently of sex chromosomes, resulting in an equal probability of inheritance among males and females (Piel et al., 2017; Rees et al., 2010). Similar observations have been reported in studies conducted in Nigeria, Tanzania, and Ghana, where SCT prevalence did not differ significantly by sex among blood donors or community populations (Jeremiah et al., 2010;

Nnodu et al., 2021). Nevertheless, although males generally constitute a larger proportion of blood donors because women are more frequently deferred due to lower hemoglobin levels, pregnancy, menstruation, and iron deficiency, these factors influence donor eligibility rather than the genetic occurrence of SCT (World Health Organization [WHO], 2021).

5.1.2 Tribal Distribution

The distribution of SCT varied across ethnic groups, with the Luo tribe recording the highest prevalence (11.64%)**,** followed by the Luhya tribe (2.27%)**,** while no SCT-positive cases were identified among donors from the Kalenjin, Kamba, Kisii, Meru, and other represented ethnic groups. This pattern is consistent with the established epidemiology of sickle cell disorders in Kenya, where communities residing around the Lake Victoria basin, particularly the Luo and parts of the Luhya population, have historically exhibited higher frequencies of the sickle cell gene because of prolonged exposure to malaria, which has maintained the HbS allele through balanced natural selection (Piel et al., 2017 ; Piel et al., 2017). However, the absence of SCT among some ethnic groups in the present study should be interpreted cautiously because the number of donors from these communities was relatively small, limiting meaningful comparisons. These findings highlight the need for targeted public health interventions, including genetic counselling, premarital screening, and routine donor screening in high-prevalence communities, to enhance transfusion safety and reduce the transmission of HbAS donor units into the blood supply (Nnodu et al., 2021; WHO, 2021).

5.1.3 Place of Birth and SCT Prevalence

The distribution of sickle cell trait (SCT) by place of birth demonstrated marked geographical variation. Donors born in Kisumu County had an SCT prevalence of 10.8%, while those from Siaya County recorded the highest prevalence at 12.9%. Smaller proportions of SCT-positive donors were also observed among individuals born in

Homabay (12.5%) and Vihiga (4.4%), whereas no cases were identified among donors from Bungoma, Kakamega, Kericho, Kisii, Kitui, Migori, Nairobi, Nandi, Nyeri, and Trans Nzoia. The relatively higher prevalence observed in Kisumu, Siaya, and Homabay is consistent with the epidemiology of sickle cell trait in the Lake Victoria basin, where malaria has historically exerted selective pressure favoring the persistence of the HbS allele because of the partial protection it confers against severe *Plasmodium falciparum* malaria (Kato et al.,2018; Piel et al., 2017; Williams & Weatherall, 2012). Nevertheless, the absence of SCT in some counties should be interpreted with caution because relatively few donors originated from these areas, limiting the ability to draw firm conclusions regarding regional differences. These findings support previous studies demonstrating that the geographical distribution of SCT closely mirrors malaria endemicity across sub-Saharan Africa (Nnodu et al., 2021; Piel et al., 2017).

5.1.4 Previous Blood Donations and Frequency of Donation

The study showed that a substantial proportion of the voluntary blood donors were first-time donors, although a considerable number had donated blood previously. Repeat donors are essential for maintaining a safe and sustainable blood supply because they are generally more familiar with donor eligibility requirements and are less likely to present with transfusion-transmissible infections than first-time donors (Allain et al., 2010; WHO, 2021). Regular voluntary non-remunerated donors also contribute to improved blood availability and are considered the safest source of blood for transfusion services (WHO, 2021). The presence of repeat donors in this study therefore reflects positive donor retention practices at Kisumu RBTC and underscores the importance of strategies aimed at encouraging donor loyalty.

5.1.5 Effect of Donor Age

The majority of blood donors in the present study were aged 18-24 years, with a mean age of approximately 26 years. This finding is consistent with blood donation trends in Kenya

and many low- and middle-income countries, where young adults, particularly secondary school and university students, constitute the largest proportion of voluntary blood donors because of organized school-based blood donation campaigns (WHO, 2021; Ministry of Health Kenya, 2022). Younger donors are generally healthier and meet donor eligibility requirements more frequently than older adults, making them a reliable source of blood for national transfusion programs. However, reliance on student donors may contribute to seasonal shortages during school holidays, highlighting the need to diversify the donor base by recruiting more community-based voluntary donors (WHO, 2021).

5.1.6 Average Hemoglobin Levels

The mean hemoglobin concentration among voluntary blood donors was approximately 13.9 g/dL, which falls within the acceptable range for blood donation and satisfies the minimum hemoglobin threshold recommended for donor eligibility. Adequate hemoglobin levels are essential for protecting donor health and ensuring that donated blood contains sufficient red blood cell mass for therapeutic use (WHO, 2021). The observed mean hemoglobin level suggests that donor selection procedures at Kisumu RBTC were effective in identifying healthy individuals who met the national and international criteria for blood donation. Similar mean hemoglobin concentrations have been reported among healthy blood donor populations in other African countries, reflecting appropriate donor screening practices before blood collection (Jeremiah et al., 2010; Nnodu et al., 2021).

5.1.7 Implications for Blood Transfusion Services

The presence of sickle cell trait among voluntary blood donors poses challenges and considerations for blood transfusion services. While individuals with SCT can generally donate blood, the potential for SCT blood to contribute to complications in recipients, particularly those with sickle cell disease (SCD), must be carefully managed (Wiencek et al., 2017). This study underscores the need for robust screening processes and the

establishment of guidelines to ensure the safety and efficacy of blood transfusions, particularly in regions with a high prevalence of SCT.

5.2 Storage-Related Haematological and Biochemical Changes

Long term storage and preservation of Red blood cells are essential for continuous supply of safe blood and blood products for clinical use (Aninagyei et al., 2018). Storage-related changes in donor blood are crucial considerations for maintaining transfusion efficacy and recipient safety. This study investigated hematological and biochemical alterations in SCT positive (HbAS) and SCT negative (HbAA) donor blood over a 28-day storage period.

5.2.1 Storage-Related Haematological Changes

The findings reveal distinct patterns between the two groups, highlighting differential susceptibility to storage-induced changes (Yurkovich et al., 2017). Previous research has demonstrated a detrimental impact of storage on the morphology and functions of blood cells (Kotla et al., 2022 & Lin et al., 2019). This study found similar trends of storage changes but they were more pronounced in sickle cell trait positive donor blood. For SCT negative donor blood, percentage differences in hemoglobin (Hb) levels were 0.73% on day 7, 1.45% on day 14, 2.38% at day 21, and 3.57% at day 28. Percentage differences in hemoglobin (Hb) levels were higher in SCT positive donor blood group compared to SCT negative, with 1.81% at day 7, 3.92% at day 14, 6.75% on day 21, and 9.72% on day 28. This indicates a greater rate of change in Hb levels for HbAS donor blood group compared to HbAA over the same storage period. The data indicates that hemoglobin (Hb) levels in sickle cell trait positive (HbAS) donor blood exhibit a greater percentage increase over time compared to sickle cell trait negative (HbAA) donor blood. By day 28, HbAS shows a 9.72% increase, significantly higher than the 3.57% increase in HbAA. This suggests that HbAS donor blood undergoes more pronounced changes in Hb levels during storage period. These findings are crucial for donor blood storage protocols, as they highlight the

need to consider the genetic background of voluntary blood donors in maintaining blood quality and effectiveness for blood and blood products transfusions.

Hematological changes**:** Hematocrit (HCT) and mean cell volume (MCV) increased in both SCT positive and negative blood, indicative of dehydration and swelling of RBCs, respectively. However, SCT positive donor blood exhibited higher baseline HCT values and more pronounced in HbAS suggesting inherent differences in erythrocyte stability (Verma et al., 2022). HCT increase depicts RBC swelling & morphological changes that kick off during blood storage (Wither et al., 2016). This aligns with Verma et al. (2022), who noted the unique characteristics of SCT erythrocytes. The alterations in HCT can be explained by the fact that the increase in hematocrit depicts the morphological alterations that kick off during blood storage, Bosman et al. (2012). The increased morphological alterations lower the potency of transfused blood through elevated rates of elimination of transfused blood cells through macrophages Oyet al. (2018). The SCT negative Groups HCT ANOVA: Significant differences were observed ($P < 0.05$) with Tukey HSD: Significant increases at all-time points compared to Day 0, with the largest increase at Day 28. The SCT positive group's HCT ANOVA: Significant differences were observed ($p < 0.05$) with Tukey HSD: Significant increases at all-time points compared to Day 0, with the largest increase on Day 28.

The decline in mean cell hemoglobin concentration (MCHC) was steeper in SCT positive donor blood, reflecting greater Hemolysis/cellular degradation resulting in loss of membrane integrity, increased osmotic fragility, and leakage of hemoglobin. HbAS are more prone to oxidative stress and hemolytic processes. For HbAA; ANOVA: Significant differences were observed ($p < 0.05$) with Tukey HSD: Significant reductions at all-time points compared to Day 0, with the most notable reduction at Day 28. With, HbAS Group; ANOVA: Significant differences were observed ($p < 0.05$) with Tukey HSD: Significant reductions at all-time points compared to Day 0, with the greatest drop at Day 28. Hemoglobin (Hb) levels increase over time in both sickle cell trait negative and positive donor blood during storage. Initially, sickle cell trait negative blood has higher Hb levels

compared to HbAS blood. However, by Week 4, the increase in Hb levels in HbAS blood (from 13.78 to 15.12) is more pronounced than in sickle cell trait negative blood (from 15.13 to 15.67). Both blood donor groups experienced storage-related hematological changes, HbAS donor blood undergoes a more significant rise in Hb concentration over the four-week period. The literature by Aninagyei et al. (2018) & Klein et al. (2008) supports the observation of storage-induced changes in RBC indices, further emphasizing the vulnerability of SCT positive donor blood RBCs. The RBCs degradation for the HbAA Group; ANOVA: Significant differences were observed ($p < 0.05$) with Tukey HSD: Significant reductions at all-time points compared to Day 0, with the largest decrease at Day 28. The RBCs from HbAS donor group; ANOVA: Significant differences were observed ($p < 0.05$) with Tukey HSD: Significant reductions at all-time points compared to Day 0, with the largest decrease on Day 28.

The haematological alterations in MCV and MCHC seem to be the result of a deregulated mechanism pathway of cell volume, which expounds the volume of Red blood cells.
These haematological alterations were accompanied by slow elevations of potassium ions and lactate dehydrogenase during storage, as well as gradual increases in plasma hemoglobin because of enhanced red cell breakdown. Plasma haemoglobin, a potent NO scavenger, can decrease NO bioavailability, organ perfusion, injury, and mortality in patients with sepsis, organ failure, and septic shock. (Yoshida et al., 2019). These findings may be related to the fragility of hemoglobin S erythrocytes after hemoglobin S polymerization, as well as decreased deformability and red cell elasticity during storage as a result of low oxygen tension and a low pH storage solution (Yoshida et al., 2019).

Platelet (PLT) count decreased significantly in both groups, but the reduction was more marked in SCT positive blood, indicating heightened susceptibility to storage-induced platelet degradation. Aninagyei et al., 2018). For Sickle cell trait negative donor blood ANOVA: Significant differences were observed ($P < 0.05$). Tukey HSD: Significant reductions in platelet count were seen at all-time points compared to Day 0, with the most significant drop on Day 28 (diff = -96.31, p adj = 0.00) whereas Sickle cell trait positive

donor blood; ANOVA: Significant differences were observed ($p < 0.05$). Tukey HSD: Significant reductions at all-time points compared to Day 0, with the largest decrease at Day 28. This phenomenon may compromise transfusion efficacy, particularly in contexts requiring platelet-rich products. Aninagyei et al. (2018) & Aubron et al. (2018) highlighted the rapid disintegration of platelets during storage, corroborating these findings.

The present study demonstrated progressive hematological deterioration in both HbAS and HbAA donor blood during 28 days of refrigerated storage, with changes consistently more pronounced in HbAS units. Platelet counts, total white blood cell counts, plateletcrit, and mean corpuscular hemoglobin concentration (MCHC) declined progressively, whereas hemoglobin, hematocrit, mean corpuscular volume (MCV), and percentage hemolysis increased with storage duration, reflecting accelerated storage lesions in sickle cell trait blood. These findings agree with Maruti et al. (2021), who reported progressive alterations in red cell indices, leukocyte degeneration, and reduced platelet viability during blood storage. Similarly, Klein et al. (2008) observed significant increases in MCV and hematocrit with prolonged storage, attributing these changes to ATP depletion, ionic imbalance, and erythrocyte swelling. Ravel et al. (2004) also documented marked reductions in leukocyte and platelet counts because of granulocyte degeneration and platelet activation during refrigeration. Furthermore, Bardyn et al. (2017) demonstrated that oxidative stress and membrane remodeling progressively impair erythrocyte deformability throughout storage, while Nestheide et al. (2023) showed that HbAS erythrocytes are particularly susceptible to membrane damage and reduced deformability under hypothermic conditions. Therefore, the present findings reinforce growing evidence that HbAS donor blood develops storage-related hematological lesions more rapidly than HbAA blood, supporting consideration of shorter storage durations and enhanced quality monitoring for SCT-positive blood units.

5.2.2 Storage-Related Biochemical Changes

Lactate dehydrogenase (LDH) levels increased steadily in both SCT positive and negative blood, indicating ongoing Red blood cells' cellular degradation, damage and hemolysis during donor blood storage. SCT positive donor blood exhibited higher LDH levels throughout the storage period, Associated RBC cellular degradation, damage and hemolysis during donor blood storage. HbAS exhibits higher LDH levels throughout storage reflecting increased erythrocyte fragility and susceptibility to oxidative stress/hemolysis (Wither et al., 2016).

For HbAA group LDH: ANOVA results**:** Significant variation in LDH levels over days of storage ($P < 0.05$). Significant increases in LDH levels from Day 0 to subsequent days, with the highest increase on Day 28 (334.60 units). Significant differences between most pairs of days, indicating progressive LDH increase over time. For HbAS group LDH: ANOVA results**:** Significant variation in LDH levels over days of storage ($P < 0.05$). Significant increases in LDH levels from Day 0 to subsequent days, with the highest increase on day 28 (424.93 units). Significant differences between most pairs of days, indicating a similar progressive increase in LDH.

Potassium levels rose progressively in both groups, with SCT positive donor blood demonstrating higher average levels. For SCT negative donor group, potassium Levels; ANOVA results: Significant variation in potassium levels over days of storage ($P < 0.05$). Potassium levels significantly increased from day 0 to subsequent days, with the highest increase on day 28 with significant differences observed between all pairs of days. For SCT positive donor group, potassium Levels; ANOVA results: Significant variation in potassium levels over days of storage ($P < 0.05$). Potassium levels significantly increased from day 0 to subsequent days, with the highest increase on day 28 with significant differences observed between all pairs of days. This elevation likely results from increased hemolysis and platelet degradation, which release intracellular potassium into plasma, potentially posing risks of hyperkalemia in transfusion-recipients (Yoshida et al., 2019)

noted similar potassium ion dynamics in stored blood. For SCT donor group, Sodium level: ANOVA results: Significant variation in sodium levels over days of storage ($P < 0.05$) whereas Tukey HSD Test: Sodium levels significantly decreased from Day 0 to subsequent days, with the most substantial decrease on Day 28 and the Significant differences observed between most pairs of days. For SCT donor group, Sodium level; ANOVA results: Significant variation in sodium levels over days of storage ($P < 0.05$) with Tukey HSD Test: Sodium levels significantly decreased from Day 0 to subsequent days, with the most substantial decrease on Day 28 and the Significant differences observed between most pairs of days. Sodium levels decreased over storage time due to: Low ATP to maintain potassium-sodium pump, altered membrane integrity, effect of anticoagulant (CPD, SAGM) & haemolysis-release of intracellular contents diluting Sodium (Aninagyei et al., 2018).

Glucose levels initially rose and then declined in both sickle cell trait positive and negative donor blood, suggesting early metabolic activity followed by utilization or degradation of glucose substrates during storage over time. The decline may reflect diminished metabolic capacity of stored RBCs in the donor blood units over time, as described by Hess et al. (2014). For HbAA donors' group, glucose: ANOVA Results: Significant variation in glucose levels over days of storage ($P < 0.05$) with Tukey HSD Test: Glucose levels significantly increased on Day 7 compared to Day 0. Decreases observed from Day 14 to day 28, with significant differences in most comparisons. For HbAS donors' group, glucose: ANOVA results**:** Significant variation in glucose levels over days of storage ($P < 0.05$) with Tukey HSD Test: Glucose levels significantly increased on day 7 compared to Day 0. Significant decreases observed from day 14 to Day 28, similar to the HbAA results.

Ferritin and bilirubin levels increased consistently in both SCT positive and SCT negative donor groups, indicating ongoing breakdown of RBCs and release of iron and heme products into blood plasma. For SCT negative donor group, Total Bilirubin Levels; ANOVA results: No significant variation in total bilirubin levels over days of storage (P

= 0.28). For SCT positive donor group, Total bilirubin level: ANOVA results: Significant variation in total bilirubin levels over days of storage ($P < 0.05$). For the SCT negative donor group, Ferritin; ANOVA results**:** No significant variation in ferritin levels over days of storage ($P= 0.106$) whereas SCT positive donor group, Ferritin level; ANOVA results**:** No significant variation in ferritin levels over days of storage ($P = 0.609$).

The present study showed progressive biochemical deterioration during storage, characterized by increasing lactate dehydrogenase (LDH), potassium, ferritin, bilirubin, and percentage hemolysis, together with declining sodium and glucose concentrations, with significantly greater changes occurring in HbAS than HbAA donor blood. These observations are consistent with Yoshida et al. (2019), who reported continuous potassium leakage, increasing plasma hemolysis, and release of intracellular enzymes during refrigerated red cell storage following sodium–potassium pump failure and membrane injury. Similarly, Hess et al. (2014) attributed progressive biochemical deterioration to anaerobic glycolysis, ATP depletion, lactate accumulation, and declining pH, which impair membrane transport systems and accelerate storage lesions. Bardyn et al. (2017) further demonstrated that oxidative stress and metabolic exhaustion promote membrane instability and increased release of intracellular constituents into plasma during storage. Reisz et al. (2016) also reported progressive oxidative injury and irreversible metabolic impairment after approximately three weeks of storage, resulting in enhanced cellular degradation. Furthermore, Kotla et al. (2022) described increasing ferritin concentrations as evidence of disturbed iron homeostasis, while Lin et al. (2019) associated rising bilirubin levels with progressive erythrocyte breakdown and oxidative damage. Collectively, these studies corroborate the present findings and suggest that HbAS donor blood experiences accelerated biochemical storage lesions compared with HbAA blood, potentially compromising transfusion quality, efficacy, and safety during prolonged storage.

5.3 Summary of Key Findings

Long-term storage of red blood cells (RBCs) is critical for maintaining a steady blood supply for clinical use. This study analyzed hematological and biochemical changes in sickle cell trait (HbAS) and non-sickle cell trait (HbAA) donor blood over 28 days. Both groups showed increased hematocrit (HCT) and mean cell volume (MCV), indicating RBC dehydration and swelling. HbAS blood had higher baseline HCT and more significant increases, reflecting different erythrocyte stability. Mean cell hemoglobin concentration (MCHC) decreased more in HbAS blood, suggesting altered oxygen-carrying capacity. Platelet and white blood cell counts declined significantly, more so in HbAS blood, impacting transfusion efficacy and immune response. Biochemical changes included rising lactate dehydrogenase (LDH) and potassium levels, indicating RBC degradation and hemolysis, with higher levels in HbAS blood. Sodium levels decreased, and glucose initially rose, then declined in both groups. Ferritin and bilirubin levels increased, with HbAS blood showing higher bilirubin. These findings highlight HbAS blood's heightened vulnerability to storage-induced changes, affecting transfusion quality.

5.4 Conclusion

These findings provide evidence supporting consideration of routine SCT screening among blood donors in malaria-endemic settings to improve blood storage quality and transfusion safety. The research findings underscore the need for meticulous blood donor screening and the implementation of strategies to manage the risks associated with sickle cell trait in the blood supply. The relatively high prevalence of HbAS among donors (10%) at the Kisumu Regional Blood Transfusion Centre calls for sustained efforts in public health education, policy development, and stringent screening protocols.

Storage-related haematological and biochemical changes were more pronounced in SCT-positive donor blood than in SCT-negative. SCT-positive blood exhibited higher hemolysis rates, increased potassium level, lactate dehydrogenase (LDH), and

haemoglobin levels (Hb), and significant reductions in platelet and white blood cell counts over the storage period. These findings suggest greater erythrocyte fragility and oxidative stress susceptibility in SCT-positive blood. The distinct storage profiles between SCT-positive and negative blood highlight the need to consider genetic factors in blood and blood products transfusion management.

5.5 Recommendations

The Kenya National Blood Transfusion Service (KNBTS) should implement routine sickle cell trait (HbAS) screening for all blood donors, particularly in high-prevalence regions, and ensure appropriate donor counseling and labeling of HbAS blood units to enhance transfusion safety and quality.

The Kenya National Blood Transfusion Service (KNBTS), through Regional Blood Transfusion Centres, should establish differential storage protocols for HbAS blood by shortening storage duration and routinely monitoring key storage lesion biomarkers, including potassium, lactate dehydrogenase (LDH), plasma bilirubin, and percentage hemolysis, to maintain optimal blood quality.

The Ministry of Health, in collaboration with County Departments of Health and KNBTS, should strengthen community awareness programmes on sickle cell trait and establish continuous surveillance of SCT prevalence among blood donors to support evidence-based donor recruitment, transfusion policies, and blood safety planning

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APPENDICES

Appendix I: Consent Form

Study Title: Storage-related hematological and biochemical changes in sickle cell trait donor blood at Kisumu Regional blood transfusion center (RBTC), Kenya.

Introduction:

You are invited to be in a research study of '**Storage-related hematological and biochemical changes in sickle cell trait donor blood at Kisumu Regional Blood Transfusion Center (RBTC) Kenya.'** You have been selected as a possible participant because you may be sickle cell trait carrier/Kisumu has a high prevalence of Sickle cell trait/disease. We ask that you read this form careful and ask any questions you may have before agreeing to be in the study.

Background Information:

The purpose of this study is to ***evaluate storage-related hematological and biochemical changes in Sickle cell trait donor blood.***

Procedures: If you agree to be in this study, we will ask you to do the following things:

1. Donate blood that may be selected for the study. Donation process may be uncomfortable because of the needle prick, but efforts will be made to ensure that only an experienced Phlebotomist draws the blood and that this will be done in the safest way possible.
2. Any information (*SCT trait positive results)* obtained will be communicated to you through your contact provided below.

Risks and Benefits of Being in the Study: One of the risks of this study is the little pain during blood donation process. We will be testing biochemical and hematological

parameters in blood you will donate, and this information may be important in the way you will be managed in the future. There are no direct benefits of this study to you, but the information will be used to help in ensuring the quality and safety of sickle cell trait donor blood, management of other sickle cell trait and Sickle cell disease related anemia and other complications.

Confidentiality: The records of this study will be kept private. In any sort of report we might publish, we will not include any information that will make it possible to identify a participant. Your record for the study may, however, be reviewed by any member of the research team, Kisumu RBTC and to that extent, confidentiality is not absolute.

Financial Considerations: There are no financial benefits from this study.

Voluntary Nature of the Study: Your decision whether or not to participate in this study will not affect your current or future relations with Kisumu RBTC. If you decide to participate in this study, you are free to withdraw at any time during blood donation process.

Contacts and Questions:

If you have any questions or concerns about the way, you have been treated as a participant in this research study, please contact Shiundu Meshack ***-0713577881*** (The researcher conducting the study). Even though he may ask for your name, information will be kept confidential.

I consent to give blood,

Name……Contact……...Date….....Signature.....…

For Official Use:

Study Researcher…….Date.....Signature…….....

Appendix II: JKUAT Institutional Ethics Review Committee Approval

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
P.O BOX 62000(00200) NAIROBI, Tel:(067) 58700001-4
(Office of the Deputy Vice Chancellor, Research Production and Extension Division)

JKUAT INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

REF: JKU/2/4/896B Date: 2nd November, 2023

Shiundu Bwakali Meshack
Department of Medical Laboratory Science, JKUAT

Dear Mr Shiundu,

RE: STORAGE –RELATED HEMATOLOGICA AND BIOCHEMICAL CHANGES IN SICKLE CELL TRAIT DONOR BLOOD AT KISUMU REGIONAL BLOOD TRANSFUSION CENTER, KENYA

This is to inform you that JKUAT Institutional Scientific and Ethical Review Committee has reviewed and approved your above research proposal. Your application approval number is **JKU/ISERC/02316/1091.** The approval period is **26th October 2023 to 27th October 2024.**

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JKUAT ISERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to JKUAT ISERC within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to JKUAT ISERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. 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.
vii. Submission of an executive summary report within 90 days upon completion of the study to JKUAT ISERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Amos Mbugua
CHAIR, JKUAT ISERC

INSTITUTIONAL ETHICS REVIEW COMMITTEE
02 NOV 2023
APPROVED
P.O. Box 62000-00200 NAIROBI
www.jkuat.ac.ke
JKUAT

Appendix III: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **797611**

Date of Issue: **12/December/2023**

RESEARCH LICENSE

This is to Certify that Mr.. MESHACK BWAKALI SHIUNDU of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisumu on the topic: STORAGE-RELATED HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN SICKLE CELL TRAIT DONOR BLOOD AT KISUMU REGIONAL BLOOD TRANSFUSION CENTER,KENYA for the period ending : 12/December/2024.

License No: **NACOSTI/P/23/31896**

797611

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix IV: Mindray BC-5000 Hematology Analyzer

Verify that the **Sample ID** is correct: Since auto numbering is on, the Sample ID is automatically entered. To override the number with another Sample ID:

i. Highlight the number in the Sample ID field.
ii. Type the desired Sample ID number.
iii. Press Ù when the Sample ID is complete.
iv. Verify that the Sample ID is correct.
v. Select the panel – CBC or CBC/DIFF: Select CBC or CBC/DIF, and Press Ù to move the cursor to the Flagging Set field.
vi. Select another flagging set if appropriate: the desired flagging set. Press Ù to move the cursor to the Collection Date and Time field.
vii. Enter the date and time that the specimen was collected: Enter the complete date, enter the time & Press Ù to move the cursor to the Comments field.
viii. Type your comments (optional) and press Ù to move to the Patient ID field.
ix. Enter the Patient ID (optional) (up to 25 alphanumeric characters and no spaces): r manually enters the Patient ID, or r scan the Patient ID from the barcode, if applicable, or r and select the Patient ID from the existing list of Patient IDs, if applicable.
x. Press Ù to move to the Patient Name field
xi. Enter the patient's name (up to 30 alphanumeric characters and spaces) and press Ù to move to the date of birth field.
xii. Enter patient gender, age, and physician's name and save details entered
xiii. Mix the specimen gently and thoroughly according to the laboratory's protocol and Verify that the Sample ID in the Sample ID Next field matches the sample to be processed.
xiv. Insert the tube into the correct position of the correct tube holder.
xv. Close the tube holder door to begin analysis and remove the tube when the tube holder door automatically opens (after aspiration).

xvi. Wait for the green LED to illuminate, which indicates the system is ready for the next sample. Information for the next sample to be processed is displayed in the Sample ID Next field on the Run screen.

Appendix V: KNBTS Questionnaire

Clinic Venue----------County----------Clinic Code: -----Donor Number-

SECTION 1: DAILY BLOOD DONOR REGISTRATION& SCREENING FORM

Surname:_____Other Names:_____**GENDER:** F/M

Student Number/ National ID Number: **Date of Birth: ------/------/-------**

Marital Status :*(Mark in the appropriate box)*

Single	Married	Divorced/Separated	Widowed

Contact Details: Postal Address (where you would like to receive your correspondence) Code

Home phone number:.....**Cell phone number:**

Email:.....Residence (county)…….

Level of education: None/Primary/ Secondary/Tertiary/Occupation**……...**

When did you last donate Blood………...**Blood Group……...**

SECTION 2: HEALTH QUESTIONNAIRE Circle the appropriate answer

1. Are you feeling well and in good health today?	Yes/No
2. Have you eaten in the last 6 hours?	Yes/No

3. Have you ever fainted? Yes/No
In the past 6 months have you:
4. Been ill, or received any treatment or any medication? Yes/No
5. Had any injections or vaccinations (immunizations)? Yes/No
6. Female Donors: Have you been pregnant or breast-feeding? Yes/No
In the past12 months have you:
7. Received a blood transfusion or any blood products? Yes/No
Do you have or have you ever had:
8. Any problems with your heart or lungs e.g. Asthma? Yes/No
9. A bleeding condition or a blood disease? Yes/No
10. Any type of cancer? Yes/No
11. Diabetes, epilepsy or TB? Yes/No

SECTION3: RISKASSESSMENTQUESTIONNAIRE

The lives of patients who receive your blood are totally dependent on your honesty & frankness in answering the questions below. Your answers will be treated in a confidential manner. Circle the appropriate answer.

In the past12 months have you:
1.Received or given money, goods or favors in exchange for sexual

activities Yes/No
2. Had sexual activity with a person whose background you do not know? Yes/No
3. Been raped or sodomized? Yes/No
4. Had a stab wound or had an accidental needle stick injury e.g. Injection needle? Yes/No
5. Had any tattooing or body piercing e.g. ear piercing? Yes/No
6. Had a sexually transmitted disease (STD)? Yes/No
7. Live with or had sexual contact with someone with yellow eyes or yellow skin? Yes/No
8. Had sexual activity with any one besides your regular sex partner? Yes/No
Have you ever:
9. Had yellow eyes or yellow skin? Yes/No
10. Injected yourself for being injected, besides in a health facility? Yes/No
11. Used non-medical drugs such as Marijuana, Cocaine, etc.? Yes/No
12. Have you or your partner been tested for HIV? Yes/No
13. Do you consider your blood safe to transfuse to a patient? Yes/No

SECTION 4: DECLARATION

(Please read this before you complete the form with your name and signature below)

I declare that I have answered all the questions truthfully and accurately. I understand that my blood will be tested for HIV, Hepatitis B&C, and Syphilis and the results of my tests may be obtained from the National Blood Transfusion Service. I understand that should any of the screening tests give a reactive result, I will be contacted by use any communication medium(s) to send me important information. Such medium(s) shall include but not limited to e-mail, post office, mobile telephone and/or fixed telephone, and offered counseling to make an informed decision about further confirmatory testing and management.

I hereby give consent to KNBTS to use the contact details provided in this form to communicate to me as the need maybe.

I understand blood may be used for scientific research, main objective being to improve the safety of the blood supply to patients.

I consent to give blood; understand that it may be used for transfusion for the benefit of others.

Signature: ----------Date: ----------

For Official Use:

Weight(kg**)**	**Hb>12.5g/dl**	**BP**	**Pulse**

Donor is Accepted	
Yes	**No**

Report:

Name of Nurse/Counselor: ----------Date: ----------

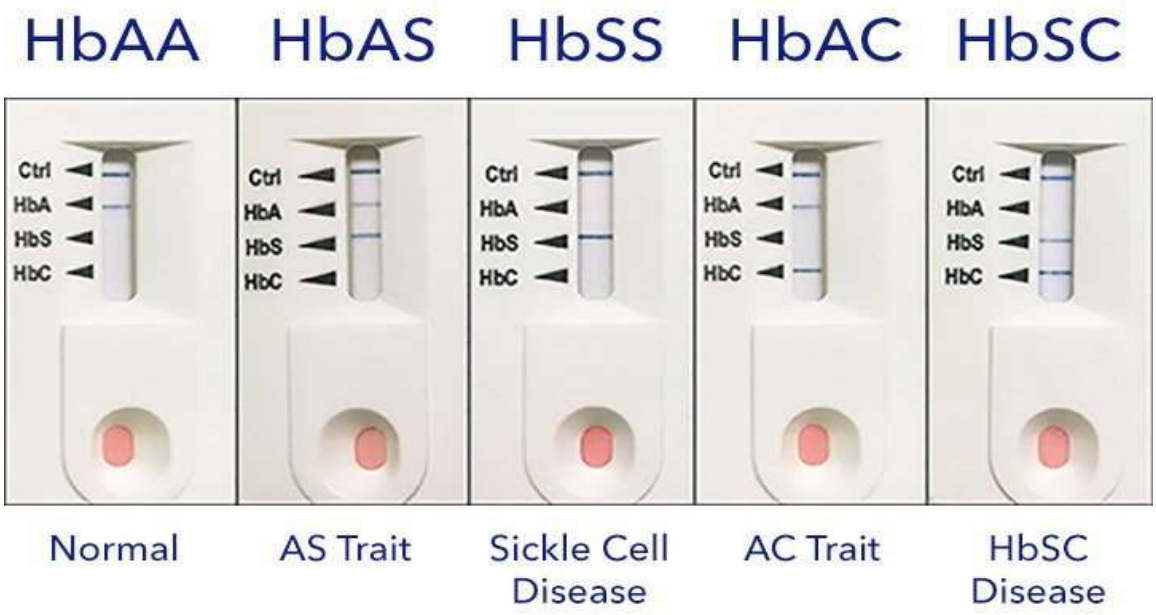
Low Volume	**>1 Venipuncture**	**Hematoma**	**Faint**		
			Mild	**Moderate**	**Severe**

Time Needle In		**Time Needle Out**	
Report: Name of Phlebotomist: ---------- Date: ----------			

Appendix VI: SoP for (BioMedomics, USA) Sickle Scan Kit

Kit operating principle, Sickle SCAN® (BioMedomics, USA) is known as a rapid, qualitative lateral flow immunoassay test for the identification of sickle cell disorder of **hemoglobin A, S,** and **C.** The test is made up of three indicators which detect the presence of that hemoglobin, allowing the user to rapidly distinguish between normal, carrier, and sickle cell samples.

Procedure: Five microliters of blood taken by fingerpick, heel stick, or venipuncture using the provided Capillary Sampler is placed into the buffered loaded pretreatment module to release hemoglobin by lysing erythrocytes. Three drops of the treated sample are dropped from the pretreatment Module and added to the sample inlet of the sickle SCAN® cartridge. The sample will interact with antibody conjugated colorimetric detector nanoparticles and travel to the capture zone. Results can be read within five minutes. The presence of hemoglobin variants **A, S,** and **C** will be indicated by blue lines in their designated regions as can be shown below.



Appendix VII: COBAS INTEGRA 400 PLUS SOP

8.1 Creating orders

8.1.1 Before creating new orders, completed orders from the previous day should be purged if the machine has not already done so.

8.1.2 An order is created as follows:

I. Add Sample's Accession# on the Sample tab of the orders work area

II. Add **Sample's Accession#** (in the ***Order ID Space***)

III. Select tests and click save

IV. Choose rack number and position for samples

8.1.3 Select tests and save the order, as follows:

a. Click the buttons for the required profiles or tests.

b. Click STAT for a high-priority order (only for STAT tests)

c. Clicks save.

8.1.4 Confirm rack positions, as follows:

a) Check the automatically assigned rack number and position for each sample. Modify the position as needed.

b) Select the Cup on the tube check box if the sample is placed on a secondary cup on a primary tube. Click ok.

8.1.5 Loading samples on board

a. Once orders are entered into the system, print the load list by clicking on File→Print→ Load list

b. Set appropriate racks with adapters for samples in Cryo

c. -vial (need at least 300-500 ul sample volume) according to the load list.

d. If using Roche Cups – must aliquot 300-500 ul using a transfer pipette - Tap cups lightly on the surface of the hood to make sure no air bubbles on the bottom of cup

e. Once all samples are loaded, load the sample rack into one of the slots in the sample area.

8.1.6 Make sure correct controls and calibrators are loaded on board in their pre-defined slots (Slot I – Rack # 20)

9.0 Start analyzing

9.1.1 If the system is on standby, press start

9.1.2 If the system is sleeping, press start. Once the system is on standby, press start to begin the processing or press F11.

9.1.3 Check the work list as the processing begins, as follows:

a) Click Orders on the navigation bar and click the work list tab.

b) Check the work list for orders that are blocked or have no samples on board. Double click blocked order to see the reason for the blockage.

c) Take appropriate actions to resolve the problem.

9.3 To rerun or repeat a test

9.3.1 Click on the validate tab in the results work area

9.3.2 Select a sample, calibration, or control order

9.3.3 Select the results in question

9.3.4 Select rerun

9.4 To accept a result

9.4.1 Click on the results in the result window, click accept

9.5 Printing of the final report

9.5.1 Once all of the results are accepted or validated, a final report will automatically be printed.

9.5.2 Final reports are printed.