TOTAL BLOOD VOLUME, IRON STATUS AND HEMATOLOGICAL PROFILES OF WHOLE BLOOD DONORS AT KENYATTA NATIONAL HOSPITAL, NAIROBI CITY COUNTY, KENYA

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DECLARATION

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

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DEDICATION

I dedicate this work to my wife Grace, my daughter Olive, family members, colleagues and friends.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome
BMI	Body mass index
BTS	Blood transfusion service
BV	Blood volume
CRP	C -Reactive Protein
CPD	cardiopulmonary bypass
EU	European Union
ESR	Erythrocyte sedimentation rate
FMH	Federal Ministry of Health
FDA	Food and Drug Administration
HGB	Hemoglobin
HCT	Hematocrit
HTC	Hospital Transfusion Committee
ID	Iron deficiency
IDA	Iron deficiency anemia
IDW	Ideal body weight
KNBTS	Kenya National Blood Transfusion Service
KNH	Kenyatta National Hospital
KU	Kenyatta University
KUHC	Kenyatta University Health Centre
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

MOH	Ministry of Health
NACOSTI	National Commission for Science, Technology and Innovation
NBTS	National Blood Transfusion Service
PBF	Peripheral blood film
PLT	Platelet
RBC	Red blood cell
RD	Replacement donors
RBTC	Regional Blood Transfusion Centers
SPSS	Statistical Package for Social Sciences
TBC	Total blood count
TBV	Total blood volume
TTIs	Transfusion-Transmitted Infections
USA	United States of America
VBD	Voluntary blood donor
VNRD	Voluntary non-remunerated donor
WBC	White blood cells
WHO	World Health Organization

ABSTRACT

The effectiveness of blood donation exercises relies on safeguarding prospective blood donors' and recipients' health. This may be realized through collection of safe and appropriate blood volume. An ineffective donor selection criterion may expose potential donors to vasovagal reactions and lead to collection of blood with hematological abnormalities. In Kenya, there is paucity of data on donors' total blood volume (TBV), iron status, hematological profiles and deferral rates. The objectives of this study were to determine: the percentage of blood volume donation, reliability of using Nadler's or Lemmens-Bernstein-Brodsky's equations to calculate TBV, donor iron status, hematological profiles and deferral rates among blood donors attending Kenyatta National Hospital. A cross-sectional study design was adopted. Using a systematic random sampling technique, 384 study participants were recruited. Donors were grouped into two categories (eligible and deferred donors) based on the donor recruitment outcome. Study questionnaires were used to collect donors' demographic information. Eligible donors were checked for the total blood volume using Nadler's and Lemmens-Bernstein-Brodsky's equations. Eight (8) ml of blood samples were collected from the donated units and halved into plain tubes and EDTA tubes. Serum ferritin levels were analyzed using Biomeriex Mini Vidas® analyzer while hematological parameters were determined using HumaCount 5D® analyzer. Ethical approval was sought from KNH-UoN ethical review committee. Non-parametric variables were analyzed using Mann Whitney U test and Kruskal Wallis H test. Intra–class Correlation Coefficient (ICC) was used to assess reliability of using the two total blood volume equations. This study identified 202 eligible donors, majority being male donors (173 vs. 29). Using Nadler's and Lemmens-Bernstein-Brodsky's equations the percentage of blood volume donated by eligible donors was 11.7% and 11.6%, respectively. Female donors donated significantly higher blood volume than their male counterparts (*P=0.01*). Reliability tests showed an excellent reliability of using either Nadler's or Lemmens-Bernstein-Brodskys' equations (average measure of 0.996 and single measure 0.991). Among eligible donors the prevalence of iron deficiency was 2.5%, whereas the prevalence of anemia was 7.4%. Female blood donors had a higher prevalence of iron deficiency (6.9% vs. 1.7%), whereas male donors had a high prevalence of anemia (7.4%vs.0%). Male donors had a significantly higher ferritin levels than female donors (*P=0.01*). The median counts of all eighteen hematological parameters analyzed were within local reference ranges. However, seven hematological parameters (RBC, Hgb, MCH, MCHC, monocytes, eosinophils and platelets) had significant variation from normal values (*P<0.05).* Additionally, male donors had significantly higher values for; red cell count, hematocrit and hemoglobin (*P=0.01*) whereas female donors had significantly higher lymphocyte and platelet counts (*P=0.05*). This study found a donor deferral rate of 47.4%. Temporarily deferred donors had a higher rate than permanent ones (93.4% vs. 6.6%). Donors with high deferral rates were first-time donors and female donors. In addition, deferral rates increased with the advancement of age. The leading causes of temporary deferrals were medication and low hemoglobin, whereas high blood pressure and diabetes were the main causes for permanent deferral. In conclusion, this study observed majority of donors met the threshold for safe blood donation. However, few donors had lower TBV, others had iron deficiency, anemia and hematological abnormalities. This study recommends a review of donor recruitment criteria to incorporate assessment of total blood volume, iron status, total blood count and advocate for blood donation awareness campaigns.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

A donor selection criterion is an essential tool used to safeguard donors' health and improve recipients' medical conditions. The effectiveness of donation service is achieved by identifying potential risk factors and collecting safe and appropriate blood volume (WHO, 2012). A prospective donor who meets donor recruitment criteria is typically allowed to donate, whereas those who fail are temporarily or permanently deferred (Mast, 2015). According to a report from the World Health Organization (WHO), countries relying on family/replacement and paid donors have higher risks of transfusion-transmitted infections (TTIs) than those depending on voluntary non-remunerated donors (WHO, 2011). Global statistics have reported the leading cause of donor deferral as low hemoglobin, accounting for about 29.7% (Kouao *et al.*, 2012). According to the WHO, donor deferral rates varies from 1% to more than 37% (WHO, 2017).

In most countries, eligible blood donors must weigh at least 50kg to donate whole blood regardless of their height or basal metabolic index (BMI). However, using weight as the only tool for approximating donors' total blood volume (TBV) is inaccurate (Salamat, 2007). Assessment of TBV was initially computed during the management of cardiac surgery and cardiopulmonary bypass but was rarely used in the selection of donors (Hilberath *et al.*, 2015). A populations' TBV is dependent on various demographic characteristics, and hence there is a need to determine limits for whole blood donation (Salamat, 2007). To minimize occurrence of any adverse / vasovagal reactions related to

excessive blood volume donation, a maximum limit for blood donation should not exceed 13% of the donors' TBV (WHO, 2012).

Chronic iron deficiency anemia (IDA) and iron depletion (ID) are known medical conditions affecting young women, first-time, and repeat blood donors (Simon, 2006). Despite this knowledge, the only invasive test prescribed during donor selection criteria is hemoglobin levels in the majority of countries including Kenya (Tailor *et al.*, 2017). According to the WHO (2020) guidelines, the recommended serum ferritin (SF) cut-off value for iron deficiency among adults is <15ng/ml. Screening for hemoglobin concentration is not sensitive in detecting early stages of iron deficiency (Patel *et al.*, 2019).

In Kenya, blood collection centers screen prospective donors for hemoglobin (Hgb) concentration and bleed donors with Hgb levels above 12.5g/dl (KNBTS, 2001). Other hematological parameters such as white blood cells, red blood cells, and platelets are not screened. Adjudged eligible donors with normal hemoglobin levels could have other abnormal hematological parameters such as thrombocytopenia, leukocytosis, leukocytopenia or even polycythemia (Abbas *et al.*, 2015). Failure to screen all hematological parameters may allow donors with other hematological abnormal profiles to donate blood which may affect the quality of blood or blood products. This study was formulated to assess the percentage of TBV donation, iron status, hematological profiles and deferral rates among blood donors presenting to donate at Kenyatta National Hospital (KNH).

1.2 Problem Statement

The current donor recruitment criteria is silent on screening donors' total blood volume (TBV) besides recommendations on the acceptable limits for donation and the dangers associated with excessive blood donation. Moreover, the existing methods for estimating TBV were initially designed for cardiac patients; this poses a great challenge in selecting a reliable tool for estimating donors' TBV. Assessment of donors' iron status is another aspect omitted in the current donor recruitment criteria. The WHO urges member countries to continually monitor donors' iron status to detect early stages of iron deficiency and iron-deficiency anemia.

The existing donor hemoglobin cut-off value was in most cases borrowed from the Caucasian adult population. Therefore, adopting such a cut-off value may be inappropriate due to variations in the country's donor populations. The current reference value needs review, validation or verification since they were adopted over two decades ago using methods and techniques that are probably absolute and outdated. Furthermore, the Clinical Laboratory Standards Institute (CLSI, 2010) recommends that screening laboratories should validate or develop their reference ranges from the population they serve before adopting ranges designed in other regions. Another challenge facing blood donation centers is the failure to screen for all hematological profiles. The practice of screening hemoglobin alone may fail to detect abnormal hematological parameters among eligible donors with normal hemoglobin levels. Finally, there is scarcity of data regarding donor deferral rates and causes especially in a hospital-based set-up.

1.3 Justification of the Study

There is scarcity of information on the Kenyan donors' total blood volume and the understanding the percentage of TBV donated is important in averting possible vasovagal reactions. The WHO Donors with TBV below 3500ml and those who donate more than 13% of their TBV are at risk of vasovagal reactions. These vasovagal reactions mainly affect young-age donors and female donors, and experiences with adverse reactions negatively affect future blood donations. Moreover, there is a need to select and adopt a reliable, easy-to-use TBV screening tool among existing methods.

Regular assessment of donors' iron status helps detect early stages of iron deficiency. Screening hemoglobin alone may not be ideal in identifying donors with iron deficiency and those with underlying medical conditions such as abnormal hematological parameters Therefore, this study aimed to understand donor iron status and hematological profiles among eligible blood donors. Globally, deferral rates and causes for deferral vary from one country to another (Kouao *et al.*, 2012). To minimize this variation, member countries are urged to balance the availability of resources, donor deferrals, and blood supply based on locally established evidence. Implementing this recommendation is faced with many challenges; such as lack of local data, poor record-keeping and inadequate finance and staff. This highlights the need to understand deferral patterns and causes in a hospital-based set-up.

1.4 Research Questions

i. What is the percentage of blood volume donated by eligible blood donors attending Kenyatta National Hospital?
ii. What is the reliability of using Nadler's equation and Lemmens-Bernstein-Brodsky's equation to calculate total blood volume among eligible blood donors at Kenyatta National Hospital?
iii. What is the prevalence of iron deficiency and anemia among eligible blood donors attending Kenyatta National Hospital?
iv. What are the hematological profiles of eligible donors attending Kenyatta National Hospital?
v. What are the rates and causes of temporary and permanent deferrals among blood donors attending Kenyatta National Hospital?

1.5 Objectives

1.5.1 General Objective

To determine total blood volume, iron status, and hematological profiles of whole blood donors at Kenyatta National Hospital, Nairobi City County, Kenya.

1.5.2 Specific Objectives

i. To determine the percentage of blood volume donated by eligible blood donors attending Kenyatta National Hospital.

ii. To determine the reliability of using Nadler's equation and Lemmens-Bernstein-Brodsky's equation to calculate total blood volume among eligible blood donors at Kenyatta National Hospital.

iii. To determine the prevalence of iron deficiency and anemia among eligible blood donors attending Kenyatta National Hospital.

iv. To determine hematological profiles of eligible blood donors attending Kenyatta National Hospital

v. To determine the rates and causes of temporary and permanent deferrals among blood donors attending Kenyatta National Hospital.

1.6 Study Significance

The data obtained from this study will provide a learning paradigm in the transfusion service by enhancing understanding of the various aspects of blood donation. The findings will inform the need to protect donors by incorporating additional screening tools in the current donor recruitment criteria. The observations will also be crucial in safeguarding recipients' health by collecting only safe and reliable blood. In addition, this study provides information on donor eligibility and deferral patterns. Finally, the information generated from this study will provide local evidence necessary for policymakers who rely on available data to develop effective blood transfusion policies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Whole Blood Donation

Whole blood and blood products are derived from persons known as blood donors (WHO, 2012). Globally, approximately 112.5 million units of blood are collected annually. Almost half of these donations are collected from developing countries which constitute the majority of the world's population (WHO, 2017). The World Health Organization (WHO) further estimates that developed countries contribute nine times more donations than developing countries, reporting (33.1 vs. 4.6 donations respectively) per 1000 persons (WHO, 2017). This variation highlights the major challenge in blood collection faced by many developing countries compared to developed countries.

In 2013, the African region collected approximately 5.6 million blood bags out of 112.5 million blood bags collected globally, translating to about 4%. In the same year, an average of 12 % deferral rate was reported among 128 member countries (WHO, 2017). According to the WHO guidelines, Kenya was expected to collect 450,000 units in 2019 but only managed to collect 164 275 units resulting in an acute blood shortage (MOH, 2019). A high rate of donor deferrals, blood discards, and acute shortage of blood highlights the importance of an effective donor recruitment criteria, reduction of deferral of suitable donors and wastage of resources, consumables and staff time (Smith *et al.*, 2013).

There are three types of blood donors, voluntary non-remunerated donors (altruistic), replacement/ family donors (coerced) and paid donors. Altruistic donors give blood regularly and voluntarily without coercion. Solemn coercion and sometimes hidden payments are primarily associated with replacement donations (Sunderam *et al.*, 2015). A previous report indicated that anonymous and regular blood donations from voluntary donors are the only assured remedy for a reliable supply of safe blood (Tonderai, 2017). A blood donor can also be described based on their donor status as a first-time or repeat blood donor. First–time donor refers to a donor donating for the first time; in contrast, a repeat donor refers to individuals who have a history of blood donation (Singh & Bhatt, 2017).

According to Padma *et al.* (2017) report, first-time donors are prone to transfusion-transmitted infections (TTIs) compared to repeat donors. This was attributed to repeat donors' understanding of donor eligibility criteria and self-deferral. Frequent screening of TTI's to blood donated by repeat donors reduces the risk of blood transfused to recipients. A study by Niazkar *et al.* (2020), mentioned that recruiting first-time donors is more expensive than retaining former or existing donors. Therefore, it is imperative to retain repeat donors to achieve reliable, safe blood and blood products.

The primary goal of the World Health Organization (WHO, 2010) is to phase out the replacement and paid blood donation and maintain 100% altruistic blood donation. It was believed by 2020, blood would be collected purely from altruistic donors. Maintaining a pool of regular voluntary unpaid donors will guarantee a reliable and stable supply of safe

blood (WHO, 2011). In sub- Saharan Africa, there is a great demand for blood due to the high prevalence of infectious diseases, malnutrition, and obstetric hemorrhage. Stringent donor recruitment criteria only allow a subset of willing prospective donors to donate (Kouao *et al.*, 2012). According to a previous study, family/ replacement donors (RD) constitute 75-80% of all blood transfused in Africa. The study further mentioned that family/ replacement donation has worked as an alternative to voluntary donation in most African countries (Tagny *et al.*, 2010).

2.2 Selection of a Suitable Blood Donor

The basic considerations in evaluating a prospective donor's eligibility to give blood include good health, age, consent, and ability to read and honestly answer questionnaires. Qualified staff should assess the donor's medical history and health status on blood donation day. Only prospective donors in good health should be allowed to donate blood or blood products (WHO, 2012). Recruitment and blood donation should be based on regularly reviewed donor selection criteria with a reliable communication channel without any form of discrimination. Blood transfusion service should also provide referral channels for further tests and treatment for donors presenting with medical conditions. The WHO recommends the following steps in the donor selection process (WHO, 2012).

2.2.1 Pre-donation Education

Pre-donation education is done through public campaigns before donors present to donate blood. The information is presented in a clear and simple language through graphics,

print media, orally, online articles, or audio-visual. All these materials aim at increasing awareness of blood donation and selection criteria. It should elaborate on the blood donation process and screening tests that will enable self-deferral. It should also help deferred donor return if the reason for deferral is temporary (WHO, 2012). In Kenya, advocacy for blood donation is rarely done. This could be a contributing factor to shortage of blood supplies. To avert this shortage there is a need for active campaigns and education on the importance of blood donation.

2.2.2 Donor Registration

All potential donors satisfying donor recruitment criteria should be registered in the donor register prior to blood donation. Vital donor registration details include; the donors' full name, nationality, area of residence, marital status, level of education, contact number, postal address, gender and date of birth. A unique donor identification number should be allotted and attached to the questionnaire, blood bag and sample tubes (WHO, 2012).

2.2.3 Filling of Donor Questionnaire

All blood donors must fill out a donor questionnaire with information relating to national blood donor selection criteria (Arslan, 2007). A donor is normally given a questionnaire to fill out before medical assessment and interview (Kissinger *et al.*, 2000). Alternatively, some blood transfusion centers send electronic questionnaires or post them to donors' residential addresses to be completed prior to donation. This method is advantageous by saving time and giving donors time to think about answers (Sanchez *et al.*, 2004).

However, some questions may need clarification and at times it may lead to self-deferral for the wrong reasons (Rahman *et al.*, 2011). Special attention is required for new or first-time donors to introduce them to the questionnaire and its importance. It is reported that regular voluntary non-remunerated donors take less time in filling out donor questionnaire (WHO, 2012).

2.2.4 Donor Interview

A filled donor questionnaire should be analyzed in an interview between donating staff and donor before donation. The purpose of an interview is to assess donors' health status, medical history and any risk of transfusion-transmitted infections. It also creates a platform to assess whether the donor read and understood the questionnaire and filled it well (Offergeld & Heiden, 2017). It has been reported that many donors do not understand medical terminologies and may not recognize the importance of their choices to their health (Sanchez *et al.*, 2004). Therefore, it is recommended that assistance be availed to anyone with challenges in filling out the questionnaire. Prospective donors should be assured of privacy and confidentiality if they disclose sensitive information (WHO, 2012).

2.2.5 Donor Informed Consent

Donor informed consent is a detailed document designed to inform the nature and processes involved in blood transfusion. Before consent, the blood transfusion service should furnish the donor with all information on the donation process and possible adverse events, screening tests on the samples collected and assurance of privacy and

confidentiality of personal data (WHO, 2012). A donor is expected to sign a consent form signifying they have understood the blood donation process and the risks involved, including adverse reactions (KNBTS, 2009).

2.2.6 Donor Deferral and Counseling

A prospective donor who fails to satisfy donor recruitment criteria should be disqualified as either permanent or temporary deferral. A disqualified donor should be handled with dignity and given a chance to ask questions regarding deferral (Kheiri & Alibeigi, 2015). Deferred donors should be told if their deferral is to protect the patient or their health. All these discussions should take place in a private location and be treated with the utmost confidentiality. An efficient donor recruitment service should ensure deferred donors are referred for further tests and treatment (WHO, 2012).

Previous studies have shown deferral of donors negatively affects future donations, especially for those deferred for 12 months and first-time donors. Unsatisfactory or unclear information to the deferred donor is also likely to negatively affect future donation (Bruhin *et al.*, 2020). Donors deferred for a period of less than 12 months should be told the reasons for deferrals and encouraged to participate in future donations. Many temporary deferred donors are likely not to donate, hence the need to recall them after deferral time elapses (Custer *et al.*, 2011).

2.2.7 Post Donation Care

All blood transfusion establishments are ambitious to minimize the incidence of adverse reactions related to blood donation. They strive to implement policies to safeguard donors' and recipients' health (Gilleta *et al.*, 2015). Adverse events associated with whole blood donation are classified as either general (vasovagal reactions) or local (venipuncture site) (AABB, 2014). Most adverse events are minor such as bruises, dizziness, or lightheadedness, but sometimes major adverse events may occur, such as loss of consciousness or nerve damage. These effects can be avoided or minimized by engaging a qualified, skilled staff, using appropriate donor recruitment criteria, and observing quality donor management care. However, blood transfusion centers must communicate to all prospective donors all risks likely to occur and measures are taken to minimize them (Anne *et al.*, 2010)

Every donor should be accorded high standards of care and assured of their well-being by the recruiting staff (WHO, 2012). Nonetheless, adverse/ vasovagal reactions may occur immediately or later following blood donation. Although adverse reactions are minimal in most centers, the prevalence of adverse reactions ranges from 1-10% (Saito *et al.*,2013; Eder *et al.*,2008). The prevalence of vasovagal reactions could be higher if the data is collected directly from donors after donation (Goldman *et al.*, 2013). Furthermore, the probability of a donor returning in subsequent donation reduces substantially in the event of an adverse reaction (Baart *et al.*, 2011). According to France *et al.* (2013) return rate among first-time donors is largely dependent on the occurrence of vasovagal reactions.

Several factors have been associated with vasovagal reactions. These factors include; donor status (repeat vs. first-time), age (young), gender (female), and donated blood volume (<3500ml) (Bravo *et al.,* 2011). Although vasovagal reactions due to blood donation are minimal, collection centers must adopt preventive measures to ensure the safety of the donor. Such measures include; applying muscle tensions (Wieling *et al.*, 2011), collection of adequate blood and administration of liquids (Wong *et al.*, 2013), training health care providers on the detection of symptoms and management of reactions (EDQM, 2013).

2.3 Total Blood Volume

2.3.1 Physiology and Blood Volume

Total blood volume (TBV) is defined as the total amount of fluid that always flows within venules, veins, capillaries, arteries, and heart chambers. Among healthy individuals plasma constitutes the highest percentage of about 60%, while other cellular components such as leukocytes, platelets, and erythrocytes account for 40% (Thibault *et al.*, 2009). An individual's TBV is dependent on their weight, gender and height, but it is presumed a healthy blood donor has more than five liters of blood circulating in their system. Physiologically women have reduced total blood volume compared to men (Ragav & Sandeep, 2020).

A potential donor is expected to have eaten 4-6 hours before blood donation, and following whole blood donation, they are supposed to take fluids. An individual's TBV is closely regulated by organ systems, hydration status, and sodium content. Systemic

dysfunctions such as liver failure, renal failure, sodium depletion, and hemorrhage can decrease or increase donors total blood volume (Ragav & Sandeep, 2020). Globally, assessment of donors' total blood volume (TBV) during recruitment is rarely practiced especially in the sub-Saharan Africa. To avert the occurrence of vasovagal reactions due to excessive donation, many countries have adopted different absolute quantities for whole blood donation based on their population anthropometrics (Karp & King, 2010). Worldwide, the volume of whole blood donations ranges from a minimum of 250ml in some Asian countries to a maximum of 525ml in the USA.

The minimum acceptable donor weight also varies globally (Karp & King, 2010). For instance, the WHO (2012) guidelines recommend a minimum donor weighing of 45kg, other countries such as Australia, India and Singapore also require a minimum donor weight of 45kg, in Hong Kong donor minimum weight is 41kg (Karp & King, 2010). In Kenya, a prospective donor weighing 50kg is expected to donate between 400ml and 500ml of whole blood per donation (NBTS, 2001). Donor weight has been an acceptable criterion used in approximating donors blood volume prior to donation. Different regulatory and scientific bodies have set safe limits for the percentage TBV donations as 10-15% (AABB, 2012). However, the WHO guidelines recommend a maximum donation limit of 13% (WHO, 2012).

2.3.2 Methods of Estimating Total Blood Volume

Assessment of a donor's TBV status is vital and sometimes a challenging area in blood transfusion. Clinically, a recruiting officer has to accurately assess whether a donor is

hypovolemic, euvolemic, or hypervolemic. For many decades, recruiting personnel in many developing countries have relied predominantly on their physical examination skills. Techniques for determining total blood volume have evolved since the nineteenth century. The standard method of determining TBV is a dilution technique that involves labeled radioactive indicators (Hilberath *et al.*, 2015). These dilution techniques were initially proposed over 70 years ago to help determine blood volume (BV) in cardiac surgery patients. Indocyanine and Evans Blue were the first dyes, which were later replaced by radioactive indicators. Chromium-51 was used to label red cells, while Iodine-125 or Iodine-131 was used to label plasma volume, the duo isotope techniques were combined to generate TBV (Stuart, 2007).

Adopting the duo isotope technique faced a myriad of challenges such as time-consuming (6-8 hours), prone to errors, labor-intensive, invasive procedures and possible side-effects of radioactive isotopes. Moreover, dilution techniques lacked acceptable reference ranges for blood volume parameters (Manzone *et al.*, 2007). Currently, clinical assessment of blood volume relies on indirect techniques such as changes in weight, lung sound, blood pressure, jugular vein distension, blood creatinine, urea and nitrogen. Sometimes clinicians use invasive central pressure monitoring or fluid challenge to assess blood volume in difficult patients (Donald, 2013).

The earliest mathematical formula used for estimating TBV was formulated by Dr. Allen and his colleagues in the year 1956. This mathematical equation was deduced from studies on blood volume (BV) involving a cohort of young healthy Taiwan, Chinese and

Asian medical students. It was initially used for heparin dose determination among patients undergoing cardiopulmonary bypass (CPD). Dr. Allen equation estimated TBV using a nomogram that relied on variables such as gender, weight and height (Lee *et al.*, 2019). However, according to Hilberath *et al.* (2015), most algorithms used in estimating BV, including the Dr. Allen formula, are unreliable, particularly in obese individuals.

The most recent mathematical equations for estimating TBV are the Nadler equation and the Lemmens-Bernstein-Brodsky's equation (Ragav & Sandeep, 2020). The Nadler formula was formulated in 1962 after a modification of Dr. Allen's work by Dr. Nadler and his colleagues (Nadler *et al*., 1962). In 2006, Drs. Lemmens, Bernstain, and Brodsky observed the existence of several methods used for estimation TBV, which were impractical to use in a clinical set-up and hence developed the Lemmens-Bernstein-Brodsky's equation (Jia *et al.*, 2013). The trio described the formula as simple, which allowed clinicians to estimate TBV over body mass indices (BMI) and a full spectrum of body weights in patients unstressed by critical illness or acute trauma (Lemmens *et al.*, 2006).

According to Ragav and Sandeep (2020), Lemmens-Bernstein-Brodsky's equation is more reliable for higher ranges of body mass index (BMI) and body weight. In Europe, a modified version of Nadler's equation was adopted to calculate donors' TBV. A reliable assessment tool for estimating total blood volume is crucial in safeguarding blood donors' health and managing cardiovascular disorders. According to Holme *et al.* (2008), TBV equations are simple and as accurate as those utilizing skinfold thickness or lean

body mass. There is a scarcity of literature on TBV estimation among prospective blood donors in Kenya and Africa in general.

2.4 Blood Donor Iron Status

2.4.1 Iron Deficiency and Anemia

According to WHO (2011), approximately 500 million people worldwide are affected by anemia which emanates from iron deficiency (ID). It develops as a result of excessive iron loss via hemorrhage, fault in hemoglobin synthesis, and the limited ability of the body to absorb iron (Hoffbrand & Moss, 2011). Moreover, in many resource-limited countries, the dietary intake of iron is inadequate in childhood (Obeagu *et al.*, 2016). Although iron is among the most abundant elements on Earth's crust, its bioavailability is scarce, making it the commonest cause of anemia globally (Obeagu *et al.*, 2016). Iron deficiency is characterized by reduced red blood cells (RBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH).

In humans, food is the main source of iron. Depending on the diet the iron content is dependent on the daily caloric intake and the type of food. Good sources of dietary iron include leafy vegetables, fortified bread, lentils, poultry, fish, red meat and black-eyed beans (Hoffbrand & Moss, 2011). Other sources of iron include; intravenous (IV) injections, oral iron supplement and blood transfusion (Andrews, 2002). Dietary iron may exist as heam or non-heam. Heam iron is available in chicken, fish and red meat, while non-heam iron is present in cereals, vegetables and grains (Sembulingam & Sembulingam, 2006). According to Hoffbrand & Moss (2011), the daily iron requirement

is dependent on weight, age, sex and state of health. A well-balanced diet contains about 10 to 15mg of iron, of which 5 to 10% is absorbed. Intake of a well-balanced diet can supplement iron requirements where there is a pathology such as hemorrhage (Hoffbrand & Moss, 2011).

Donating whole blood removes a significant quantity of donors' iron stores. To safeguard a prospective donor's health, iron stores are indirectly gauged by screening the donor's hemoglobin and weight (Baart *et al.*, 2014). Prospective blood donors with comparatively reduced hemoglobin values without any signs or symptoms of anemia are typically disqualified from donating blood to prevent advancement to iron deficiency anemia (IDA). Additionally, deferral of prospective donors with reduced hemoglobin values guarantees hematological profiles of donated units meet the standard thresholds (Mast *et al.*, 2010).

2.4.2 Epidemiology of Iron Deficiency

Iron deficiency accounts for about 50% of all anemia cases in sub-Saharan Africa (Crawley, 2004). In Kenya, few studies have been done on iron deficiency anemia among various groups. According to Foote *et al.* (2013), the prevalence of anemia among children aged 6-35 months in the Nyanza region was 71.8%, while Leenstra *et al.* (2004) found a prevalence of 19.8% among adolescent school girls in Western Kenya. This study aimed to determine the prevalence of iron deficiency anemia among eligible blood donors in Kenya.

According to the WHO (2011) the prevalence of anemia among women of reproductive age in the European region was 19.9%. This prevalence was predominantly due to ID affecting women aged 15-49 years. Non-pregnant women in developing countries are suspected of having reduced or depleted iron stores (Eder & Dyb, 2008). Another study by Karin *et al.* (2018) reported a prevalence of iron deficiency at 17.5% among the South African blood donors. However, the study showed more male donors were affected by iron deficiency than female donors (ID of 18.6% and 16.3% for males and females, respectively). According to Cable *et al.* (2011), 16.4 % of males and 27.1% of female repeat donors had an iron deficiency in the United States with ferritin concentrations below 12ng/ml. Another study on the Danish population documented the prevalence of iron deficiency (<15ng/ml) among female and repeat male donors at 39% and 9%, respectively (Rigas *et al.*, 2014).

According to Schotten *et al.* (2016), the prevalence of iron deficiency anemia can be reduced by the provision of iron supplements or extension of donation intervals. Extension of donation interval is preferred over iron supplements due to side effects associated with taking iron tablets. The side effects of iron supplements include; poor compliance, negative perceptions and gastrointestinal disturbances (Moore *et al.*, 2014). According to Angelantonio *et al.* (2017), extending the donation interval is associated with a reduced risk of low hemoglobin deferral. The study further observed donation intervals of 8- 12 weeks for men and women, respectively, would lead to significantly reduced ferritin and hemoglobin concentration compared to 12-16 weeks intervals for men and women respectively.

2.4.3 Etiology of Iron Deficiency among Blood Donors

In resource-limited countries, chronic blood loss emanating from uterine bleeding and the gastrointestinal tract are the main causes of ID among adults (Baart *et al.*, 2013). A blood bag of 450 – 500 ml contains approximately 200 mg to 250 mg of heme iron, but the actual loss of iron is proportional to the hemoglobin concentration of a prospective blood donor (Brittenham, 2011). Chronic blood loss is characterized by a negative iron balance, despite increased absorption of dietary iron at the initial stages of iron deficiency (Hoffbrand & Moss, 2011).

There are several reasons supporting regular blood donation. Some of the benefits include promoting blood supplies, hindrance of accumulation of iron which can form free radicals in the body and challenging bone marrow to sustain hematopoietic activities which will continuously produce red cells (Adediran *et al.*, 2013). Although donating blood is advantageous, donating a single unit of 450 ml leads to iron loss of about 247± 17 mg. However, first and second donations do not adversely affect donors' iron stores (Mozaheb *et al.*, 2011). Donors with a history of multiple donations have their iron stores decreased due to negative iron balance. In addition, continuous /regular donation can lead to ID that would eventually turn into anemia (Gullbring, *et al.*, 2015). According to Mozaheb *et al.* (2011), the number of donations within one year is more predictive for decreased ferritin concentrations than the number of donations in a donors lifetime.

2.4.4 Iron Overload

Iron overload (haemosiderosis) is a condition occasioned by abnormalities in the intestinal epithelium that results in uncontrolled absorption of dietary iron in the gut (Levy *et al.,* 2000). The human body lacks a physiological mechanism for excreting excess iron. Therefore, uncontrolled absorption of iron results in unnecessary accumulation of iron (Hoffbrand & Moss, 2011). Excessive accumulation of iron deposits in tissues can cause severe damage to body organs, particularly the endocrine organs, liver, and heart (Lichtman & Beutler 2010).

Hemosiderin deposition is a common feature in tissues and organs affected by iron overload characterized by a deep brown color (Garg *et al.*, 2012). According to Lichtman & Beutler (2010), clinical features associated with iron overload include cardiomyopathies, darkening of the skin, and cirrhosis of the liver. Symptoms attributed to haemosiderosis include impotence, loss of libido, fatigue, lethargy, weakness, and arthopathies. Other causes of iron overload include; increased iron intake and infusion of iron via repeated red cell transfusions and medicinal iron (Garg *et al.*, 2012).

2.4.5 Iron Absorption, Transport and Storage

The human body has no mechanism for excreting iron and thus, the control of dietary iron absorption plays a vital role in iron metabolism in the body. Dietary iron is present in food as a heme protein complex, ferric protein and ferric hydroxides. Absorption of dietary iron involves a very complex process that occurs in the upper jejunum and duodenum (Hoffbrand & Moss, 2011). Organic dietary iron is partly digested in the gut to

inorganic iron and partly absorbed as heme. Heme is absorbed on the apical membrane through a receptor yet to be established. Heme is then broken down to release iron. Absorption of inorganic iron in the gut is favored by factors such as reducing agents and acids that maintain iron in the gut in a ferrous state (Fe^{2+}) rather than a ferric state (Fe^{3+}). The quantity of dietary iron absorbed is controlled by changing levels of ferroportin (Andrews, 2002).

Ferroportin concentration is majorly controlled by hepcidin. Iron deficiency with low hepcidin levels triggers increased ferroportin levels allowing more iron to enter the portal plasma (Hoffbrand & Moss, 2011). Apical membrane harbors ferrireductase that converts iron from ferric state (Fe^{3+}) to ferrous (Fe^{2+}) state and another enzyme, ferrioxidase (hephaestin), convert's iron from ferrous (Fe^{2+}) state to ferric state (Fe^{3+}) at the basal surface before binding to transferrin. Three proteins mainly mediated the storage and transport of dietary iron, ferritin, transferrin receptor 1 (TfR1) and transferrin *(*Adeniran *et al*., 2013).

Transferrin is composed of up to two atoms of iron. It delivers iron to transferrin receptors such as erythroblast that incorporate iron into hemoglobin. After the maturity of the red blood cells, they are broken down by macrophages in the reticuloendothelial system releasing iron from the hemoglobin (Watt, 2010). Iron then enters the plasma providing most of the iron on transferrin. Dietary iron provides only a small proportion of plasma transferrin absorbed via the jejunum and duodenum. Macrophages stores iron as

haemosiderin and ferritin, and the quantity varies widely depending on the overall body iron status (Reddy *et al*., 2020).

According to Obeagu *et al.* (2016), all cells have the ability to synthesize proteins, and store excess iron. Approximately, 100-1000mg of iron is preserved in the liver hepatocytes and reticuloendothelial cells as haemosiderin and ferritin. Another study by Kurz *et al.* (2011), reported that iron had two functions. First, it is used for cellular needs. Secondly, the excessive iron stored as ferritin shields the cells from possibly toxic reactions catalyzed by iron.

2.4.6 Pathogenesis of Iron Deficiency

According to Obeagu *et al.* (2016), iron deficiency develops in stages. The development of iron deficiency involves the depletion of iron stores in different compartments in a sequential and overlapping manner. Iron deficiency can be broadly classified into three stages: iron deficiency anemia, iron-deficient erythropoiesis, and negative iron balance (Tailor *et al.*, 2017). Stage I (negative iron balance) is characterized by decreased bone marrow iron store but normal hemoglobin and red cell indices (Ferritin > 15µg/l but <20µg/l). In stage II (iron-deficient erythropoiesis), body iron stores are depleted but Hgb formation is not affected (Ferritin <15µg/l with normal Hgb) and in stage III (iron deficiency anemia), hemoglobin synthesis is affected, leading to anemia (Ferritin <15µg/l with Hgb <12.5g/dl) (Chung *et al.*, 2013).

A recent publication from the World Health Organization (2020) defines ferritin levels < 15ng/ml as iron deficiency, ferritin concentrations between >15ng/ml - <200ng/ml as healthy individuals and ferritin levels above >200ng/ml as individuals at risk of iron overload. A previous study by Tailor *et al.* (2017) defined ferritin levels below 15 ng/ml as iron deficient, 15 - 20 ng/ml as borderline, and those greater than 20 ng/ml as normal.

2.4.7 Assessment of Iron Status

Several laboratory tests can be used to determine iron deficiency states. Unfortunately, there is no single standard technique/test to date that can accurately screen for iron deficiency without anemia. Furthermore, diagnostic tests related to iron fail to correlate due to diverse iron metabolism stages (WHO, 2011). For decades, the assessment of iron status has relied on simple tests such as; serum ferritin, transferrin receptors, serum transferrin saturation, serum iron, red cell indices (MCH and MCV), peripheral blood film, bone marrow iron stain, and total iron-binding capacity (Hershko, 2018). Other tests include the measurement of red cell protoporphyrin levels, reticuloendothelial iron stores, and reticulocyte hemoglobin (Obeagu *et al.*, 2016).

Serum ferritin concentration has shown to be a good and well-studied single indicator of iron status in blood donors (Goldman, 2015). The relative total body iron stores correlate well with the serum ferritin levels making it the most specific biochemical test. Depleted iron stores (early stages of iron deficiency) are reflected in a low serum ferritin concentration (WHO, 2001). Consequently, a normal range for serum ferritin reflects adequate iron stores in healthy individuals without any inflammatory or infectious

conditions (WHO, 2001). Reference ranges for most laboratories range from 30ng/ml to 300ng/ml, with a mean of 88ng/ml for males and 49ng/ml for females. According to Hoque *et al.* (2016), testing blood donors' serum ferritin concentrations varies globally. For instance, Denmark screens all new donors, while Italy and Cyprus tests all repeat donors once a year (Vuk *et al.*, 2017). The most reliable and sensitive tool for screening donor iron status is plasma/serum ferritin concentration (Mozaheb *et al.*, 2011). This study aimed to determine the iron status of all eligible blood donors attending KNH for whole blood donation.

2.4.8 Limitations of Serum Ferritin as a Biomarker of Iron Status

Serum ferritin is an acute-phase protein. It is significantly affected by inflammations and infections, which may limit its interpretation (Catharine, 2017). Screening for iron deficiency using ferritin concentrations alone, may give normal results in the presence of inflammations/infections. Therefore, additional confirmatory tests such as CRP are recommended. According to Borel *et al.* (2011), serum ferritin concentrations may vary by 27% in women and 15% in men daily. Furthermore, serum ferritin assays may also be influenced by difference in the reference ranges and technical variations such as calibration procedures (Dignass *et al.*, 2018).

2.5 Hematological Profiles

A hematologic profile is a screening test that evaluates a blood sample for a variety of blood parameters. It gives a tally for each cell type present in a given blood sample (Lugos *et al.*, 2019). Hematological profiles are commonly used to diagnose blood

disorders among individuals exposed to diverse environmental conditions and from different ancestral origins. Acceptable reference ranges for hematological parameters vary with age, gender and ethnic population (Mast *et al.*, 2010). Genetic characteristics of study populations are also believed to account for variations of most hematological indices (Buchanan *et al.*, 2010).

A hematology profile will determine the amount of red blood cells, hemoglobin, white blood cells and platelets among others cells. Tests for red blood cells and their indices such as hemoglobin are crucial in identifying anemia, a condition characterized by insufficient iron in the blood (Lugos *et al*., 2019). Tests for white blood cells and its subsets such as lymphocytes are used to indicate different medical conditions. For example, reduced or elevated white blood cells besides the normal may be an indication of; clonal disorders, hematologic pre-malignant, failure of production or infections. Generally, an abnormal increase in total white blood cell counts is an indication of bacterial infection. Hemostatic disorders are mainly associated with elevated or reduced platelet counts (Hoffbrand & Moss, 2011).

There are variations between acceptable reference ranges obtained from different donor populations (Yalew *et al.*, 2016). For instance, a study on the American donor population reported that African-Americans have lower average mean corpuscular volume (MCV), hematocrit and hemoglobin levels than their Caucasian compatriots living in the same environment, with the same sex and age (Beutler & West, 2005). However, according to a transfusion research group report, the screening of all hematological parameters is very

low during the recruitment of donors (Tagny *et al.,* 2010). Therefore, adopting appropriate local reference ranges for hematological profiles is important for donor screening, diagnosis, treatment and follow-up.

The reference ranges for hematological parameters currently used in most African and Asian countries were heavily borrowed from developed countries and may not be applicable in most geographical locations (Haileamlak *et al.,* 2012). Implementing borrowed hematological reference values, may fail to detect an underlying disease or cause unessential further investigations (Dosoo *et al.*, 2013). Furthermore, previous studies conducted in African and Asian countries documented differing hematological profiles than those from populations in developed countries (Roshan *et al.*, 2013; Kueviakoe *et al.*, 2011). Due to variations in hematological parameters, the WHO (2017) has suggested consideration of sex, age, ethnicity and other local evidence when formulating appropriate reference ranges for hematological parameters.

Despite the recommendations to regularly review reference ranges based on local demographic factors, there is limited information on the hematological profile among eligible blood donors in Kenya. The existing reference range used for screening hemoglobin in donor recruitment was heavily borrowed from the American and European populations over two decades ago (KNBTS, 2001). These reference ranges may not be appropriate for the Kenyan population. This study aimed to determine the hematological profiles of presumably healthy blood donors allowed to donate whole blood at Kenyatta National Hospital, Kenya.

It is essential to adopt reference ranges that are population or region-specific to improve accuracy in interpreting laboratory results. Previous studies had reported that Kenyan hematological reference ranges are generally lower than those used in Europe and American States (Geoffrey *et al.*, 2018; Rose *et al.*, 2018). Another study in western Kenya mentioned if US-derived reference ranges were used in Kenya, more than 58.0% of participants in a clinical trial would have been deemed unfit (Zeh *et al.*, 2011). Therefore, this study aimed to determine the hematological abnormalities among eligible blood donors at KNH.

2.5.1 The Red blood Cells

Human make approximately 10^{12} new red blood cells (erythrocytes) each day through a finely regulated process of erythropoiesis (Segal *et al*., 2012). In adult life, erythropoiesis is mainly confined to humeri and femurs proximal ends and central skeleton. The formation of red blood cells begins with stem cells and involves various stages of development. The erythropoiesis process is controlled by a hormone called erythropoietin. Production of this hormone is triggered by the inability of hemoglobin to release O_2 and in cases of anemia (Murphy, 2014).

A normal red cell has a flexible biconcave shape with a diameter of 8µm; this size facilitates its movement through microcirculation, whose minimum pore size is 3.5µm. It carries hemoglobin to tissues for successful gaseous exchange. Red cells have a lifespan of 120 days, and it is estimated to cover a distance of 300 miles (Hoffbrand & Moss, 2011). To fulfill its functions, it can generate its' own energy (adenosine triphosphate)

anaerobically through the Embeden-Meyerhof pathway. The pathway also generates nicotinamide adenine dinucleotide hydrogen (NADH) which methaemoglobin reductase uses to reduce dormant oxidized hemoglobin to actively reduced hemoglobin. Methaemoglobinaemia is a clinical state characterized by circulating oxidized (ferric) hemoglobin instead of the usual reduced (ferrous) state (Orlov & Karkouti, 2015).

2.5.2 Hemoglobin

Hemoglobin is a protein molecule in the red cells that carries carbon dioxide from the tissues to the lungs and returns oxygen from the lungs to the tissues. A normal red cell carries about 640 million hemoglobin molecules (Hoffbrand & Moss, 2011). The adult hemoglobin consists of an iron-porphyrin compound called heme and four polypeptide chains ($\alpha_2\beta_2$). The iron-porphyrin group is responsible for the red color of the red cells. Among healthy adults, Hgb A is the most dominant hemoglobin, with a molecular weight of 68000 Da, other hemoglobin molecules are Hgb $A_{2,}$ and Hgb F. Hemoglobin molecule plays a crucial role in maintaining the shape of red cells. Abnormal hemoglobin structure can disrupt red cell functions and shape (Desmarets *et al.*, 2016).

Anemia is a condition characterized by reducing hemoglobin concentration in the blood below the acceptable reference range for sex and age (Osungbade & Oladunjoye, 2012). Anemia may be classified based on the cell morphology (microcytic-hypochromic, macrocytic), their erythropoietic response (ineffective, hyperproliferative) or etiology (hemolytic, hemorrhagic) (Kenneth *et al.*, 1992). Although the accepted reference ranges

for hemoglobin vary between countries, the WHO (2008) defines Hgb thresholds for anemia as 13g/dl for adult men and 12g/dl for adult women.

2.5.3 Red Cells Indices

In 1929, Wintrobe introduced red cell indices, which were mean cell hemoglobin (MCH), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) (Hoffbrand & Moss, 2011). The mean cell volume is defined as red blood cell average size; it is expressed as femtoliters. The other two MCHC and MCH quantify hemoglobin per unit volume and per red cell, respectively. Red cells indices are crucial in interpreting the etiology of anemia. These indices can be calculated if the values of red blood cell count (RBC), hemoglobin, (Hgb) and packed cell volume (PCV) is known. With the advent of advanced cell counters these indices are now calculated automatically (D'Ambrosio, 2013).

2.5.4 White Blood Cells Parameters

White blood cells are also known as leucocytes, are cellular elements responsible for the body's defense mechanism (Abbas *et al.*, 2015). Leucocytes are produced in the bone marrow. They are broadly classified into granulocytes and agranulocytes based on the presence or absence of intracellular granules. Granulocytes are further sub-classified into basophils, eosinophils and neutrophils. The other cells that lack granules in their cytoplasm include B-lymphocytes, T-lymphocytes, macrophages and monocytes (Gordon, 2013). The various types of white blood cells serve different functions. The distribution and the number of leucocytes in the body fluctuate; however, each parameter

has a specific percentage and a specific reference range. According to Faris *et al.* (2020), the acceptable references range for the various WBC parameters varies according to the individual's age. This study aimed to establish the percentage distribution of white blood cells among eligible donors based on their demographic characteristics.

Leucocyte count is a common measure used as a diagnostic test to detect diverse medical conditions. Eligible blood donors should be from a population group with low infections (Abbas *et al.*, 2015). According to a review study by Urquhart *et al.* (2008), obese people have a higher leucocyte count than non-obese individuals. A recent study by Taha *et al.* (2018) found a significant difference between body mass index (BMI) and age, and there was an insignificant difference between females and males in white blood cell count. Another study by Roshan *et al.* (2013), observed a considerable difference in hematological values between African and Caucasian people. To date, no recent study has been conducted on the leucocyte count in apparently eligible blood donors in Kenya. Therefore, this study aimed to determine white blood cells profiles and differential counts among eligible blood donors.

2.5.5 Screening of Hematological Parameters among Blood Donors

Complete blood count (CBC) is a common test done when screening for diseases. A CBC analyzer uses whole blood sample preserved in an anticoagulant known as EDTA (Bursey *et al.*, 2013). It has the potential of giving useful information that assists in the diagnosis, screening and management of diseases. In a blood donation set-up, an abnormality in the CBC report will inform the recruitment officer of the presence of a

hematological disorder. Hematological abnormalities can also be checked by individual flags provided by the analyzer which indicate values outside the reference ranges (Carey, 2015). The utilization of a complete blood count analyzer in the screening of blood donors is limited to resource-rich countries. In Kenya, only a hemoglobin test is done when screening prospective blood donors for anemia (KNBTS, 2001).

There are various methods used to estimate hemoglobin levels among potential blood donors. Some of these methods include; HemoCue, portable hemoglobinometer, sahli's, cyanmethemoglobin, and colorimetry-hemoglobinmeter (Adam *et al.,* 2012). At KNH blood donation unit, a portable hemoglobinometer (NEO Immuno-Gamma) was used in screening donors' hemoglobin. Screening hemoglobin protects anemic donors from donating whole blood (Boulton, 2008). Accepting donors with normal Hgb levels ensures the safety of donors and recipients (Eder, 2011).

2.5.6 Hematological Reference Intervals

In Kenya, few studies have been carried out to establish local hematological reference intervals. In addition, comparison studies have found variation in local reference intervals despite being carried out in the same Country (Sing'oei *et al.,* 2021). For instance, a comparison study on hematological reference intervals among infants living in Kisumu and Kilifi counties found Kisumu ranges were higher for hemoglobin, hematocrit and platelets. This variation was attributed to the difference in ethnic composition, altitude, environmental factors and exposure to infectious diseases (Sing'oei *et al*., 2021).

This study aimed to understand the hematological profiles of eligible whole blood donor noting that the Kenyan policy is silent on screening hematological profiles. This study adopted hematological reference intervals derived from the adult urban population in Kenya (Omuse *et al*., 2018). The reference intervals were deduced from subjectively healthy black African adults aged 18 to 65 years. The participants were drawn from Kenyan urban towns located in Nairobi, Kiambu, Nakuru and Thika. Recruitment was largely done from shopping malls, corporations, hospitals, churches, universities and colleges. Moreover, the CLSI (2010) guidelines were used in inclusion and exclusion of participants (Omuse *et al*., 2018). These reference intervals were used in comparing hematological profiles of whole blood donated by eligible donors at KNH. Table 2.1 below shows hematological reference intervals derived from adult urban population in Kenya.

Table 2.1: Complete blood count reference intervals for adult population in Kenya.

Parameter	Combined (Male & Female) (LL – UL)	Male (LL – UL)	Female (LL – UL)
RBC (x10^12L)	4.41-6.48	4.94-6.52	4.31-5.76
HGB (g/dL)	12.8-19.0	14.5-18.7	12.0-16.5
HCT (%)	38.0-55.0	43.0-55.0	36.0-49.0
MCV (fL)	75.7-95.6	76.5-95.5	73.4-95.8
MCH (pg)	24.8-33.8	25.1-32.8	24.4-32.7
MCHC (g/dL)	32.2-35.2	32.4-35.4	32.0-35.0
WBC (x10^9L)	3.08-7.83	3.13-8.10	2.89-7.72
Lym (%)	27.2-60.0	28.2-60.3	25.2-59.3
Lym # (x10^9L)	1.29-3.40	1.36-3.58	1.22-3.24
Neu (%)	28.0-63.3	27.4-60.3	29.5-65.4
Neu # (x10^9L)	1.05-4.08	1.02-3.92	1.07-4.42
Mon (%)	3.4 -13.3	3.5-14.3	3.2-11.0
Mon# (x10^9L)	0.14-0.74	0.15-0.76	0.14-0.68
Eos (%)	1.1-11.9	1.2-11.8	0.8-9.4
Eos # (x10^9L)	0.04-0.59	0.05-0.64	0.04-0.49
Bas (%)	0.30-1.10	0.40-1.20	0.30-1.00
Bas # (x10^9L)	0.0-0.07	0.01-0.08	0.01-0.06
PLT (x10^9L)	144-409	133-356	152-443

KEY: # = absolute count, % = differential, LL=lower limit, UL= upper limit, RBC = red blood cells (erythrocytes), Hgb = Hemoglobin, Hct = Hematocrit, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, WBC = white blood cells (leucocytes), Lym= lymphocytes, Net= neutrophils, Mon= Monocytes, Eos= eosinophils, Bas= basophils and PLT= platelet (**Source:** Omuse *et al*., 2018).

2.6 Blood Donor Deferral

2.6.1 Types of Donor Deferral

A prospective donor willing to give blood may be deferred from donating whole blood for various reasons relating to the recipient or donors' safety (Birjandi *et al.,* 2013). A donor may be deferred temporarily or permanently owing to suspicion or confirmation of having a medical condition such as hematological disease or infectious condition that may affect donors' own health or influence the safety of the donated blood (Bruhin *et al*., 2020). Temporary deferral implies a potential donor is disqualified based on time-bound, removable factors such as medication, hemoglobin and more. In contrast, permanent deferral connotes that a prospective donor has a long-lasting, non-removable factor such as chronic diseases (Aneke *et al.,* 2016). Although there is a need to ensure adequate blood supply, the caveat relies on the availability of donors and eligibility criteria (Chauhan *et al.,* 2015).

All prospective blood donors deferred for various reasons are expected to be treated with decorum and given a clear explanation of the cause of deferral. They should also be allowed to ask questions (WHO, 2012). Deferral rates among prospective blood donors vary widely, for instance; studies in Asia report that blood donor deferral rates differ from one region to another (Abdelaal, 2016; Kasraian & Negarestani, 2015; Ngoma *et al.,* 2014). Other studies done in European counties revealed a slightly lower donor deferral rates than in Asia counties (Smith *et al*., 2013). This study aimed to explore temporary and permanent deferral rates at Kenyatta National Hospital in Kenya.

2.6.2 Causes of Donor Deferral

Studies analyzing causes of blood donor deferral have mentioned variation of causes between countries and continents (Kasraian and Negarestani, 2015). In many studies, low hemoglobin concentrations have been cited as the leading cause of temporal deferrals (Smith *et al*., 2013). For instance, a study done in Turkey reported low Hgb as the main cause of temporary deferral at 20%. The same trend was revealed in studies done in the Netherlands and Asian countries (Baart *et al.*, 2013; Meinia, 2016). According to Njenga *et al.* (2019) Kenyan donors deferred due to low hemoglobin stood at 10.1%. The current study aimed to determine the causes of donor deferral at Kenyatta National Hospital.

Two independent studies observed hepatitis B and HIV as the leading cause of permanent deferral (Kasraian & Negarestani, 2015; Tufail *et al.*, 2013). A recent study done in Kenya reported hepatitis B as the leading cause of permanent deferral in Nakuru and Bomet counties (Bartonjo *et al.*, 2019). Other studies conducted in Africa showed that more males are deferred than female donors. According to Valerian *et al.* (2018) more males in Tanzania are deferred than females (12.0 % vs. 15.6%, respectively). Another study in Ivory Coast reported higher deferral rates (75.2%) among male donors with the majority being repeat donors (Kouao *et al.*, 2012). Understanding specific causes for temporary and permanent deferrals is crucial in establishing appropriate mitigation measures and referral systems.

The prevalence of transfusion-transmitted infections also varies in different countries, with developed countries recording lower rates than developing states. According to a

recent study done in Kenya, the overall prevalence of TTIs was 14.1% with family replacement recording the highest prevalence of 25% (Bartonjo *et al.,* 2019). The risks of transfusion-transmitted diseases are higher in countries practicing replacement or paid donation (Kiba *et al.,* 2012). However, this widely accepted belief was challenged with evidence by four studies in sub-Saharan counties neighboring Burkina Faso that observed insignificant difference between voluntary and replacement donors (Loua & Nkoure, 2010; Mbanya *et al.,* 2010; Diarra *et al.,* 2009; Allain *et al.,* 2010).

2.6.3 Policies on Blood Transfusion

To alleviate the acute shortage of whole blood and its products, most of African countries often rely on replacement or family donors. However, family replacement donation has been cited to pose a greater risk for TTIs (van *et al.,* 2010). Donor funds from developed countries have helped many African countries like Kenya establish structures to promote reliance on voluntary donation, donor screening, laboratory infrastructure, accreditation, continuous quality improvements, information systems and development of national blood transfusion policies (Chevalier *et al.,* 2016).

Countries in Sub-Saharan Africa have continuously improved access to adequate and safe blood supplies. To maintain these gains and achievements continued commitment and funding are required. Although blood collection has increased gradually since 2004, collected blood units are yet to meet the national demand (WHO, 2016). Recent studies show that about 50% of African countries supported by PEPFAR lack a computerized information system for tracking blood donors and screening transmission of transmitted

infections. As of 2016, only four African countries (Tanzania, Rwanda, South Africa, and Namibia) had achieved accreditation of their National Blood Transfusion Service (WHO, 2016).

The Kenya National Blood Transfusion Service (KNBTS) was launched in 2000 under the Ministry of Health. The existing Kenya policy document on donor selections and blood donation is limited in content. Other policy documents include; Guidelines on Appropriate use of Blood and Blood Products, and Hemovigilance Manual for Transfusion Service in Kenya. The KNBTS has stratified its services into; regional blood transfusion centres and satellite centres. Satellite centres collect blood and transfer them to regional blood centres where they are tested, grouped, stored, and distributed to hospitals. The KNBTS guidelines recommend screening of all donated units for HIV, syphilis, hepatitis B, and hepatitis C virus. The same model has been used in some African states like Nigeria (FMH, 2006). The Kenyan donor recruitment questionnaire has been in use since 2009, when it was last reviewed.

According to Kenyan guidelines, a donor will be allowed to donate blood if they weigh above 50 kg, with Hgb above 12.5g/dl, age between 16- 65 years (< 18 years consent is required from parent or guardian). The blood volume collected shall not exceed 500ml (KNBTS, 2001). Though the current donor eligibility criteria has been in existence for close to two decades, it is too general and misses guidelines on some questions captured in the donor questionnaire. Assessment of donor total blood volume, iron status and hematological profiles is not captured in the current donor recruitment guidelines.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the Kenyatta National Hospital-Blood Transfusion Unit (KNH-BTU). The facility is located in Nairobi County, Kenya. The KNH-BTU works in conjunction with the National Blood Transfusion Services (NBTS) to bridge the gap by mobilizing replacement donors and conducting blood drives. The KNH serves as the largest referral hospital providing specialized medical care to the country's population, and referrals from neighboring countries (Salah, 2014). The hospital has 24 theatres, 22 out-patient clinics, and an accident and emergency department, with over 1800 beds in the wards (Salah, 2014). A blood donation unit was created to meet the hospital's high demand for blood. The blood donation unit coordinates blood donation, screening, storage, and dispatch. The transfusion unit mainly targets replacement donors who present to donate blood to their relatives and friends.

3.2 Study Design

This study adopted a cross- sectional study design targeting whole blood donors attending Kenyatta National Hospital-Blood Transfusion Unit.

3.3 Study Population

This study targeted whole blood donors attending Kenyatta National Hospital-Blood Transfusion Unit for whole blood donation.

3.3.1 Inclusion Criteria

This study recruited blood donors who consented to the study. The blood donors were aged 18 to 65 years.

3.3.2 Exclusion Criteria

Potential blood donors not consenting to this study and blood donors aged below 18 years and above 65 years were exempted from this study.

3.4 Sample Size Determination

The study sample size was determined using Fisher's *et al.*, (1998) formula. In the absence of existing reference studies on donors TBV, a prevalence of 50% was used to calculate sample size of the study population.

$$n = \frac{z^2\, pq}{e^2}$$

n= represent samples size,

z = confidence interval 1.96 (95%)

p = 50% (Taking 50% as the proportion of donors attending in KNH-BTU

q= calculated as 1 - p

e = level of precision (± 5%)

$$n_0 = \frac{1.96^2\ (0.5 \times 0.5)}{0.05^2}$$

= 384 participants.

Therefore, this study recruited 384 whole blood donors.

3.5 Sampling Technique

Study participants were sampled using a systematic random sampling technique. The sampling was done at the KNH-BTU blood donor registration desk. The KNH-BTU receives approximately 2100 blood donors per month. Sampling interval K^{th} was calculated as; $= \frac{N}{n}$. Where, population size N is (2100), and the sample size is n (384), giving a sampling interval (*K*) of 5. Every day, sampling started from a donor registered as number two, intervals of five were used to identify potential study participants. The recruitment process was done continuously until 384 study participants were recruited. Those who agreed to the study were requested to fill out a recruitment/ enrolment form (Appendix II or V).

3.6 Recruitment and Consenting Procedures

All prospective blood donors identified by the sampling technique were introduced to the study. After recruitment, each participant made an informed consent and answered a screening questionnaire (Appendix I or IV and Appendix III or VI), respectively. A screening questionnaire was used to group study participants into categories A and B. Category A comprised participants who were allowed to donate. In contrasts, category B encompassed of participants who were deferred from blood donation.

3.7 Study Flowchart

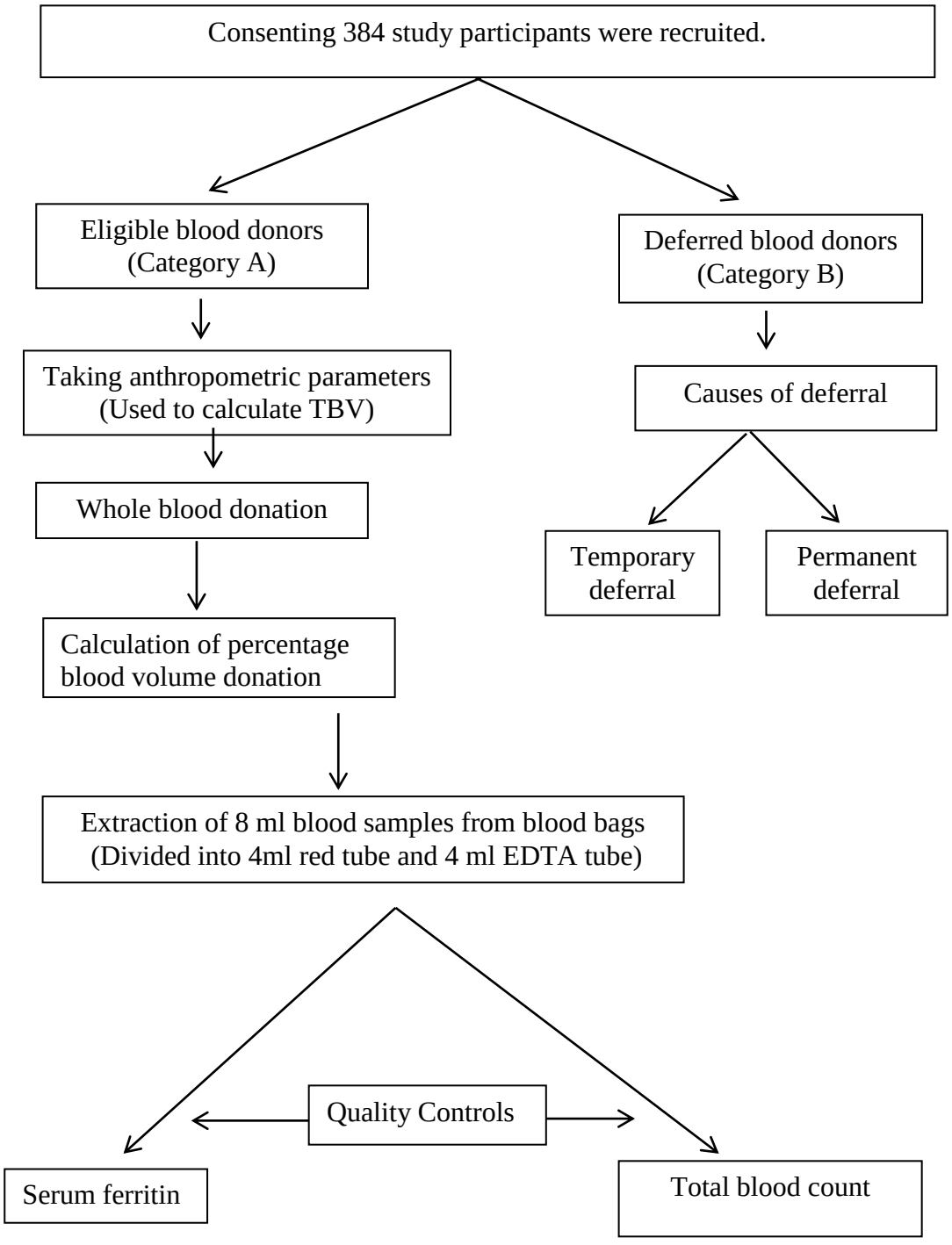

Figure 3.1: Study flowchart illustrating the study stages

3.8 Data Collection

3.8.1 Demographic Data

Filled study questionnaires (Appendix II and III) were used to collect participants' demographic information such as donation type (voluntary or replacement donation), gender, donor status (first-time or repeat), age, marital status, frequency of donation, last time of donation, type of deferral and reason/s for deferral.

3.8.2 Temporary and Permanent Deferrals

Prospective donors who failed to meet donor eligibility criteria (category B) were recorded as deferrals. Deferred donors were broadly classified as temporary or permanent deferrals. The maximum deferral time for those who were temporarily deferred was a period not exceeding one year ($\leq$12 months).

3.9 Measurement of Anthropometric Parameters among Eligible Donors

All prospective donors who met donor recruitment criteria and consented to the study were screened for anthropometric parameters; height (cm), weight (kg), and basal metabolic index (BMI) using calibrated scale. Donors' height was measured without shoes, and weight was assessed with only indoor clothing and correlated to the nudity state by subtracting 1kg. The basal metabolic index categories were classified using WHO classification (underweight <18.5kg/m^2, normal weight 18.5-24.9 kg/m^2, overweight 25-29.9 kg/m^2, and obese >30 kg/m^2) (WHO, n.d.). Data collected was used to calculate total blood volume (TBV) using Lemmens-Bernstein-Brodsky's equation (Lemmens & Bernstein, 2006) and Nadler's equation (Nadler *et al.*, 1962). Table 3.1

shows mathematical formulae for calculating TBV using Nadler's and Lemmens-Bernstein-Brodsky equations and basal metabolic index.

Table 3.1: Total blood volume and basal metabolic index equations.

Equations	**Formula**
Nadler's equation	Male TBV=03669h^3+0.03219w+0.6041 Female TBV = 03561h^3 + 0.03308w+0.1833
Lemmens-Bernstein-Brodsky's equation	TBV= 70 ÷ √(BMI÷ 22)
Basal Metabolic Index (BMI)	BMI= w÷ h^2

TBV= total blood Volume, w= weight (Kg) and h= height (cm /m)

Source: (Ragav & Sandeep, 2020)

3.10 Blood Donation, Samples Collection and Transportation

All eligible donors were positioned on a blood donation couch with the donating arm extending straight from the shoulder to the wrist. Phlebotomy was done by selecting donors' arm with a prominent ante-cubital vein. The venipuncture site was cleaned with cotton wool soaked in a surgical spirit. A tourniquet was tied 3-4 inches above the venipuncture site. Phlebotomy was done with minimal trauma. Donors were instructed to press a sponge ball with the same hand continuously. Blood flow was maintained with continuous mixing with the anticoagulant (Citrate Phosphate Dextrose Adenine 1). At the end of the donation, the tourniquet was removed and a gauze pad was applied on the

venipuncture site with slight pressure. After 2-3 minutes the venipuncture sight was sealed with a fresh bandage. Donors were instructed to continue lying on the donation couch for at least 15 minutes as they were being observed for any vasovagal reactions.

A normal blood donation exercise takes 8 to 10 minutes to be complete. Donors who showed signs of any adverse reactions such as unconsciousness, muscular twitching, syncope or vomiting were exempted from continued donation. After successful donation, blood bags from consenting participants were weighed using a calibrated weighing balance. Approximately 8 ml of blood was drawn from each bag and divided into 4 ml in the red top vial (plain tube) and 4ml in the purple top vial (EDTA tube). The EDTA samples were mixed gently by inverting the tubes 8-10 times. Specimen's identifiers were participants' enrolment numbers.

Blood samples were transported from KNH-BTU to Kenyatta University Health Centre (KUHC) laboratories. Authority to transport biological samples was sought from the host institution (KNH) and the analyzing laboratories (KUHC) via the Material Transfer Agreement form (Appendix XIV). All blood samples for laboratory analysis were packed using a three-part packaging system. Specimen vials were wrapped in biohazard bags using an absorbent material (cotton wool) and then packed with cold packs in a specimen transportation box. Study participants' identification forms were placed in the outer pouch of the biohazard bags. The package was labeled "BIOLOGICAL SUBSTANCE." It was transported to the analyzing laboratories at Kenyatta University Health Centre within one and half hours.

3.11 Calculation of Percentage blood Volume Donation

Every donated pint weighed in grams was converted to volume (ml) using a conversion factor of 1.06. In Kenya the acceptable blood volume range is between 405 – 495ml. The percentage of blood volume donated was determined using this formula.

Percentage of blood volume donated = $\frac{\text{Actual blood volume donated (mL)}}{\text{Total blood volume(ml)}} \times 100$

3.12 Laboratory Procedures

3.12.1 Hematological Analysis

Blood samples collected in EDTA vials were analyzed within 8 hours of collection. The total blood count (TBC) was analyzed at KUHC by Huma Count 5D® analyzer using K3EDTA blood samples. The analyzer utilizes 3D laser light scatter (fluorescence) for counting WBC differential, light impedance for counting RBC and platelet counts, and cyano-hemoglobin at 540 nm for counting hemoglobin. The analyzer uses approximately 20µl of blood to analyze 29 hematological parameters; Human, 2004. (SOP Appendix VII).

In this study, a total of 18 hematological parameters were analyzed. These parameters were: platelets (PLT), red blood cell count (RBC) and it indices which included; hemoglobin (Hgb), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC). White blood cell count (WBC) and its subsets which involved: absolute and differential count for lymphocytes, neutrophils, monocytes, eosinophils and basophils. All specimens analyzed were preserved for one week before disposal. All biological wastes, including the remaining

blood samples, were placed in the appropriate segregation waste bins prior to disposal. Figure 3.2 illustrates how the hematological sample was processed.

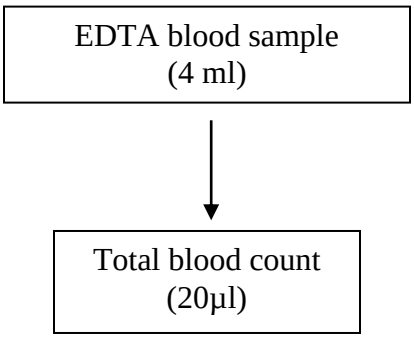

Figure 3.2: Flowchart of hematology Analysis

3.12.2 Biochemistry Analysis

Red top samples were left to stand for 30-90 minutes at room temperature to ensure complete clotting. Clotted specimens were centrifuged at 3000 rpm for 10-15 minutes and then transferred into a labeled micro-vial tube. The collected serum samples were refrigerated in a freezer awaiting bulk analysis. Thawed serum samples were used to analyze serum ferritin concentrations and C -reactive protein (CRP) (SOP Appendix VIII and IX respectively).

Serum ferritin tests and C -reactive protein tests were analyzed using Biomerieux Mini Vidas® and Veda Lab® analyzers, respectively. Biomerieux Mini Vidas® utilizes enzyme-linked fluorescent assay technology to analyze ferritin concentrations. Approximately 100µl of serum was used to analyze a sample of serum ferritin. Veda Lab® analyzer utilizes immunochromatographic techniques to quantitatively detect C-

reactive protein in whole blood, plasma or serum. A single run uses 25 µl of serum sample. Figure 3.3 shows the analytic usage of the clotted blood sample.

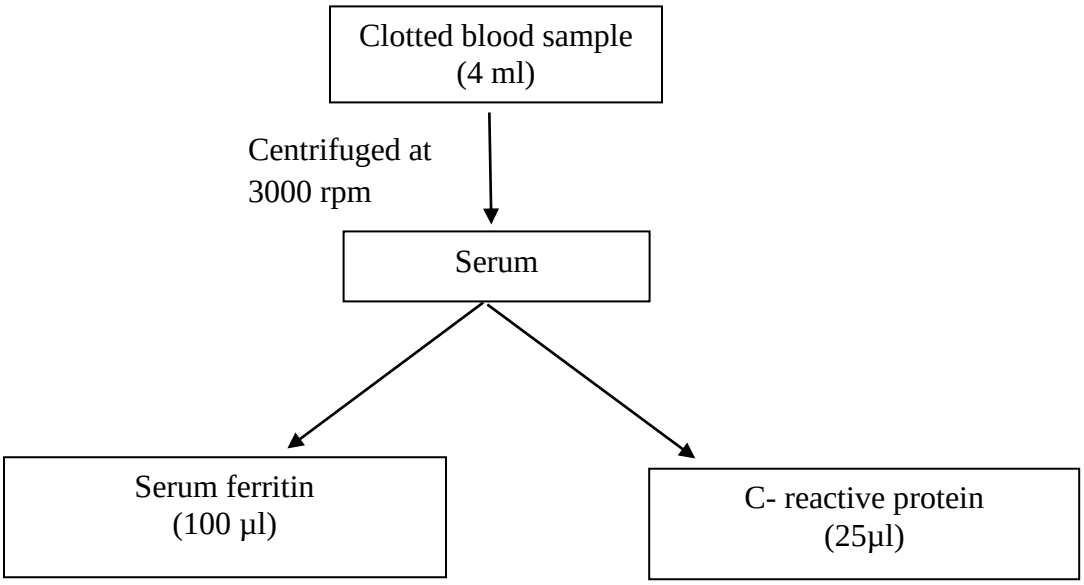

Figure 3.3: Flowchart of the biochemistry analysis.

3.12.3 Quality Assurance

All blood samples collected for this study were processed within the recommended time. Blood samples collected in the purple tube (EDTA) and red tube (clot activator) were preserved in a specimen transportation box fitted with ice packs before transportation. Anticoagulated (EDTA) blood samples were processed within 8 hours. The stability of the serum sample for ferritin analysis can be maintained at 20 - 26 ºC for 24 hours, at 2 - 6 ºC for seven days, and at -20 for several years. Samples were preserved at -20 awaiting bulk analyses. All procedures were done by qualified personnel using approved standard operating procedures. All analyzers were assessed for efficiency by running internal quality controls and calibration. All abnormal results were repeated for confirmation, and

an average of the values was taken. All samples taken from eligible donors for TTI's screening were negative based on KNH immunology laboratory reports.

3.13 Ethical Approval and Permissions

Ethical approval was sought from KNH-UoN ERC (Appendix XII). License to conduct this research was obtained from the National Commission for Science Technology and Innovation (Appendix XIII). Authorization to collect blood samples at Kenyatta National Hospital, Blood Transfusion Unit was sought from the hospital management (Appendix XV). Principles of beneficence, justice, and respect for persons were observed when handling all study participants. Besides the normal blood donation process, study participants were not subjected to any other invasive procedures.

All study subjects were at liberty to withdraw at any phase in the study. All personal data such as filled study questionnaires and laboratory results were handled with the utmost confidentiality. Study participants were identified with a coded number. Collected data were stored in a computer database protected with a password. All filled questionnaires were kept in a lockable file cabinet. Study results were released to study participants based on their preferred channel such as email, phone call or SMS.

3.14 Data Analysis

All requisite data was cleaned, coded, and typed into Microsoft Excel Spreadsheets before they were transferred into SPSS version 26.0. All statistical analyses on the distribution, measures of central tendency, dispersion and prevalence studies were

analyzed by descriptive statistics such as medians, interquartile ranges, frequencies and percentages. Tests of normality both numerical and graphical were assessed by Kolmogorov-Smirnov and Q-Q plot. Inferential statistics comparing two independent non-parametric variables such as gender and donor status were analyzed using the Mann Whitney U test. Kruskal Wallis H test was used in inferential studies comparing more than two independent non-parametric variables such as donor age groups and number of donations. Intra-Class Correlation Coefficient (ICC), T-test, and Bland and Altman Plot were used to analyze the reliability and consistency of using Nadler's equation and Lemmens-Bernstein-Brodskys' equation. The level of significance was set at $p = 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of study participants

A total of 384 prospective blood donors comprised of 103 (26.8%) females and 281 (73.2%) males, who consented to enroll in the study. These participants were broadly divided into two categories (category A and B) based on the outcome of donor recruitment criteria. Category "A" comprised of 202 (52.6%) eligible blood donors who were allowed to donate whole blood, while category "B" consisted of 182 (47.4%) deferred donors who were disqualified and did not donate blood for various reasons (Figure 4.1).

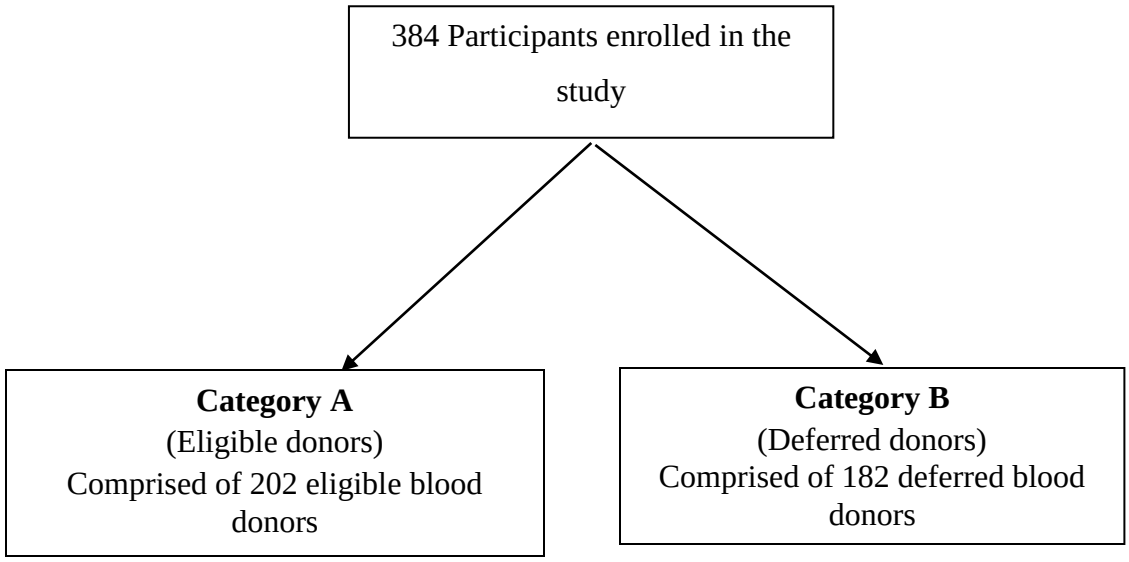

Figure 4.1: Categories of study participants

4.1.1 Socio-demographic characteristics of eligible blood donors at Kenyatta National Hospital

A total of 202 blood donors met donor recruitment criteria and were allowed to donate whole blood. Of these, male donors were the majority constituting of 173 (86.6%). The median age was 28 years, with the age group 20-29 years having the highest number of participants 99 (49%). First-time donors were the majority compared to repeat donors (119 vs. 83, respectively). This study found only 2 (1%) of participants were voluntary non-remunerated donors while the rest were family replacement donors 200 (99%). Regarding marital status, married participants were the majority compared to single donors (62.9% vs.37.1%, respectively). Based on the history of last donation time, donors who had donated over two years ago were the majority 63 (31.2%), whereas donors with the shortest donation intervals below a span of 6 months were 5 (2.5%) as shown in Table 4.1.

Table 4.1: Socio-demographic characteristics of eligible blood donors

Characteristics		Frequency	Percentage (%)
Gender	Male	173	86.6%
	Female	29	14.4%
Age group	<19	11	5.4%
	20-29	99	49%
	30-39	68	33.7%
	40-49	19	9.4%
	>50	5	2.5%
Donor status	First-time	119	58.9
	Repeat	83	41.1%
Marital status	Single	75	37.1%
	Married	127	62.9%
Donor type	Replacement	200	99%
	Voluntary	2	1%
Number of donations	First –time	119	58.9%
	Second-time	70	34.7%
	Third-time	9	4.5%
	Fourth-time	4	2%
Last donation time	First –time donors	119	58.9%
	< 6 Months	5	2.5%
	6-12 Months	15	7.4%
	>12 Months	63	31.2%

KEY: % = percentage, >= above, < =below

4.1.2 Anthropometric parameters of eligible blood donors

Three anthropometric parameters, weight, height, and basal metabolic index (BMI) were measured. The overall median and interquartile range for weight was 68.5 (12) kg, height was 172 (11) cm and BMI was 23.4 (5) kg/m^2. The three anthropometric parameters were matched with the donor's gender. Male and female donors differed significantly in height and BMI (P-value 0.001 and 0.001, respectively) but not in weight (P-value = 0.988), (Table 4.2)

Table 4.2: Anthropometric parameters based on demographic characteristics of eligible blood donors

	Median (IQR)			Mann Whitney U test	
Variables	**All participants N=202**	**Male N=173**	**Female N=29**	**Statistics**	**P-value**
Weight (Kg)	68.5 (12)	68 (13)	72(21)	2504	0.988
Height (cm)	172 (11)	173 (12)	164 (15)	761	0.001*
BMI	23.4 (5)	23 (4)	25 (8)	1418	0.001*

KEY: N= number, IQR = interquartile range, BMI= basal metabolic index, Kg= kilogram, cm= centimeters, * statistically significant

This study further found a majority of male and female donors had normal BMI (67.3% and 55.2%, respectively). The overall prevalence of obesity and overweight was 5.5% and 23.5%, respectively. The prevalence of overweight was higher among male donors than female donors (24.9% vs.13.8%, respectively), while the prevalence of obesity was high among females than male donors (31% vs. 1.2%, respectively), as shown in Figure 4.2.

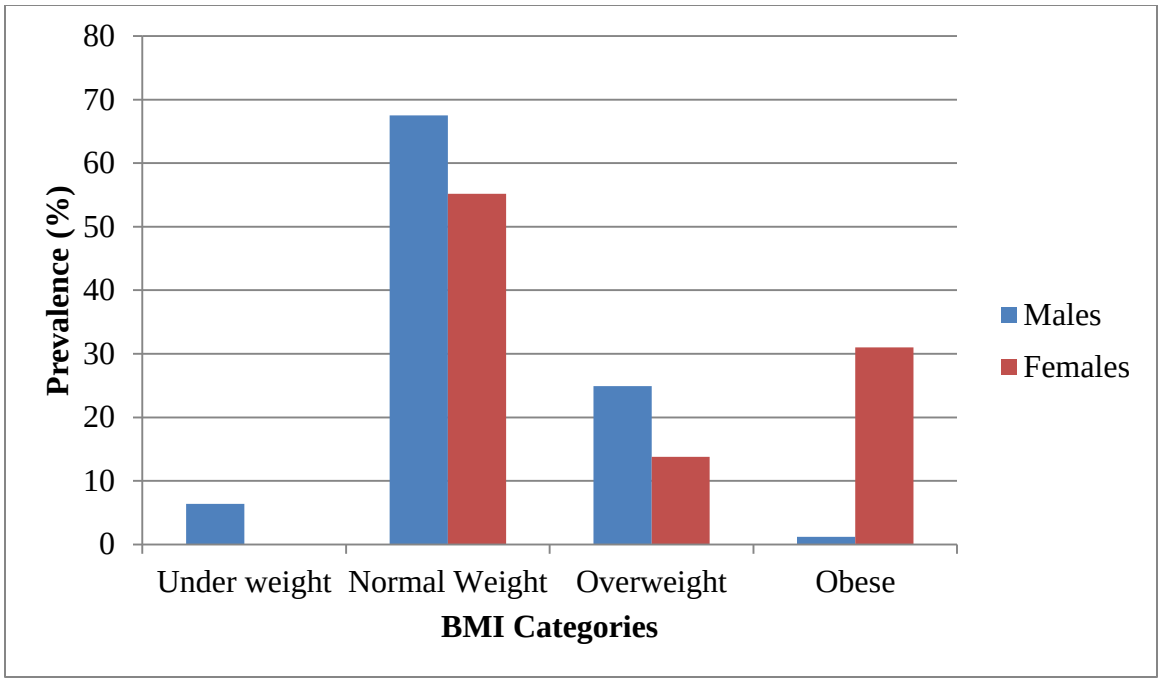

Figure 4.2: Distribution of BMI categories based on donors' gender

4.2 Total blood volumes donated by donors attending Kenyatta National Hospital Blood Transfusion Unit

4.2.1 Percentage of blood volume donated using Nadler and Lemmens-Bernstein-Brodsky equations

Of the 202 eligible blood donors, the percentage TBV donated was 11.7% and 11.6% using Nadler's equation and Lemmens-Bernstein-Brodsky's equation, respectively. Using the two equations, the lowest percentage of the blood volume donated was 5.8% and 5.9%, while the highest percentage donation was 17.4% and 16.8%, respectively. With Lemmens-Bernstein-Brodsky equation Mann Whitney U test found that female donors contributed a significant percentage of their TBV than their male counterparts (U value= -3.920, P= 0.001), but insignificant differences between first-time and repeat donors (U

value -0.617, P= 0.537). Using Nadler's equation and Lemmens-Bernstein-Brodsky's equation this study observed insignificant differences between donated blood volumes and donors-age groups, (P >0.05), the same scenario was seen with Nadler's equation as shown in Table 4.3.

Table 4.3: Percentage of total blood volume donated using Nadler's and Lemmens-Bernstein-Brodsky equations

Equations		**Nadler**				**Lemmens-Bernstein-Brodsky**			
Characteristics		**Median (IQR) (%)**	**Min (%)**	**Max (%)**	**P-value (statistic)**	**Median (IQR) (%)**	**Min (%)**	**Max (%)**	**P-value (statistic)**
Eligible donors (N=202)		11.7 (3)	5.8	17.4		11.6 (3)	5.9	16.8	
Gender	Male	11.3 (2)	5.8	15.1	0.001* (-4.723)	11.3 (2)	5.9	14.8	0.001* (-3.920)
	Female	12.6 (3)	9.9	17.4		12.2 (3)	9.7	16.8	
Age	<19	11.1 (1)	9.6	12.1		11.3 (2)	9.7	12.4	
	20-29	12.2 (2)	6.7	17.3	0.316 (4.732)	12.5 (3)	6.8	16.4	0.205 (5.927)
	30-39	12.3 (3)	5.8	17.4		12.3 (3)	5.9	16.7	
	40-49	10.1 (4)	8.2	15.4		10.2 (4)	8.3	15.0	
	>50	12.1 (4)	8.1	12.4		12.4 (4)	8.2	12.4	
Donor status	First-time	11.6 (3)	6.0	17.3	0.651	11.8 (3)	6.1	16.4	0.537 (-0.617)
	Repeat	11.7 (2)	5.8	17.4	(-0.452)	11.6 (2)	5.9	16.8	

KEY: N= number, IQR= interquartile range, Min= Minimum, Max= Maximum, Statistic= U or H value, * = statistically significant.

4.2.2 Distribution of blood donors' total blood volume based on their demographics

A total of 202 eligible whole blood donors were screened for total blood volume (TBV) using Nadler's and Lemmens-Bernstein-Brodsky's equations. The median and interquartile range for TBV was 4628.5(655) ml and 4633(655) ml using Nadler's and Lemmens-Bernstein-Brodsky's equation, respectively. The established TBV were compared with blood donors' demographics using Mann Whitney U test and Kruskal Wallis H test. Using both equations, male donors had a significantly higher TBV than female blood donors (P = 0.001). Donor status and donors age groups showed insignificant differences with the established TBV (P > 0.05), as shown in Table 4.4.

Table 4.4: Distribution of blood donors' TBV based on their socio-demographics

		Nadler's equation				Lemmens-Bernstein-Brodsky equation			
Characteristics		**Median (IQR) (ml)**	**Min (ml)**	**Max (ml)**	**P-value (statistic)**	**Median (IQR) (ml)**	**Min (ml)**	**Max (ml)**	**P-value (statistic)**
Eligible donors (N=202)		4628.5(655)	3270	6322		4633.3(655)	3450	6984	
Gender	Male	4692 (682)	3329	6322	0.001*	4676 (634)	3505	6984	0.001*
	Female	4084 (727)	3270	4873	(-6.213)	4194 (650)	3450	5054	(-5.583)
Age	<19	4518 (977)	3545	6197	0.385	4424(934)	3685	6234	0.575
	20-29	4632 (606)	3270	6197	(2.465)	4632 (479)	3450	6984	(2.895)
	30-39	4573 (640)	3857	6164		4588 (629)	3505	5921	
	40-49	4696 (996)	3857	6164		4664 (1168)	3960	6079	
	>50	4661 (774)	4501	5760		4680 (749)	4462	5698	
Donor status	First-time	4628 (699)	3270	6322	0.528	4588 (649)	3450	6234	0.313
	Repeat	4660 (553)	3329	6197	(-1.004)	4666 (607)	3505	6984	(-1.008)

KEY: N=Number, IQR= inter-quartile range, Min= Minimum, Max= Maximum, TBV=Total Blood Volume, Statistic= U or H value, * statistically significant.

4.2.3 Blood donors at risks of vasovagal reactions

Using Nadler's and Lemmens-Bernstein-Brodsky equations, this study found that 18.8% of eligible blood donors at KNH were at risk of vasovagal reactions. This is according to the WHO guidelines that stipulate donating more than 13% of donors' TBV predisposes donors to vasovagal/adverse reactions. Another factor predisposing eligible donors to adverse reactions is blood volume below 3500ml. In this study, Nadler's equation observed 5 (2.5%) donors had TBV below 3500ml while Lemmens-Bernstein-Brodsky's equation found a female donor 1(0.5%) had TBV below 3500ml. Female donors were exposed to vasovagal reactions (donating >13%) more than male donors (44.8% vs. 14.5%, respectively). First-time donors were likely to be exposed to adverse reactions than to repeat donors (20.2% vs. 16.9%, respectively). Blood donors aged below 19 and those above 50 years were not exposed to vasovagal reactions compared to other age groups (Table 4.5).

Table 4.5: Blood donors at risk of vasovagal reactions

Variable		N	Nadler's equation		Lemmens-Bernstein-Brodsky	
			Donation >13% (%)	TBV < 3500 ml (%)	Donation >13% (%)	TBV < 3500 ml (%)
Eligible donors		202	38 (18.8%)	5(2.5%)	38 (18.8%)	1(0.5%)
Gender	Male	173	25 (14.5%)	1(0.6%)	25(14.5%)	0
	Female	29	13(44.8%)	4(13.8%)	13 (44.8%)	1(0.3%)
Donor status	First-time	119	24(20.2%)	4(3.4%)	24(20.2%)	1(0.8%)
	Repeat	83	14(16.9%)	1(1.2%)	14(16.9%)	0
Age group	<19	11	0	0	0	0
	20-29	99	19(19.2%)	4 (4%)	19(19.2%)	1(1%)
	30-39	68	13(19.1%)	1(1.5%)	14(20.6%)	0
	40-49	19	4(5.9%)	0	4(5.9%)	0
	>50	5	0	0	0	0

KEY: N=number, TBV= Total blood volume, % = percentage, >= above, < =below

4.3 Reliability of using Nadler's and Lemmens-Bernstein-Brodsky's equations to estimate blood donors' total blood volume (TBV)

Intra-Class Correlation Coefficient (ICC) was used to assess the reliability of screening donors' TBV using both Nadler's and Lemmens-Bernstein-Brodsky's equations. Single and average measures showed excellent reliability of using Nadler's and Lemmens-Bernstein-Brodsky's equations (ICC of 0.985 and 0.992, respectively, with a P value <0.001). The lower and upper bound limits for average measures were 0.990 and 0.994, respectively, as shown in Table 4.6.

Table 4.6: Reliability of estimating blood donors TBV using Nadler's and Lemmens-Bernstein-Brodsky's equations

	Intra-class Correlation Coefficient	95% Confidence interval		Statistic	
		Lower Bound	Upper Bound	F value	P-value
Single measure	0.985	0.980	0.989	131.50	0.000*
Average measure	0.992	0.990	0.994	131.50	0.000*

KEY: * Statistically significant

One sample T-test found an insignificant difference in TBV established by Nadlers' and Lemmens-Bernstein-Brodsky equation, suggesting the two equations are in agreement. This study observed the mean difference and standard deviation of TBV between the two equations as 8.98 and 103.86, respectively, with lower and upper limits of -194.58 and 212.55, respectively (t =1.229 and P= 0.221) as shown in Table 4.7.

Table 4.7: Difference in TBV established by Nadler equation and Lemmens-Bernstein-Brodsky equation

					95% confidence interval	
	N	Mean	SD	T	P-value	Lower	Upper
Difference	202	8.98	103.86	1.229	0.221	-194.58	212.55

KEY: N= number, SD= standard deviation

Figure 4.2 below further illustrate the insignificant difference in total blood volume established by established by Nadlers' and Lemmens-Bernstein-Brodsky equations.

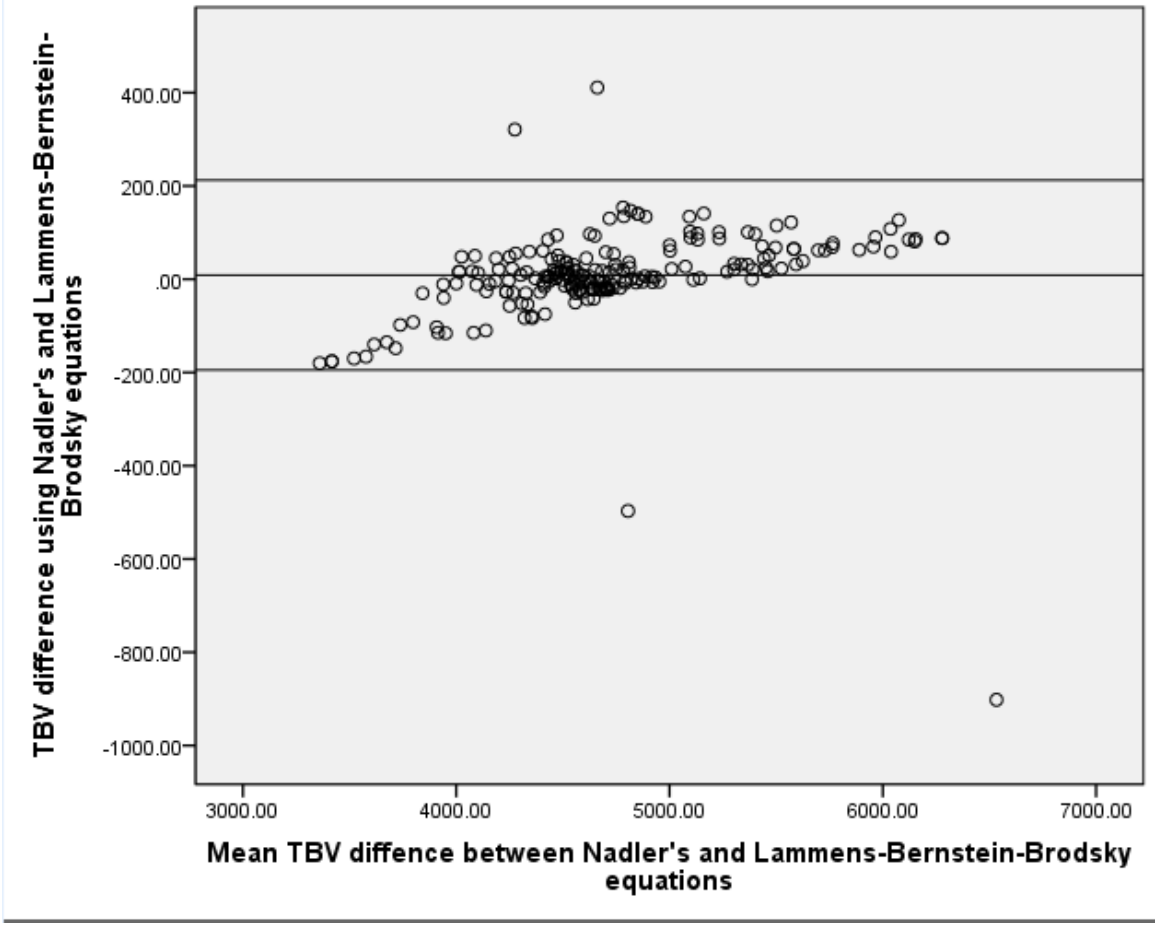

Figure 4.3: Bland and Altman Plot showing TBV mean and difference of using Nadler's and Lemmens-Bernstein-Brodsky's equations

4.4 Prevalence of iron deficiency and anemia among eligible blood donors attending Kenyatta National Hospital

4.4.1 Prevalence of iron deficiency among eligible blood donors

According to the WHO guidelines, the cut-off value for iron deficiency is serum ferritin <15 ng/ml. In this study, prevalence of iron deficiency among eligible donors was 2.5%, with male and female donors recording 1.7% and 6.9%, respectively. Majority of donors (70.3%) had normal iron status. Blood donors at risk of iron overload were 27.2%

(ferritin levels > 150 ng/ml and >200ng/ml for female and males, respectively) as shown in Table 4.8 below.

Table 4.8: Prevalence of iron deficiency based on WHO guidelines

Iron status	Variables (Cut-off values)	N	Prevalence %
Iron deficiency Donors	Combined (<15ng/ml)	202	5(2.5%)
	Male	173	3 (1.7%)
	Female	29	2 (6.9%)
Healthy donors	Combined	202	142(70.3%)
	Male (>15 –200ng/ml)	173	122 (70.5%)
	Female (15-150ng/ml)	29	20 (70%)
Iron overload	Combined	202	55(27.2%)
	Male (>200ng/ml)	173	48 (27.6%)
	Female (>150ng/ml)	29	7 (14.1%)

KEY: Combined= Male and female, N= number, % = percentage, >= above, < =below

4.4.2 Distribution of serum ferritin concentrations based on socio-demographics characteristics

A total of 202 eligible blood donors were screened for serum ferritin concentrations. The median level for serum ferritin concentration was 128ng/ml with an interquartile range of 96.7 ng/ml. The minimum serum ferritin level recorded was 11.91 ng/ml while the maximum was 488.18 ng/ml. All samples had CRP levels of less than 2.5 µg/ml. Male

donors had significantly higher serum ferritin concentrations than female donors (U=1759.0, P = 0.010).

This study compared serum ferritin concentrations with donors' age groups and found a significant difference (H= 13.92, P=0.008). Age group 40-49 years recorded the highest (152.48ng/ml) median count for ferritin concentrations whereas donors under 19 years recorded the least (67.45 ng/ml) concentrations. Comparison of serum ferritin levels based on blood donor status showed an insignificant difference between first-time donors and repeat blood donors (U=4179.5, P=0.063) as shown in Table 4.9.

Table 4.9: Distribution of serum ferritin levels based on blood donors' demographics

Attribute	Category	Number (N)	Median (IQR) (ng/ml)	Min (ng/m)	Max. (ng/ml)	Mann W/ Kruskal	P-value
Eligible donors		202	128 (96.7)	11.91	488.88		
Gender	Male	173	131 (85.22)	11.91	488.11	U=1759	0.010*
	Female	29	84.65 (147.14)	13.21	349.57		
Age group	<19	11	67.45(61.42)	11.98	198.43	H=13.92	0.008*
	20-29	99	119.76(101.7)	11.91	472.12		
	30-39	68	140.09(74.94)	13.21	488.18		
	40-49	19	152.48(84.83)	66.52	462.10		
	>50	5	93.29(160.91)	35.48	243.50		
Donor status	First-Time	119	134.34(144.2)	11.91	472.12	U=4179.5	0.063
	Repeat	83	125.45(71.9)	13.21	488.11		

KEY: N= number, IQR= interquartile range, Min= Minimum, Max= Maximum, Mann W= Mann Whitney U test, Kruskal= Kruskal Wallis H test * statistically significant

4.4.3 Distribution of serum ferritin concentrations among repeat blood donors

Inferential statistic (Kruskal Wallis H test) compared serum ferritin concentrations with blood donors' history of last donation time and the number of donations and found an insignificant difference (H= 3.810, P=0.283 and H= 4.396, P=0.222, respectively) as shown in Table 4.10 below.

Table 4.10: Distribution of serum ferritin concentrations based on last donation time and the number of donations

Attribute	Category	(N)	Median (IQR) (ng/ml)	Min	Max	Kruskal	P-value
Last donation Time	First-time	119	134.34 (144.2)	11.91	472.12	H=3.810	0.283
	< 6 months	6	126.85(46.05)	11.98	198.43		
	6-12 months	14	87.05(157.06)	11.91	472.12		
	>12 months	63	125.45(63.77)	30.24	488.18		
No. of donations	First –time	119	134.34 (144.2)	11.91	472.12	H=4.396	0.222
	Second-time	70	124..65(69.09)	13.21	488.18		
	Third-time	8	84.65(174.97)	14.32	200.30		
	Fourth-time	5	135.01(45)	112.72	157.30		

KEY: N= number, IQR= interquartile range, Min= Minimum, Max= Maximum, Kruskal= Kruskal Wallis H test

4.4.4. Prevalence of anemia among eligible blood donors

According to the WHO guidelines, the cut-off values for anemia among adult males is Hgb <13.0g/dl and adult females is Hgb <12.0g/dl. In this study, the prevalence of anemia among eligible donors was 7.4%, with male donors recording 8.7%. The

minimum hemoglobin level recorded among male donors was 12.5g/dl. All eligible female donors had hemoglobin above 12.0g/dl as shown in Table 4.11.

Table 4.11: Prevalence of anemia based on WHO guidelines

Anemia	Variables (Cut-off values)	N	Prevalence %
Anemia	Combined	202	15(7.4%)
	Male <13.0g/dl	173	15(8.7%)
	Female< 12.0g/dl	29	0

KEY: N= number, % = percentage, >= above, < =below.

4.4.5 Correlation of serum ferritin levels with hemoglobin, last donation time and the number of donations

Spearman's correlation test showed a significant positive correlation between blood donors' hemoglobin levels and serum ferritin concentrations (r_s= 0.317, P <0.001). It also revealed a significant negative correlation between serum ferritin concentrations and the number of donations (r_s= -0.14, P< 0.049). However, no correlation was seen between serum ferritin concentrations and last donation time (r_s= 0.948, P= 0.083), as shown in the Table 4.12.

Table 4.12: Correlation of serum ferritin levels with hemoglobin, last donation time and number of donations

Correlation	Number	Spearman's correlation (r_s)	P Value
Ferritin and hemoglobin	N= 202	0.317	0.000*
Ferritin and last donation time	N= 202	0.948	0.083
Ferritin and number of donations	N= 202	-0.14	0.049*

KEY: *Correlation is significant at the P< 0.05 level (2-tailed), N = number

4.5 Hematological profiles of eligible blood donors at Kenyatta National Hospital

4.5.1 Comparison of hematological profiles between blood donors' gender and local reference intervals

A total of eighteen (18) hematological parameters were analyzed, these were; total red blood cell count (RBC), hematocrit (Hct), hemoglobin (Hgb), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), total white blood cell count (WBC), differential and absolute WBC counts (lymphocytes, neutrophils, monocytes, eosinophils and basophils) and platelets (PLT). In this study, hematological parameters for males, females and combined (male and female hematological values) were within local reference intervals as shown in Table 4.13.

Table 4.13: Comparison of hematological profiles between gender and local reference intervals

	Male donors (N=173)		Female donors (N=29)		Combined donors (N=202)	
Parameter	**Median (IQR)**	**Reference intervals**	**Median (IQR)**	**Reference intervals**	**Median (IQR)**	**Reference intervals**
RBC (x10^12L)	5.06(0.7)	4.9 4-6.52	4.96(0.5)	4.31-5.76	4.9 (.74)	4.41-6.48
Hgb (g/dL)	14.8(1.7)	14.5-18.7	13.3(1.5)	12.0-16.5	14.3 (1.8)	12.8-19.0
HCT (%)	45.3(5.0)	43.0-55.0	42.4(5.0)	36.0-49.0	44.9 (5.1)	38-55
MCV (fL)	89.8(5.1)	76.5-95.5	90.8(4.3)	73.4-95.8	90.0 (5)	75.7-95.6
MCH (pg)	29.4(1.8)	25.1-32.8	29.3(1.6)	24.4-32.7	29.4 (1.9)	24.8-33.8
MCHC (g/dL)	32.9(1.5)	32.4-35.4	33.1(1.2)	32.0-35.0	33(1.4)	32.2-35.2
WBC (x10^9L)	4.9(1.3)	3.13-8.10	5.34(1.30	2.89-7.72	4.9 (1.4)	3.08-7.83
Lym (%)	41.8(12.0)	28.2-60.3	43.1(11.0)	25.5-59.3	41.9 (12.1)	27.2-60.0
Lym #(x10^9L)	2.06(0.7)	1.36-3.58	2.37(0.5)	1.22-3.24	2.1 (.67)	1.29-3.40
Neu (%)	47.3(12.1)	27.4-60.3	47.3(12.6)	29.5-65.4	47.8 (12.1)	28.0-63.3
Neu #(x10^9L)	2.25(0.88)	1.02-3.92	2.34(1.1)	1.07-4.42	2.3 (.89)	1.05-4.08
Mon (%)	6.8(3.1)	3.5-14.3	6.3(3.7)	3.2-11.0	6.7 (3.1)	3.4 -13.3
Mon# (x10^9L)	0.35(0.53)	0.15-0.76	0.36(0.3)	0.14-0.68	.35 (.7)	0.14-0.74
Eos (%)	2.7(2.5)	1.2-11.8	3.1(3.3)	0.80-9.40	2.8 (2.8)	1.1-11.9
Eos # (x10^9L)	0.13(0.1)	0.05-0.64	0.16(0.2)	0.04-0.49	0.14 (.14)	0.04-0.59
Bas (%)	0.3(0.2)	0.40-1.20	0.3(0.2)	0.30-1.00	0.3 (.2)	0.30-1.10
Bas # (x10^9L)	0.01(0.1)	0.01-0.08	0.01(0.1)	0.01-0.06	0.01(0.1)	0.01-0.07
PLT (x10^9L)	225(62)	133-356	262(57)	152-443	234 (64)	144-409

KEY: # = absolute count, % = differential, IQR= interquartile range, Max=maximum, Min= minimum, RBC = red blood cells (erythrocytes), Hgb = Hemoglobin, Hct = hematocrit, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, WBC = white blood cells (leucocytes), Lym= lymphocytes, Net= neutrophils, Mon= Monocytes, Eos= eosinophils, Bas= basophils and PLT= platelet, N=number

4.5.2 Difference between normal and abnormal hematological parameters among eligible blood donors

Out of eighteen (18) hematological parameters analyzed, seven (7) variables (RBC, Hgb, MCV, MCHC, monocytes %, eosinophil's %, and platelets) showed significant differences between normal and abnormal hematological values. The majority of the participants had normal hematological values. Mean corpuscular hemoglobin concentration (MCHC) and red cell count (RBC) had the highest percentage of abnormal values (27.2% and 15.3%, respectively), whereas basophils and mean cell hemoglobin (MCH) had the least percentage of abnormal values (Table 4.14).

Table 4.14: Difference between normal and abnormal hematological values

Parameters	Normal (median)	Percent. (%)	Abnormal (median)	Percent (%)	U-value	P-value
RBC (x10^12L)	5.08	84.7	4.23	15.3	684	0.000*
Hgb (g/dL)	14.3	96.5	12.7	3.5	-4.49	0.000*
HCT (%)	44.9	96.5	37.9	3.5	585	0.521
MCV (fL)	89.45	93.1	96.8	6.9	188	0.000*
MCH (pg)	29.4	99	22.8	1	-	-
MCHC (g/dL)	33.2	72.8	31.7	27.2	588	0.000*
WBC (x10^9L)	4.945	95	7.940	5	768	0.287
Lym (%)	2.087	92.1	3.522	7.9	930	0.013
Lym #(x10^9L)	41.85	97	47.1	3	588	1.00
Neu (%)	2.279	94	3.795	6	950	0.333
Neu #(x10^9L)	47.3	95.5	64.9	4.5	772	0.573
Mon (%)	0.347	97	0.122	3	0.00	0.000*
Mon# (x10^9L)	6.8	97.5	3.15	2.5	198	0.087
Eos (%)	3.10	92.6	0.90	7.4	354	0.000*
Eos # (x10^9L)	0.114	93.6	0.034	6.4	759	0.021
Bas (%)	0.012	100	0	0	-	-
Bas # (x10^9L)	0.4	98.5	0.2	1.5	-	-
PLT (x10^9L)	239	96	128	4	194	0.000*

KEY: # = absolute count, % = percentage, RBC = red blood cells (erythrocytes), Hgb = Hemoglobin, Hct = hematocrit, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, WBC = white blood cells (leucocytes), Lym= lymphocytes, Net= neutrophils, Mon= Monocytes, Eos= eosinophils, Bas= basophils and PLT= platelet, *=statistically significant.

4.5.3 Erythrocyte profiles of whole blood donors at KNH based on gender

Mann Whitney U test showed that male donors had significantly higher median counts for red blood cell (U=1446.6; P<0.01), hematocrit (U=1530.5; P=001) and hemoglobin concentration (U=1466.4; (P<0.01) than female donors. The other red blood cells indices did not differ significantly between female and male donors as shown in Table 4.15.

Table 4.15: Comparison of red blood cells parameters between males and female donors

Variables	**Gender**		**Mann Whitney U test**	
Erythrocytes parameters	**Female (N=29) Median (IQR)**	**Male (N=173) Median (IQR)**	**U –value**	**P-value**
RBC (x10^12L)	4.96 (0.5)	5.06 (0.7)	1446.5	0.000*
HCT (%)	42.4 (5.0)	45.3 (5.0)	1530.5	0.001*
Hgb (g/dL)	13.3 (1.5)	14.8 (1.7)	1466.4	0.000*
MCV (fL)	90.8 (4.3)	89.8 (5.1)	2050	0.116
MCHC (g/dL)	33.1 (1.2)	32.9 (1.5)	2412.5	0.742
MCH (pg)	29.3 (1.6)	29.4 (1.8)	2322.0	0.521

KEY: N= number, IQR= interquartile range, RBC = red blood cells, Hgb = Hemoglobin, Hct = hematocrit, MCH = mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, * = statistically significant

4.5.4 Leucocytes and platelet profiles of whole blood donors at KNH based on gender

This study compared white blood cells parameters (absolute and differential) and platelets between female and male donors. The median absolute lymphocyte count was significantly higher in female donors than male donors (U=1765; P =0.011). Female

donors had slightly higher white blood cells levels than male donors. The Mann Whitney U test further revealed that female blood donors had a significantly higher platelet count than male donors (U=1450, P < 0.05) as shown in Table 4.16.

Table 4.16: Leucocytes and platelet profiles of whole blood donors based on gender

	Gender		**Mann Whitney U test**	
Leucocyte parameters	**Female (N=29) Median (IQR)**	**Male (N=173) Median (IQR)**	**U – value**	**P-value**
WBC (x10^9L)	5.34 (1.3)	4.91(1.3)	1952	0.056
Lymphocytes (%)	2.37 (0.5)	2.06 (0.7)	1765	0.011*
Lymphocytes #(x10^9L)	43.1 (11.0)	41.8 (12.0)	2315	0.507
Neutrophils (%)	2.34 (1.1)	2.25 (0.88)	2231	0.341
Neutrophils #(x10^9L)	47.3 (12.6)	47.3 (12.1)	2476.5	0.913
Monocytes (%)	0.36 (0.3)	0.35 (0.53)	2401	0.469
Monocytes # (x10^9L)	6.3(3.7)	6.8 (3.1)	2297.4	0.712
Eosinophils (%)	0.16(0.2)	0.13 (0.1)	2433	0.379
Eosinophils # (x10^9L)	3.1 (3.3)	2.7 (2.5)	2253	0.712
Basophils (%)	0.01(0.0)	0.01(0.1)	2494	0.446
Basophils # (x10^9L)	0.3 (0.2)	0.3 (0.2)	2286.5	0.962
Platelets (x10^9L)	262 (57)	225 (62)	1450	0.000*

KEY: # = absolute count, % = percentage, N= number, IQR= interquartile range,
* = statistically significant

4.5.5 Hematological profiles of eligible blood donors based on age group

This study analyzed the difference between hematological parameters and donors' age group and found an insignificant difference (P > 0.05), as shown in Table 4.17.

Table 4.17: Hematological profiles of eligible blood donors based on age group

Parameter	Medians across age groups (years)					Kruskal Wallis H	
	< 19 (N=11)	20-29 (N=99)	30-39 (N=68)	40-49 (N=19)	>50 (N=5)	H value	P-value
RBC(x10^12L)	4.81	4.93	4.94	5.03	5.29	6.06	0.195
Hgb (g/dL)	13.8	14.4	13.8	14.6	14.8	7.84	0.098
HCT (%)	44.3	45.3	44.1	46.1	45.7	6.28	0.179
MCV (fL)	93.1	90.3	89.1	90.4	89.4	6.16	0.187
MCH (pg)	30.0	29.4	29.3	28.8	27.3	5.18	0.270
MCHC (g/dL)	23.4	33.0	33.1	33.1	32.2	3.21	0.523
WBC (x10^9L)	4.22	4.97	5.19	4.98	5.34	2.22	0.696
Lym (%)	1.82	2.14	2.09	2.08	2.26	3.03	0.554
Lym #(x10^9L)	39.4	42.7	41.2	40.8	48.9	4.24	0.374
Neu (%)	2.11	2.30	2.29	2.55	2.10	2.51	0.643
Neu #(x10^9L)	50.4	46.2	49.2	49.9	39.7	8.27	0.082
Mon (%)	6.90	6.80	6.60	5.50	6.40	3.15	0.533
Mon# (x10^9L)	0.31	0.36	0.35	0.28	0.34	2.48	0.648
Eos (%)	2.40	2.90	2.80	2.00	3.10	2.63	0.623
Eos # (x10^9L)	0.12	0.14	0.14	0.12	0.22	1.54	0.819
Bas (%)	0.30	0.30	0.30	0.20	0.30	3.04	0.552
Bas # (x10^9L)	0.01	0.01	0.01	0.01	0.02	2.72	0.066
PLT (x10^9L)	230.0	239.0	231.5	253.0	178.0	8.19	0.085

KEY: # = absolute count, % = percentage, N= number, RBC = red blood cells, Hgb = Hemoglobin, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume.

4.6 Rates and causes of temporary and permanent deferrals among blood donors attending Kenyatta National Hospital

4.6.1 Deferral rates among deferred blood donors at KNH

The demographic distribution of the deferred donor population was stratified by gender, donor status, age group and donor type as presented in Table 4.18 below. Out of 384 prospective blood donors enrolled in this study, 182 donors failed to meet donor selection criteria and were deferred for various reasons. This translated to an overall deferral rate of 47.4%. Female donors had a higher deferral rate than male donors (71.8% vs. 38.4%, respectively). Donors aged below 19 years recorded the lowest deferral rates compared to donors above 50 years. In addition, first-time donors, and temporary deferrals constituted the highest deferral rates (53.7% and 93.4%, respectively). All deferred donors were replacement donors.

Table 4.18: Distribution of deferral rates based on socio-demographic characteristics

Variables		Frequency	N. deferred	Deferral rates
Overall deferral	All donors	384	182	47.4%
Gender	Male	281	108	38.4%
	Female	103	74	71.8%
Age group	<19	16	5	31.3%
	20-29	185	86	46.5%
	30-39	121	53	43.8%
	40-49	45	26	57.8%
	>50	17	12	70.6%
Donor status	First time	257	138	53.7%
	Repeat	127	44	34.6%
Donor type	VNRD	2	0	0%
	FRD	382	182	47.6%
Deferral type	Temporary	182	170	93.4%
	Permanent	182	12	6.6%

KEY: N=number, VNRD= voluntary non-remunerated donor, FRD=family replacement donor

4.6.2 Causes of temporary and permanent deferral among blood donors attending Kenyatta National Hospital

Out of 182 deferred blood donors, 170 (93.4%) were temporarily deferred and 12 (6.6 %) were permanently deferred. The leading causes for temporary deferral for male and female donors were medication and low hemoglobin (33.5% vs.31.3%, respectively). Other reasons for temporary deferral included vaccination, hard drugs, multiple sexual

partners, low weight, breast feeding and body tattooing. High blood pressure and diabetes were the leading causes of permanent deferral (Table 4.19).

Table 4.19: Causes for temporary and permanent deferment of whole blood donors

Causes	Male (n = 108)	Female (n = 74)	Combined (n=182)
	Temporary causes		
Medication	35 (32.4%)	26 (35.1%)	61 (33.5%)
Low hemoglobin	35 (32.4%)	22 (29.7%)	57 (31.3%)
Vaccination	9 (8.3%)	5 (6.8%)	14 (7.7%)
Hard drugs	8 (7.4%)	4 (5.4%)	12 (6.6%)
multiple sexual partners	6 (5.6%)	2 (2.7%)	8 (4.4%)
Menstruation	0 (0.0%)	6 (8.1%)	6 (3.3%)
Low weight	1 (0.9%)	4 (5.4%)	5 (2.7%)
Breast feeding	0 (.0%)	3 (4.1%)	3 (1.6%)
Tooth extraction	2 (1.9%)	0 (0.0%)	2 (1.1%)
Tattoo	2 (1.9%)	0 (0.0%)	2 (1.1%)
Total	**98 (90.7%)**	**72(97.3%)**	**170 (93.4%)**
	Permanent causes		
High BP	5 (4.6%)	1 (1.3%)	6 (3.3%)
Multiple conditions (diabetes, Abnormal BP, asthmatic and ulcers)	3 (2.8%)	1 (1.3%)	4 (2.2%)
Diabetes	2 (0.9%)	0 (0.0%)	2 (1.1%)
Total	**10 (9.3%)**	**2 (2.7%)**	**12 (6.6 %)**

KEY: N=number, BP= blood pressure

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio–demographic and anthropometric characteristics of eligible blood donors at Kenyatta National Hospital

In this study, the inclination towards blood donation was significantly high in males compared to female donors (85.6% vs. 14.4%), respectively. This scenario could be attributed to previous observations that showed female donors have numerous deterrents to blood donation. Such deterrents include difficult veins, ailments and medical reasons as barriers to blood donation (Erhabor *et al*., 2013). According to Quader, (2021), the main reason for low numbers of eligible female donors is anemia that emanates from; blood loss during childbirth and menstruation, lactation, superstition and socio-cultural beliefs. Male dominance in blood donation resonates with other observations made in African countries (Buseri *et al.*, 2010; Ritter *et al.*, 2012; Orkuma *et al.*, 2015).

Other studies from great Britain and European countries have found a different picture where female donors donate more than male donors (Lattimore *et al.*, 2015; Bani, 2011). This finding could be attributed to female being more altruistic than men. It may also be due to increased awareness campaigns on blood donation among prospective donors. According to the WHO, female blood donors contribute 30% of all donations across 118 member states. The report further mentioned female donors in the African region contribute about 25%, with a global variation of 2% to 72 % (WHO, 2016). The present study found female donors contributed about 14.4%. This percentage may be influenced

by decreased education and awareness campaigns on donor eligibility in a majority of African countries like Kenya.

The current study found donors aged 20-29 years recorded the highest numbers compared to other age groups. These findings agree with a study in Ghana that demonstrated donors aged 21-30 years were the majority (Addai-Mensah *et al.*, 2015). This phenomenon may be explained by a previous report that revealed a majority of blood donations in resource limited countries emanate from young donors WHO (2016). Stratification of donors based on age group showed that donors aged 18 to 19 years had a donation rate of 5.4%. This low rate could be due to the low numbers of consenting participants in this age. Moreover, the current study was done in a hospital-based set-up, mainly targeting family members and friends. This finding differs greatly from other studies done in developing countries that target learning institutions for blood donation. For instance, a report from the WHO (2016) asserted donors aged 18 years and below in developing countries had the highest donation rate.

Blood donation rates in resource-rich countries such as New Zealand, Netherlands and Australia are higher among older donors (45-65 years). However, some developed countries, such as France, Singapore and Poland, have high donation rates among young people (WHO, 2016). This scenario may be explained by the difference in the age group structure of the population. Understanding demographic information of prospective donors' populations is crucial in formulating and monitoring donor selection criteria to meet blood demands.

This study found that most participants were donating blood for the first-time compared to repeat donors (58.9% vs. 41.1%, respectively). This finding is agrees to a study by Shamsudeen and Harry (2018) that observed a similar prevalence among Ghanaian donors. However, our results contradict findings by Wiersum-osselton *et al.* (2014) and Suemnig *et al.* (2017) that cited higher numbers of repeat blood donors in Poland and Netherlands, respectively. The differences may be due to the later studies being done in resource-rich countries. One challenge facing hospital-based donation is the inability to maintain a constant supply of safe blood, mainly due to financial challenges. Despite this, previous authors have argued that first-time donors are prone to TTIs compared to repeat donors (Padma *et al*., 2017; Allain *et al.*, 2012).

A reminder to donate, a desire to help people and a good attitude among staff at blood donation centers are cited as the main motivating factors for repeat donation (Shamsudeen & Harry, 2018). In addition, a study on Tanzanian donors established that repeat donation was significantly associated with a good donation experience and a good altitude among staff (Mauka *et al.,* 2015). Another study in Cameroon showed that incentives like money, recognition, time off work and gift items were the leading motivators for donation (Koster, 2011). The concept of motivation towards blood donation may be of less importance in our study site, where the majority of donor were donating for the first-time after being requested to donate blood to their relatives or friends.

A previous study has shown that repeat and first-time donors can be motivated to donate if the exercise consists of minimal disruption to their normal duties (Shaz *et al.*, 2014). Another study described the top deterrent to blood donation as the inconvenient location and operating time (Jemberu *et al.*, 2016). Our research did not consider the inconveniences faced by prospective blood donors at the donation unit. However, variation between first–time and repeat donors could be attributed to the availability of a good transport system, proximity to the town and nature of the donation center (fixed hospital location vs. mobile donation drives) (Shaz *et al.*, 2014)

This study has established that the availability of voluntary blood donors at KNH is almost nonexistent (1%) as most prospective donors were family/replacement donors (99%). This observation could be attributed to the nature of the blood collection set-up where most donors were compelled to give blood to their family members and friends. The current findings contrast with a previous study done in the former eight provinces of Kenya that observed 36% replacement donors and 64% voluntary donors (Kimani *et al.*, 2011). This huge variation could have resulted from the former using mobile blood donation centers, differences in population sampling criteria and the effects of the Covid 19 pandemic. A study on Egyptian donors reported family replacement donation at 87.7% and voluntary donation at 12.3% (Ibrahim *et al.*, 2014). A recent study on the Pakistan donor population reported spiritual satisfaction and helping a friend or family member as that the primary motivating factors for reliance on replacement donation (Tamkeen *et al.*, 2019).

The current observations indicate over-reliance on family/ replacement donors at the KNH blood donation center. These are donors who strive to replace blood utilized by their hospitalized relatives or friends and not necessarily help strangers. Additionally, some recipients prefer blood from their lineage because they believe blood transfusion can transfer character traits. This belief is supported by a study on the Cameroonian population that found donation to a family member was more acceptable than a donation by a stranger or institution (Koster, 2011). Although family replacement donation is cited for having higher transfusion-transmitted infections (TTIs), some studies have reported contrary findings on the prevalence of TTI's among replacement donors (WHO, 2011; Quader, 2021).

In the present study, married donors were more than single donors (62% vs. 24%, respectively). This scenario could have resulted from the hospital-based donation adopted in the current study. Other studies conducted on mobile donation centres (blood donation drives) targeting learning institutions have reported a higher number of single donors. The predominance of married donors in our study correlates to a study by Bugdorf *et al.* (2017), but differs from studies by Pule (2014) and Orkuma *et al.* (2015) where singles donors had more donations than married donors.

This study observed the prevalence of obesity and overweight as 5.5% and 23.5%, respectively. Female blood donors had a higher prevalence of obesity than male donors at 31% and 1.2 %, respectively. These findings agree with another study done on Kenyan women that showed the prevalence of obesity at 9.1% and overweight at 20.5% (Mkuu *et*

al., 2019). Another study by Ettarh *et al.* (2013), found that Kenyan women were more prone to obesity than men (43.5% vs. 34%, respectively). Globally obesity and overweight affect more women than men (WHO, 2016). Obesity and overweight are associated with a higher prevalence of cardiovascular diseases, diabetes and major cancers (Mkuu *et al.*, 2019). Additionally, obese and overweight women are at greater risk of maternal outcomes such as postpartum hemorrhage, pre-eclampsia, and gestational diabetes (Kulie *et al.*, 2011).

Globally, the prevalence of obesity and overweight has tripled since 1975. In 2016, close to 1.9 billion adults were either obese or overweight. Living in urban towns, using contraceptives, not working, having higher education levels, and increased consumption of high fats diets and high-calorie foods are cited as high risks of obesity among women in Kenya (Mkuu *et al.*, 2019). Currently, there is no cut-off value for donors' height, necessitating the need to consider weight and height scales as proposed by Wiltbank *et al.*, (2010). Previous Wiltbank *et al.* (2010) and Rios *et al.* (2010) observed that American guidelines on minimum donor weight and maximum blood loss do not sufficiently protect younger blood donors. Following these observations, Blood System Inc. instituted new weight and height restrictions for all young donors to ensure donors with total blood volume below 3500ml do not participate in whole blood donation (Benjamin, 2010).

5.1.2 Percentage of blood volume donated by eligible blood donors attending Kenyatta National Hospital

In the current study, the median percentage of total blood volume donated was 11.7% and 11.6% using Nadler's equation and Lemmens-Bernstein-Brodsky equation, respectively. These statistics show compliance with the WHO (2012) recommendations of a maximum of 13%. The findings also agree with safe limits of 10-15% set by regulatory and scientific bodies (AABB, 2012). In addition, both equations showed the percentage of TBV donated ranged from 5.8% to 15.5% for men and 9.7% to 17.4% for women. This observation shows some donors exceeded the maximum donation limits. This could be attributed to the challenge of using weight alone in estimating donors' TBV. These findings are comparable to a study by Karp & King (2010), which reported that TBV ranged from 11.1% to 15.8% for men and 12.2% to 17.4% among women, suggesting an existing global variation of the percentage blood volume donation.

This study observed female donors donated a significantly higher percentage of their TBV than their male counterparts, suggesting that female donors have lower blood volumes than male donors which could be attributed to menstrual blood loss among women and the effect of testosterone among men (Ally *et al*., 2018). This observation is similar to a report by Karp & King, (2010), who compared blood volumes donated in 17 counties. However, in these studies, TBV was estimated using specific values for blood volume as 60 ml/kg in women and 66 ml/kg in men. Our study also found eligible male donors donated 11.3% of their TBV, in agreement with a study in Pakistan which

documented 10.2% and 10.8% as percentage blood volume donated by male do using two different equations (Salamat, 2007).

The present study also found the median TBV established by Nadler's equation and Lemmens-Bernstein-Brodsky's equation as 4628.5 ml and 4633.4 ml, respectively. These statistics shows that most donors had TBV above the minimum recommended threshold of 3500ml. These finding differ from a study done in Pakistan that reported a median TBV of 4819.2 ml. The considerable difference could be attributed to only male participants in their study (Salamat, 2007). The minimum TBV established by Nadler's equation and Lemmens-Bernstein-Brodsky's equation was 3270 ml and 3450 ml, respectively. This could be due to the failure to assess TBV when recruiting donors. According to the AABB (2012) standards, donating 500 ml ± 10% of blood represents 15% blood loss from a donor with TBV of 3500 ml.

This study observed that 18.8% of donors were at risk of vasovagal reactions as a result of donating more than 13% of their TBV. This prevalence is mainly attributed to the failure to incorporate TBV screening in donor selection. However, other scientific and regulatory bodies have set 15% as the maximum safe limit (AABB, 2012). The American Red Cross has initiated guidelines to prevent prospective donors aged below 19 years with blood volumes <3500 ml from donating whole blood (Eder *et al.*, 2008). The impact of this move is currently assessed for consideration of involving older donors. Although our country lacks policies on limits for TBV donation, losing more than 13% of blood

volume during donation may expose donors to adverse effects such as fainting or becoming anemic.

Using Lemmens-Bernstein-Brodsky and Nadler's equations, this study found 0.5% and 2.5% of eligible donors at risk of vasovagal reactions due to TBV below 3500 ml. This is also due to the failure to screen for TBV when selecting donors. This observation is relatively similar to other studies published globally. For example, a study by Hasan *et al.* (2020) in Malaysia showed the risks of vasovagal reactions at 1.5%. Similarly, another study in India reported donors were at risk of vasovagal reactions at 1.2% (Philip *et al.*, 2014). Our observations are slightly lower than a study by Benjamin (2010),which reported that approximately 5% of American donors aged 16-60 years had estimated blood volume below 3500 ml. The difference in vasovagal rates may be due to variation in donor selection criteria and sample size.

A multivariate analysis of blood volumes documented that TBV below 3500 ml is the strongest indicator of vasovagal reactions among young donors donating for the first–time (Benjamin, 2010). According to Salamat (2007), several factors can cause adverse reactions/ vasovagal reactions. These factors include; the volume of blood, weight, gender, and age (Salamat, 2007). Currently, no local evidence has explored these risk factors among the Kenyan donor population.

In this study, female donors had higher risks for vasovagal reactions than male donors. According to Hasan *et al.* (2020) report, this scenario may be attributed to different

emotional states and females being more anxious than male donors during blood donations. The current observation also agrees with studies done in India by Tondon *et al.* (2008) and Philip *et al.* (2014). Additionally, this study observed that first-time donors had higher risks of adverse reactions than repeat donors. This finding agrees with a study in Malaysia that reported first-time donors had a significantly increased rate of vasovagal reactions compared to repeat donors. This phenomenon was attributed to anxiety and lack of experience (Hasan *et al.*, 2020).

The current study found that donors below 19 years and those above 50 years were not exposed to vasovagal reactions. This association may be attributed to younger donors having higher carotid-aortic baroreceptor sensitivity than older donors. Simulation of these baroreceptors causes vasovagal reactions during and after the donation process. As age advances, baroreceptor sensitivity becomes damped (Hasan *et al.*, 2020). This observation contradicts other studies that reported a positive association between increasing participants sample size and age groups (Hasan *et al.*, 2020; Sultan *et al.*, 2016). According to a previous study by Rios *et al.* (2010), excluding prospective donors in their early 20s could defer almost 99% of all female donors and approximately 2.7% of available donors due to low TBV.

Effective donor recruitment criteria and blood donation processes are the initial steps toward blood safety, provided all the guidelines and standards are strictly observed. The demographic characteristics of the donor population may vary based on geographical location. Therefore, it is advisable to develop and adopt standards and guidelines that

match the features of the indigenous people based on local evidence. One of the critical areas likely to be affected by the existing regional characteristics is the amount of blood collected during blood donation. Ideally, the volume of blood collected from a donor should not cause vasovagal/adverse reactions but avail adequate quantities of blood and blood components (WHO, 2012).

5.1.3 Reliability of using Nadler's and Lemmens-Bernstein-Brodsky equations in calculating donors' total blood volume

The present study compared the reliability of using two conventional methods, Nadler's and Lemmens-Bernstein-Brodsky equations in estimating donors' TBV and found excellent reliability. To date, there is no consensus among several conventional methods used to estimate TBV. The two equations were selected based on their recommendation in transfusion medicine literature and their ease of use (Polly *et al.*, 2017; Ragav & Sandeep, 2020). These equations are simple to use and can be ideal for estimating blood donors' total blood volume.

Earlier conventional methods assumed the existence of a constant ratio between blood volume (BV) and surface area/body weight (Lemmens *et al.*, 2006). According to Lee *et al.* (2019), some methods use the mean value for indexed blood volume as 70 ml/kg for normal-weight adults and other methods use gender-specific mean value for indexed blood volume (normal male adults as 70 ml/kg and 65 ml/kg for normal female adults). Computing TBV with these methods resulted in significant errors marked with systematic

bias. To avert systematic errors and bias, some mathematical equations have been developed and others modified to help determine accurate TBV among adult humans.

In this study, one sample T-test showed a statistically insignificant difference in blood volumes estimated by the two equations (Nadler's and Lemmens-Bernstein-Brodsky equations). This suggests that any of the equations may be used to approximate blood donors' TBV. However, a previous study on the analysis of methods used to calculate TBV reported that Nadler's equation overestimates blood volume (Newman, 2014). This was attributed to ideal body weight (IBW) which is partially affected by the difference in the body composition related to age and sex (Strugnell *et al.*, 2014).

A previous study that focused on the difference between ideal and actual body weight in estimating TBV showed that using ideal body weight would give lower blood volume. This was attributed to excluding the influence of skeletal muscle and adipose tissue (Muraki *et al.,* 2018). Estimating TBV based on actual body weight tends to give higher TBV because of the effects of adipose tissues. According to Kortbeek *et al.* (2008), the obesity status of a prospective donor should be factored in when estimating donors' TBV. In obese donors, TBV can seriously be overestimated from equations based on weight alone. Overestimating donors' TBV may exposes donors to vasovagal / adverse reactions. Therefore, it is imperative to establish donors' body metabolic index when estimating their blood volume.

5.1.4 Prevalence of iron deficiency and anemia among eligible blood donors at Kenyatta National Hospital

The current study found the prevalence of iron deficiency (ID) among eligible donors as 2.5%. The main reasons for this finding could be attributed to low numbers of female donors and donor status, where the majority of donors were donating for the first-time. This prevalence is consistent with other Saudi Arabia and Iran studies that observed a prevalence of 2.17% and 2.14%, respectively (Abdullah, 2011; Yousefinejad *et al.*, 2010). These findings were attributed to an increase in donations that resulted in a decrease in iron stores among repeat donors. Other studies with the same serum ferritin cut-off values found a higher prevalence of ID. For example, studies by Gunnarsdóttir *et al.* (2017) and Mozaheb *et al.* (2011) observed a prevalence of 11% and 20% among Iceland and Iranian blood donors, respectively. Other reasons for variation in ID prevalence could be differences in sample size, population iron status, the number of donations per year and last donation intervals.

The World Health Organization (WHO, 2020) defined iron deficiency as serum ferritin concentration <15 ng/ml. Previous studies with a higher prevalence of iron deficiency had reduced ferritin threshold values (serum ferritin concentration <12 ng/ml). For instance, Ali *et al.*, (2015) reported a prevalence of 24% among eligible blood donors in Sokoto, Nigeria. Another study with the same serum ferritin cut-off values (<12 ng/ml) found a prevalence of 20.6% among regular blood donors in Port Harcourt, Nigeria (Jeremiah *et al.,* 2010). The most vital indicators for ID in a blood donor population are menopausal status, gender, number of donations and time since the last donation (Rigas

et al., 2019). According to Reddy *et al.* (2020), chronic ID among the Indian donor population is mainly attributed to donation frequency of more than three times a year.

In our study, female donors had a higher prevalence of ID than male donors (6.7% vs. 1.7%, respectively). This observation is mainly attributed to menstrual iron loss among females. Eligible iron deficient donors gave a unit of blood, further reducing their iron stores by 200-250 mg. This observation is similar to a study by Ali *et al.*, (2015), who reported the prevalence of ID among female and male donors at 11.8% and 7.4%., respectively. Another study by Gunnarsdóttir *et al.*, (2017) observed the prevalence of iron deficiency among female and male donors at 22% and 1%, respectively. The probability of an eligible donor developing iron deficiency varies globally; this is due to differences in the capacity to absorb iron, use of supplemental iron, frequency of blood donation, nutritional iron intake as well as implications of geographic residence and socio-economic status (Goldman, 2015).

The present study found males had a significantly higher serum ferritin concentration than female donors. This observation is similar to previous studies done in Iceland and Nigeria, which reported female donors had lower serum ferritin concentrations than their male counterparts (Gunnarsdóttir *et al.*, 2017; Ali *et al.*, 2015). This observation is mainly due to menstrual iron loss in females. According to Deepa *et al.* (2017), there is a definite relationship between female donors of reproductive age and low ferritin concentrations. This finding was attributed to their inability to restore dietary iron lost from physiological demands.

This study observed a significant difference between serum ferritin concentrations and donors' age groups. It further showed a gradual increase in ferritin concentrations as donors advance in age. This observation differs from a study by Mozaheb *et al.*, (2011), who published an insignificant difference between ferritin levels and donors' age. Still, they also observed a downward trend in all ages. In pre-puberty life, no significant difference can be observed between ferritin concentrations, ages and the sexes. The difference emerges only after the onset of menstruation, and then the situation reverts ten years after menopause when hemoglobin and ferritin levels become similar to that of age-matched men (Ali *et al.*, 2015).

The current study found an insignificant difference between serum ferritin concentrations and donor status (first-time and repeat blood donor). This phenomenon could be attributed to inconsistencies in blood donation among repeat donors. Our finding disagrees with an investigation by Abdullah (2011), who reported that first-time donors in Saudi Arabia had significantly higher mean ferritin concentration compared to repeat donors. Another study by Adediran (2013) observed that mean serum ferritin levels were significantly lower in regular blood donors in Lagos. They argued that repeat donors might be having a pre-latent or latent ID when they present to donate.

This study observed an insignificant difference in serum ferritin concentrations between the last donation time and the number of donations. This phenomenon may be attributed to the lack of regular blood donation among repeat blood donors in this study. Our finding agrees with a study by Rigas *et al.*, (2019) but differs from a study by Deepa *et*

al. (2017), which reported first-time donors have low serum ferritin concentrations, probably due to their low socioeconomic status and dietary habits.

The present study found the prevalence of anemia at 7.4%, with male donors recording a prevalence of 8.7%. None of the eligible female donors was anemic. This observation was mainly attributed to the Kenya policy that prescribes an eligible donor should have a pre-donation hemoglobin level above 12.5g/dl regardless of gender. Hemoglobin level below this cut-off value has been cited as the leading cause of donor deferral in several countries. A previous report by Njenga *et al.* (2019), documented a low hemoglobin account for about 11.6% of all donor deferral cases in Kenya. According to Mast (2014), measuring hemoglobin concentration alone is not satisfactory to qualify blood donors' fitness to donate blood. However, screening for iron status and hemoglobin concentrations enables a good prediction of donors exposed to anemia (Moaheb *et al.*, 2011).

According to the WHO (2012) guidelines for defining anemia, the hemoglobin cut-off value for adult men should be below 13.0 g/dl and for non-pregnant adult women 12.0 g/dl. There are variations regarding the acceptable minimum hemoglobin cut-off values for female and male donors. For instance, countries like Kenya and Nigeria have adopted a uniform hemoglobin cut-off value for both genders as 12.5 g/dl; in Brazil, the minimum hemoglobin requirement for male donors is >13 g/dl and for females >12g/dl. According to the European council, the cut-off values for blood donation are 13.5g/dl and 12.5 g/dl for male and female donors, respectively (Deepa *et al.*, 2017). The rationale for a single

cut-off value for hemoglobin is that female donors represent the highest number of donors deferred due to low hemoglobin. In addition, most of them are likely to have hemoglobin levels near 12.5 g/dl.

The present study showed a significant correlation between the number of donations and the serum ferritin levels. This observation agrees with the findings by Mozaheb & Khayami, (2011) and Deepa *et al.* (2017), who reported a statistically significant correlation between frequency of donation and serum ferritin levels. This phenomenon was attributed to repeat blood donation, which significantly affects iron stores and hemoglobin concentration in all donors (Mozaheb *et al.*, 2011). A study by Omeara *et al.* (2011), revealed an insignificance difference between serum ferritin levels and frequency of blood donation a scenario that may be due to variation in demographic characteristics among the donor population.

Low hemoglobin has been cited as the leading cause of donor deferral. Therefore, blood donation centers are tasked with safeguarding blood donors' health against developing iron deficiency and anemia (Adediran *et al.,* 2013). They also have a crucial role in promoting voluntary non-remuneration donation in societies, like Kenya, where hospital-based donation is primarily from family replacement donors. Early detection of ID and anemia among prospective donors would advocate for use of iron supplements and necessary changes in donation intervals (Abdullah, 2011).

5.1.5 Hematological profiles of eligible blood donors attending Kenyatta National Hospital

In this study, the median results for all hematological parameters derived from male and female donors were within the local reference intervals for adults in the urban population. This observation might be attributed to several factors such as similarities in demographic characteristics, geographic location and a subjective donor recruitment criterion similar to the one used in formulating reference intervals. This finding differs from a study by Sing'oei *et al.* (2021), which reported marked differences in some hematological reference values developed in Kericho and Kisumu counties in Kenya. This variation was attributed to the difference in ethnicity, geographical locations and age group distribution among the study participants.

This study found some donors recorded abnormal hematological values (below and others above reference intervals). Seven hematological parameters (red cell counts, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentrations, monocytes, eosinophils and platelets) had significant variation from normal. The finding may be attributed to the act of screening only hemoglobin concentration when selecting donors. The variation in hemoglobin levels may have resulted from different screening techniques employed. (Capillary blood vs. venous blood). This observation implies that some eligible donors could be having normal hemoglobin levels with abnormalities such as erythrocytopenia, leucocytopenea or thrombocytopenia that is not detected when selecting donors.

Hematological abnormalities have also been mentioned in other parts of Kenya (Kibaya *et al.,* 2008) and African countries. For instance, a Nigerian study found a significant proportion of eligible donors had abnormalities in hematocrit, lymphocyte, neutrophil, eosinophils, white blood cells and platelet (Lugos *et al.*, 2019). Another study on Sudanese blood donors reported platelet abnormalities at 13.3 % (Abbas *et al.*, 2016). Other African countries with the same observations include; Botswana (Mine *et al.*,2012), Uganda (Eller *et al.*, 2008) and Tanzania (Ally *et al.*,2018). Abnormalities in monocytes and eosinophils counts could be attributed to the higher prevalence of infections such as parasitic infestation associated with eosinophilia and leukocytosis. In addition, reduced platelets count could have resulted from genetic factors and increased utilization of platelets due to the high prevalence of malaria.

The current study established that male blood donors had significantly higher red blood cell count, hemoglobin and hematocrit values than female donors. The marked difference was mainly attributed to the effect of the testosterone hormone on erythropoiesis and menstrual blood flow in females (Ally *et al.,* 2018; Sing'oei *et al.,* 2021). Similar observations were reported in other studies worldwide, including in African countries (Ali *et al.,* 2015; Ally *et al.,* 2018; Miri-Dashe *et al.,* 2014; Tekkeşin *et al.,* 2015; Murphy, 2014; Yalew *et al.,* 2016). Comparable reports have also been observed in Kenya (Sing'oei *et al.*, 2021; Geoffrey *et al.,* 2018; Rose *et al.,* 2018). According to Zeh *et al.* (2011), higher values of red cell indices among male donors is attributed to mass of muscle fibers and body size. In the early stages of human development, no significant

difference in hemoglobin or red cell count can be seen in both genders. Only after the onset of puberty do differences emerge (Ali *et al.*, 2015).

This study found female participants had a significantly higher lymphocyte count than male donors. This difference could be attributed to innate variability in immune cell behavior (Andersen & Vance, 2019). Another study reported women to have a greater risk for adverse responses to vaccines, viral infections and autoimmune disorders, while exhibiting cell-mediated and humoral responses compared to men. The current findings agrees with studies done in Kenya and other African countries (Miri-Dashe *et al.*, 2014; Tembe *et al.*, 2014; Ali *et al.*, 2015; Omuse *et al.*, 2018; Odhiambo *et al.*, 2015).

According to Miri-Dashe *et al.* (2014), increased leucocyte counts among female donors is attributed to contraceptive use. In addition, the variability in immune characteristics between males and females may not solely be attributed to hormonal variation, but also to differences in triglycerides, cholesterol and body fat distribution (Palmisano *et al.*, 2018). Another study in Kenya reported that females had higher monocyte counts than males, a phenomenon that they attributed to exposure to environmental conditions and endemic parasitic infections (Rose *et al.*, 2018).

This study also found female blood donors had significantly higher platelet counts than male donors. The variation in platelet count has been associated with the difference in hormonal profiles between females and males (Elderdery & Alshaiban, 2017). It may also occur due to the simulation of megakaryopoiesis by erythropoietin hormone in response

to menstruation (Sing'oei *et al.*, 2021). Other researchers made similar observations (Miri-Dashe *et al.*, 2014; Ally *et al.*, 2018; Rose *et al.*, 2018; Tembe *et al.*, 2014). In contrast, some scholars documented an inverse relationship between platelet count and hemoglobin concentrations (Kulnigg-Dabsch *et al.*, 2013; Park *et al.*, 2013). An analysis research on platelet concentrations mentioned that the African population has relatively lower values than the Western population. The cause of this variation is unknown, but probable causes may include genetics, environmental factors, malaria infection and diet (Daniel *et al.*, 2019).

This study found an insignificant difference between hematological profiles and blood donors' age groups. A similar observation was reported on Ethiopian blood donors (Gessese *et al*., 2020). On the contrary, a study on Tanzanian blood donors reported only mid-sized cells (basophils, eosinophils and monocytes) were significantly higher among donors aged 18 to 20 years (Ally *et al*., 2018). Another study on Iranian blood donors observed a significant difference in the red cell count and mean cell hemoglobin between age groups 30-40 and 41-50, respectively (Rasouli *et al*., 2017). These observations were used to justify the importance of age-specific reference ranges.

5.1.6 Rates and causes of temporary and permanent deferrals among blood donors at Kenyatta National Hospital

This study found a donor deferral rate of 47.4%. This rate may be attributed to the majority of participants being family/replacement donors. A previous study on the prevalence of infections observed a significant difference between voluntary and

replacement donors. This phenomenon was explained by the fact that replacement donors believe they must donate to save their relative's life whereas voluntary donors donate at their own will (Ibrahim *et al.,* 2014). These findings resonate with other studies that reported replacement donors were likely to be deferred compared to voluntary donors (Okoroiwu *et al.*, 2018; Valerian *et al.*, 2018; Meinia *et al.*, 2016). According to WHO (2010), family/replacement donors are believed to have higher risks of TTIs compared to voluntary donors.

A high deferral rate among blood donors has been associated with a high-risk sexual activities, prevailing endemic conditions, lack of accurate hemoglobin screening tools, poor knowledge of donor eligibility criteria, and variation of motivation factors towards blood donation (Chenna *et al.*, 2015). This study observed a relatively higher deferral rate compared to other countries in Africa and beyond. For instance, the deferral rate of a Tanzanian study was 12.7% (Valerian *et al.*, 2018), in Nigeria, it was 8.69% (Okoroiwu *et al.*, 2019) and Ethiopia was 14.2 % (Birhaneselassie, 2016). Moreover a study on the American donor population observed a donor deferral rate of 15.6% (Shaz *et al.*, 2012).

Availability of stricter donor selection criteria and high levels of keenness among recruiting officers may lead to an increase in deferral rates (Valerian *et al.*, 2018). Variation in socio-economic status may also contribute to the difference in deferral rates. For instance, donors in high and middle-income countries have higher hemoglobin and nutritional status and a lower prevalence of infectious diseases than donors in developing countries (WHO, 2011). The World Health Organization recommends member countries

to develop policies on blood transfusion that will define donor deferral, recruitment, screening for diseases, donor notification of results and referrals (WHO, 2012).

In this study, the donor deferral rate was higher among female donors than male donors (71.8% vs. 38%, respectively). Some of the reasons for this observation include; greater caution among physicians when selecting female donors, stricter donor eligibility criteria among female donors, and lastly the probability of a female donor deferral is about 15 times more compared to a male donor (Agnihotri, 2010). The findings agree with studies in Nigeria (Ekwere *et al.*, 2014) and India (Agnihotri *et al.*, 2015). Other studies associated high deferral rates among females donors with cultural beliefs that refrain female donors from donating blood owing to their monthly menstrual flow (Okoroiwu *et al.*, 2018).

This study further observed donor deferral rates increase with the advancement of age. This finding agrees with studies done in northern Tanzania (Valerian *et al*., 2018) and Turkey (Arslan, 2007). On the contrary, a study done in Saudi Arabia reported low deferral rates (7.9%) among donors aged 40 years above and high deferrals (87.9% among donors aged 18-30 years (Salah, 2020). Another study in India observed a high deferral rate (65%) among donors aged 18-30 years and a lower rate (3%) among donors aged above 60 years (Manish *et al*., 2022). Higher deferral rates among young blood donors may be caused by food fading, leading to low micronutrients and under-nutrition (Reddy *et al.,* 2020). The difference in deferral rates may be due to variations in the incidence of infections and the age structure of a population.

The present study found higher donor deferral rates among first-time donors than repeat donors (53.7% vs. 34.6%, respectively). This disparity may be attributed to regular checks during each donation and greater awareness of blood donation criteria among repeat blood donors. According to Ekwere *et al.* (2014), the leading cause of first-time donor deferral is risk factors relating to hepatitis B and HIV infection. This observation agrees with another study by Kasraian & Negarestani, (2015), which reported higher deferral rates among first-time Iranian blood donors (48.1% vs. 13.1%). This finding indicates the importance of creating awareness of eligibility criteria for the general population.

Temporarily deferred donors had a higher deferral rate than permanent deferral (93.4% vs. 6.6%, respectively) in this study. This scenario may be attributed to demographic factors such as a high number of first-time donors and replacement donors. Other studies with high permanent deferrals mentioned high numbers of paid/remunerated donors (WHO 2010). These findings agree with a study on Iranian blood donors that found that short-term deferral accounted for 95.5% (Kasraian & Negarestani, 2015). In contrast, some studies reported higher rates for permanent deferrals than short-term deferrals. For example, a study on Nigerian blood donors found permanent deferral account for 68.9% (Okoroiwu & Uchechi, 2019). The same trend is comparable to studies from Valerian *et al.* (2018) and Rehman *et al.* (2012). This scenario was associated with donors concealing their lifestyles and health status for money or the desire to save their relatives. As a consequence, it might be hard to gather relevant information for temporary deferral except for hemoglobin levels that are checked during screening.

The main causes of temporary deferrals in this study were medication and low hemoglobin (33.3% and 31.3%, respectively). These rates may have resulted from donor ignorance, poor nutrition, repeated pregnancies and low social-economic status. Several studies have documented anemia as the main cause of donor deferral. Ahmad *et al.* (2020) reported comparable results at 34.1%, Chauhan *et al.* (2015) at 24.1% and Sareen *et al.* (2012) at 39.4%. Another study by Kasraian & Negarestani (2015) found that 31.9% of the Iranian blood donor population had underlying medical conditions agreeing with our findings.

The leading cause of permanent donor deferral in this study was abnormal blood pressure accounting for 3.3%. Other reasons for permanent deferrals were diabetes and co-morbidities with chronic conditions; the same observation was mentioned in a study by Padma *et al.*, (2017). This finding illustrates the importance of sensitizing potential donors on donor selection criteria and deferral duration. According to Kasraian & Negarestani (2015), there have been variations in donor deferral rates and thus recommend further studies to evaluate reasons and deferral rates in various geographical locations.

Finally, some sections in the Kenyan donor eligibility criteria differ from those used in other countries. For instance, the Kenyan policy defines deferral time for a tattoo as 12 months, whereas the American Red Cross guidelines allow a donor to donate if the tattoo was applied by state-regulated agencies (American Red Cross, n.d). For chronic conditions such as blood pressure and diabetes, the Kenyan policy recommends a

permanent deferral, but according to American Red Cross guidelines, donors who have controlled these conditions should be allowed to donate (American Red Cross, n.d). In addition, the Kenyan policy on donor recruitment lacks some aspects observed in other countries. For instance, the policy lacks criteria for antibiotic use, infection with viruses such as Zika and Ebola, and travel to other countries, among others observed by the American Red Cross.

5.2 Conclusions

1. The percentage of TBV donated by eligible donors at KNH was 11.7% and 11.6% as determined using Nadler's and Lemmens-Bernstein-Brodsky's equations, respectively. Female donors donated a significant higher percentage of their TBV than male donors ($p<0.001$).
2. The Nadler's and Lemmens-Bernstein-Brodsky equations showed excellent reliability when estimating donors' TBV (average and single measures ICC =0.985 and 0.992, respectively).
3. The prevalence of iron deficiency and anemia was 2.5% and 7.4%, respectively. Female blood donors had a higher prevalence of iron deficiency (6.9% vs. 1.7%) whereas male donors had a high prevalence of anemia (7.4%vs.0%). Male donors had significantly higher ferritin levels than female donors.
4. The median counts of all hematological parameters analyzed were within the acceptable local reference intervals. However, seven hematological parameters (RBC, Hgb, MCH, MCHC, monocytes, eosinophils and platelets) significantly varied from normal values. In addition, male donors had significantly high hematocrit,

hemoglobin and red cell count while female donors had significantly high lymphocytes and platelets.

5. The donor deferral rate was 47.4%. Temporarily deferred donors had a higher rate than permanent deferral (93.4% vs. 6.6%, respectively). The common causes for temporary deferrals were medication and low hemoglobin, whereas the leading reasons for permanent deferrals were high blood pressure and co-morbidities.

5.3 Recommendations

1. Total blood volume test is recommended in the Kenyan donor selection criteria to avert exposing prospective donors with low TBV to vasovagal/ adverse reactions
2. Nadler's or Lammens–Bernstein–Broadsky equations may be used in estimating blood donors' total blood volume.
3. Iron status test should be included in the Kenyan donor selection criteria and a review of donor hemoglobin cut-off values to align with WHO definition of anemia.
4. Incorporation of total blood count test in the Kenyan donor selection criteria in addition to hemoglobin, only donors with normal hematological profiles should be allowed to donate.
5. Awareness campaigns on Kenyan donor selection criteria, targeting those with high deferral rates such as replacement donors, first-time donors and female donors should done.

5.3.1 Recommendations for further studies

This study further recommends;

1. Studies to establish total blood volume reference interval among eligible blood donors in Kenya
2. Studies on blood donor selection criteria, deferrals and its effect on blood availability in Kenya.

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APPENDICES

Appendix I: Study Information and Consent form

Study title

Total blood volume, iron status, and hematological profiles of whole blood donors at Kenyatta National Hospital, Nairobi County, Kenya.

Principle investigator

My name is **Mr. Njenga K. John**, a student pursuing a Ph.D. at Kenyatta University School of Medicine, Department of Medical Laboratory Science.

Co-investigators and institutional affiliation

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Introduction

This is to introduce you to the study conducted by the principal investigator and Co-investigator listed above. The purpose of this form is to educate you to make an informed decision to either participate or not in this study. This document contains information detailing potential risks and benefits of participation. Your participation is voluntary. You are at liberty to agree or disagree with enrolling in the study. Disagreeing with this study will not affect your participation in blood donation processes. You can withdraw from the study at any time. You are also encouraged to ask questions regarding your role.

Nature of the study

This research project targets the total blood volume, iron status, and hematological profiles of whole blood donors at Kenyatta National Hospital, Nairobi County. This study seeks to recruit prospective whole blood donors presenting to donate whole blood at Kenyatta National Hospital. A total of 384 consenting participants will be included in the study. Consenting participants will be requested to fill an enrolment form, screening questionnaire, and informed consent. Participation in this study will be grouped into two categories A and B. Category A will comprise participants who will be allowed to donate.

In contrast, category B will comprise participants who will be deferred from donation. Category, A participants, will have their anthropometric parameters; height (cm), weight (kg), and basal metabolic index (BMI) measured using calibrated scales. Approximately 8 ml of their donated blood will be screened for serum ferritin, total blood count, and CRP. Reasons and deferral time for category B participants will be documented and analyzed.

Study procedures

As a study participant, you will be interviewed by an expert in a private location to enhance confidentiality. This session will last approximately 5 minutes. Anthropometric parameters, height (cm), and weight (kg) will be taken before blood donation. About 8 ml of the collected blood will be extracted from donated units for laboratory analysis. You will be asked for your preferred channel of results communication, including phone number and email address.

Study Participants

Consenting to participate in this study is voluntary. Before enrolment, you will be fully informed about the nature of this study. All benefits and risks likely to occur in the study will also be explained to you. Study participants will be at liberty to withdraw at any stage.

Types of specimen

Approximately 8 ml of whole blood that you donate will be used for this study. The sample will be collected in vials 4 ml (red and purple vacutainer tube) before transportation to the analyzing laboratory.

Benefits

Study participants who will donate blood (Category A) will receive free medical testing (serum Ferritin, TBC and CRP) at no cost. Participants with confirmed abnormal hematological parameters will be given a referral form to use when seeking management. Study participants who will be differed from donating whole blood (Category B) will get a chance to understand the reasons for deferral and, where possible, when to donate. This study will also be crucial to the Ministry of Health by providing evidence on donors' blood volume, iron status, and hematological profiles. These findings will also be vital in formulating guidelines and policies on donation volume, blood donation frequencies, and additional tests necessary for donor screening.

Potential Risks

There is no direct physical risk that is likely to occur following the measurement of anthropometric parameters (weight and height) and the extraction of blood samples from already donated pints. Qualified registered health personnel will do the recruitment, screening, and dispatch of study results to minimize the occurrence of potential perils such as emotional, social, or psychological risks.

Participants' confidentiality

All personal data you will fill in the study questionnaires and laboratory results will be handled with the utmost confidentiality. You will be identified with a coded number, and your data will be stored in a computer database protected with a password. All filled questionnaires and paper works will be kept in a lockable file cabinet. Research materials will be retained at Kenyatta University Health Center. After completing this study and publication (about 2 years), all material generated in this work will be destroyed by shredding all paperwork and soft copy data destroyed by deleting all information stored in the computer.

For more information contact

KNH-UON ERC: Cell 020- 2726300,

Principal Investigator: 0728306923

Consent Statement

I agree to participate in this study, having read/informed the nature of this research. I am aware that my participation is purely voluntary, and I am at liberty to withdraw from the study at any stage. I am also informed my profile will be treated with confidentiality.

Participant's name	**Sign**	**Date**

Witness (applicable to illiterate study participants**)**

Witness name	**Sign**	**Date**

Investigator's statement

I ascertain I have elaborated the nature of this research work to all eligible participants, and they are at liberty to accept or refuse.

Investigator's name	**Sign**	**Date**

Please choose your preferred channel of results dissemination by giving your contact details below.

SMS ……

Phone call……

Email..……

Appendix II: Participant Enrolment Form

Study title: Total blood volume, iron status and hematological profiles of whole blood donors at Kenyatta National Hospital, Nairobi County, Kenya.

Participant enrolment no …….

Participants' Demographic information

(Please answers all questions)

1. Age _____
2. Gender _____
3. Marital status _____
4. Contact / cell number _____
5. Email address……..
6. Donation type? ☐ Voluntary ☐ Replacement
7. Donor status? ☐ First-time donor ☐ Repeat donor

(Your information will be confidential)

Appendix III: Participant Screening Form

Study title: Total blood volume, iron status and hematological profiles of whole blood donors at Kenyatta National Hospital, Nairobi County, Kenya.

Participant enrolment number…….

Study research questions

1. Have you ever participated in whole blood donation?

   Yes ☐ No ☐

   Kindly answer question 2 and 3 if you have answered YES in question 1

2. How many times have you donated blood…….

3. Tick where appropriate the last time you donated whole blood

   - Less than four months ago
   - More than or equal to 6 months ago
   - More than or equal to 12 months
   - More than two years ago

4. Have you been accepted to donate blood? Yes ☐ No ☐

   If NO in question 4, kindly answer question 5 and 6

5. For how long have you been deferred (*kindly tick where appropriate*)

   - For one month and below
   - For three months
   - For six months
   - For one year
   - Permanent deferral
   - I don't know

6. What is/ are the main reason/s for deferral

……..……

……..……

……..……

……..……

For official use only

Participant enrolment category Category A ☐

Category B ☐

Appendix IV: Fomu ya Idhini

Mada ya utafiti

kiwango cha damu mwilini, madini mwilini na wasifu wa hematolojia kati ya wafadhili wa damu katika Hospitali Kuu ya Kenyatta, Nairobi Kaunti, Kenya.

Mtafiti mkuu

Jina langu ni **Njenga K. John** mwanafunzi wa chuo kikuu cha Kenyatta idara ya maabara, katika shule ya udaktari.

Watafiti wenza

Dr. Scholastica Mathenge	Kenyatta University	0722936884
Dr. Nelson Menza	Kenyatta University	0725011570
Prof. Jessie Githanga	University of Nairobi	0721245721

Utangulizi wa utafiti

Mtafiti mkuu analenga kuwahoji washiriki ambao wamejitolea kutoa damu. Lengo la utafiti ni kujua kiwango cha damu mwilini, madini mwilini na wasifu wa hematolojia kati ya wafadhili wa damu Hospitali Kuu ya Kenyatta. Utapimwa uzito wa mwili, urefu na kiwango cha damu mwilini. Ukifaulu kutoa damu kiwango kidogo 6-8ml kitachukuliwa kuangalia kiwango cha ferritin, na CRP .

Kushiriki

Iwapo utajiunga katika utafiti huu utaulizwa maswali na mhudumu aliyehitimu. Majibu yako yatakuwa siri kati yako na sisi. Mahojiano hayo yatadumu kwa muda wa dakika tano. Baada ya mahojiano tutakuomba ujaze maswali machache baada ya kukupima uzito wa mwili na urefu.

Sampuli husika

Ikiwa utakubali kusiriki katika utafiti huu. Kiwango cha damu (6-8ml) kitachukuliwa kutoka kwenye painti utakayo toa. Sampuli hii itatumika kufanya uchunguzi wa kisayansi.

Faida

Ukikubali kushiriki katika utafiti huu utafanyiwa uchunguzi a kiafya bila ya kulipa chochote. Utafiti huu utasaidia kuelewa mathara ya kutoa damu kwanye kiwango cha damu mwilini, na madini mwilini. Mshiriki atakaye patikana na ugonja wowote atashawishiwa kupata matibabu kwa kliniti ya damu kwa matibabu.Utafiti huu utasaida wizara ya afya kuelewa kiwango cha damu mwilini, na madini mwilini . utafiti huu pia utasaidia kupendekeza kubadilisha sharia kuhusu viango vya damu milini

Hatari za kushiriki

Kutoa damu ni jambo la kawaida nchini Kenya. Hatari yeyote inaweza kutokea wakati wa kutoa damu. Kwa kawaida hakuna hatari yeyote inayoweza kutokea . Ikiwa kuna hatari za kushiriki kwenye utafiti huu ni kidogo sana na ikiwa itatokea utasaidiwa na matibabu.

Kudumisha siri

Yale tutakayozungumza yatabaki kuwa siri. Habari hiyo haitafichuliwa kwa mtu yeyote yule ambaye si mmoja wa wenye kufanya utafiti. Mshiriki ana uhuru wa kutoshiriki katika utafiti wakati wowote unaotaka bila madhara yeyote. Maelezo yanayokuhusisha kama mshiriki yataekwa kwa sehemu iliyo salama. Ripoti ya maabara zitaekwa kwa muda a miaka tano ambapo zitaharibiwa kwa njia za kisasa.

Haki ya kukataa na kujiondoa kwenye utafiti

Sio jambo la lazima wewe kushiriki katika utafiti huu. Una uhuru wa kujuinga au kujiondoa katika utafiti huu.

Mawasilianao

Iwapo una swali kuhusu utafiti au unajihisi kwamba umeathirika, unaweza kuwasilia na: mtafiti mkuu; Mr. John Njenga 0728306923

Idhini ya kukubali

Nakubali kuwa maswali yote ambayo nilikuwa nayo juu ya utafiti huu yamejibiwa kwa njia ya kuridhisha. Naelewa kushiriki katika utafiti huu ni kwa kujitolea na habari nitakayo toa ni siri.

…… …….. ……..……..…

Mshiriki Sahihi Tarehe

Utaha (Kwa wahusika wasio na uwezo wa kusoma na kuandika**)**

Nathibitisha kumweleza mshiriki kuhusu utafiti huu na amejiamulia kujiunga katika utafiti huu bila kushawishiwa.

…… ……..…… ……..……

Mtafiti Mkuu Sahihi Tarehe

Tafadhali changua njia unayopenda kupokea majibu ya utafiti huu kwa kueleza nambari yako

Barua pepe…….

Simu-…….

Ujunbe mfupi……

Appendix V: Fomu ya Uandikishaji

Mada ya utafiti

kiwango cha damu mwilini, madini mwilini na wasifu wa hematolojia kati ya wafadhili wa damu katika Hospitali Kuu ya Kenyatta, Nairobi Kaunti, Kenya.

Namba ya kujiandikisha .....

Eleza majibu yote unavyoagizwa

Tafadhali jibu maswali yafuatayo.

1. Eleza jinsia yako.....
2. Eleza umri wako (miaka).....
3. Eleza kama uko kwa ndoa au la……..
4. Nambari ya simu …….
4. Aina ya ufadhili ? ☐ Kwa hiari ☐ Mbadala
5. Hali ya ufadhili? ☐ Ufadhili wa kwanza ☐ Kurudia

Appendix VI: Maswali ya Mchujo

Mada ya utafiti

Kiwango cha damu mwilini, madini mwilini na wasifu wa hematolojia kati ya wafadhili wa damu katika Hospitali Kuu ya Kenyatta, Nairobi Kaunti, Kenya.

Namba ya kujiandikisha .....

Maelekezo

Eleza majibu yote unavyoagizwa

Historia ya kutoa damu

1. Je! Umetoa damu? Ndio ☐ La ☐

   Ikiwa unejibu Ndio tafadhali jibu swali la 2 na 3

2. Ni mara ngapi umetoa damu.....
3. Mara ya mwisho kutoa damu ilikuwa lini?
   - Miezi nne iliyopita
   - Miezi sita iliyopita
   - Miezi kumi na mbili iliyopita
   - Zaidi ya miaka mbili
4. Umekubaliwa kutoa damu? Ndio ☐ La ☐

   Iwapo umejibu LA tafadhali jibu swali la 5 na 6

5. Umeelezwa unaweza kutoa damu baada ya muda upi?
   - Baada ya mwezi 1
   - Baada ya miezi 3
   - Baada ya miezi 6

- Baada ya miezi 12
- Siwezi toa damu milele
- Sijajua

6. Ni kwa sababu gani haujaruhusiwa kutoa damu?

……..……

……..……

……..……

……..……

Matumizi rasmi

Participant enrolment category

Category A ☐

Category B ☐

Appendix VII: Standard Operating Procedure for Total Blood Count

Purpose

HumaCount 5D hematology analyzer performs automated complete blood count. It is fully automated with no manual operations.

Sample requirement

Approximately 2-3 ml of EDTA blood

Samples will be analyzed at room temperature 18^{o} C to 26^{o} C and refrigerated at 2^{o} C to 8^{o}C for a maximum duration of 6 hours. Refrigerated samples will be brought to room temperature for 30 minutes before analysis.

Reagents and equipment

HumaCount 5D hematology analyzer
HC5D Diluent
HC5D Clean
HC5D CBC lyse
HC5D Diff lyse
HC5D Controls
HC Calibrator

Procedure

1. Switch on the analyzer by pressing the switch button
2. Allow the instrument to finish initialization phases
3. Perform 3 levels of quality control and confirm the analyzer is operating within the acceptable ranges.
4. Feed sample details on the analyzer
5. Mix the sample well, check for a blood clot; if it's ok to remove the tube cap and place the sample beneath the aspirator needle

6. When the analysis is complete, verify the result generated from the hematology analyzer.
7. Dilute samples if WBCs are above 100,000 and platelets above 1,000,000

Expected results will be as per the urban adult population reference intervals
(Omuse *et al*., 2018)

Appendix VIII: Standard Operating Procedure for C-Reactive Protein

Purpose

CRP is a medical test used to detect the quantity of CRP in the blood. Elevated levels are observed in various medical disorders such as inflammation, malignancies, and conditions associated with immunoglobulins, increased fibrinogens and plasma proteins.

Specimen requirements

Serum collected in plain red top or serum separator tube also heparinized plasma can be used. The blood sample is stable for 11 days at room temperature (15-25^{o}C), and refrigerated blood (2-8^{o}C) can last for two months

Equipment and reagents

- Serum
- Veda Lab analyzer
- Veda Lab CRP cuvettes
- CRP diluent
- Pipettes

Procedure

1. Ensure the analyzer is calibrated and controls are done for CRP test.
2. Remove cuvette from the storage foil.
3. Pipette 150ul of serum into the cuvette.
4. Place the cuvette vertically in the measurement rack.
5. Feed the analyzer with the sample cuvette
6. Select the test on the display and click start and follow the instructions given.
7. When testing is complete, select the sample details and command to print
8. Confirm the results, then report and interpret the results

Expected normal values for adults

CRP < 0.25 mg/dl

Elevated levels indicate inflammation

Appendix IX: Standard Operating Procedure for Ferritin test

Purpose

Ferritin is a significant protein that stores body iron. A ferritin concentration test is a quantitative analysis that indicates the quantity of stored iron. It is essential in evaluating iron stores in healthy individuals and those with iron overload and iron deficiency.

Specimen requirement

Serum collected in plain red top or serum separator tube also heparinized plasma can be used. The blood sample is stable for 24 hours at room temperature, and refrigerated blood (2-8^{o}C) can last for seven days.

Equipment and reagents

Biomerieux Mini Vidas analyzer
Calibrated Pipettes
Centrifuge

Procedure

1. Switch on the analyzer and allow it to warm up for about 45 minutes.
2. Bring cooled reagents strips to room temperature and let them stand for 30 minutes before use.
3. Dispense blood sample into the reagent strip wells and repeat the procedure for each sample
4. Select the Ferritin test and User ID
5. Select the Start option. The lighting of a green LED shows the reaction has started, and flashes of green LED indicated the end of the reaction

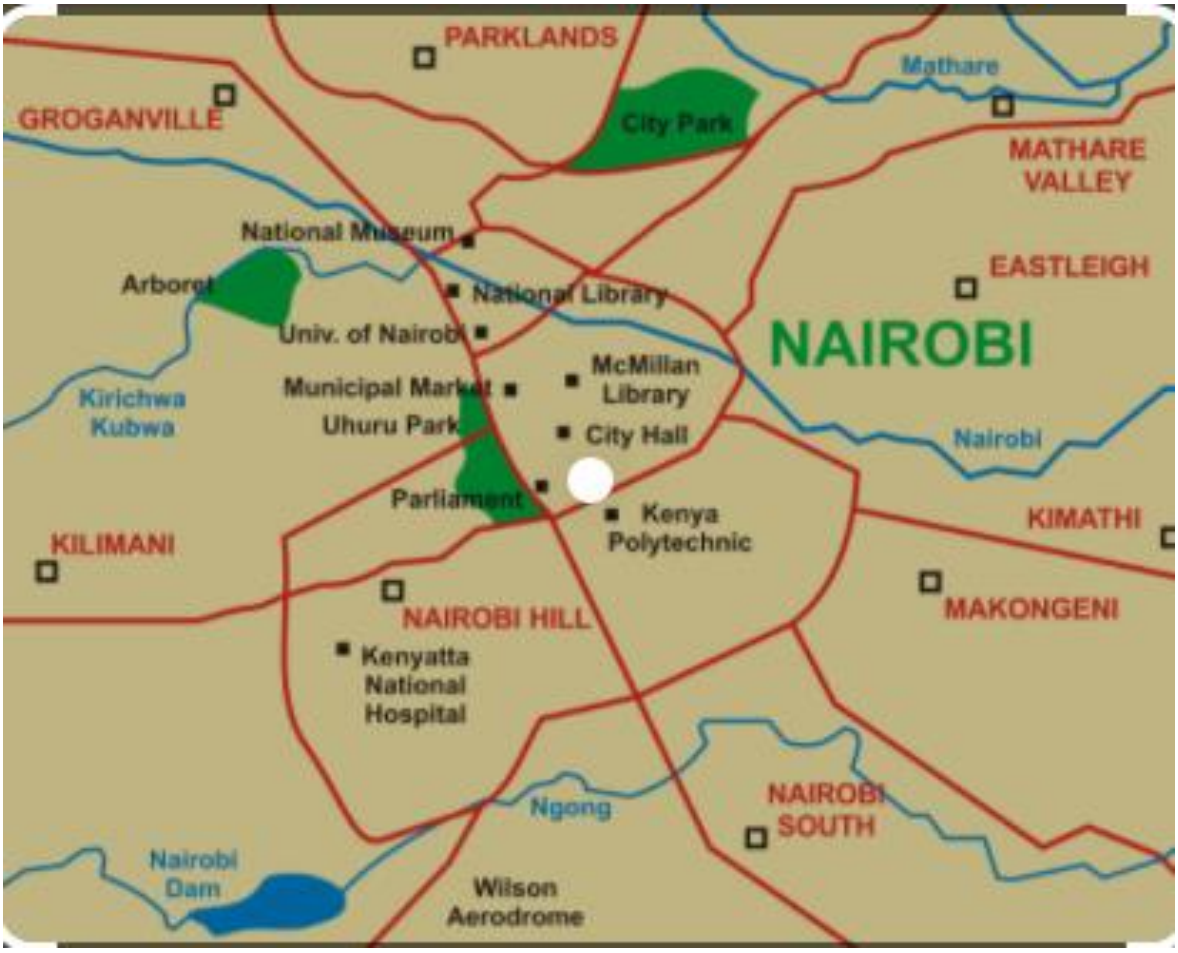
6. Calibrate the analyzer every time you introduce a new reagent lot and after 14 or 28 days.
7. Quality Control Vidas must be performed in each section and every position.

Expected normal values (5 years and above)

Female 15 - 150 ng/ml

Male 15 - 200 ng/m

Appendix X: Map of Nairobi County



Appendix XI: Graduate School Approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 4150

Internal Memo

FROM: Dean, Graduate School

DATE: 30th November, 2020

TO: Njenga Kariuki John
C/o Medical Laboratory Sciences Dept.
Kenyatta University

REF: P97/27900/2019

SUBJECT: APPROVAL OF RESEARCH PH.D PROPOSAL

We acknowledge receipt of your revised Proposal as per our recommendations raised by the Graduate School Board at its meeting of 18th November, 2020, Entitled, **"Total Blood Volume, Iron Status and Hematological Profiles of Whole Blood Donors at Kenyatta National Hospital, Nairobi City County, Kenya".**

You may now proceed with your Data Collection, Subject to Clearance with Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D. studies.

Thank you.

KENYATTA UNIVERSITY
30 NOV 2020
GRADUATE SCHOOL
P.O. BOX 43844 - 00100 NAIROBI

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

C.c. Chairman, Department of Medical Laboratory Sciences
Registrar (Academic) Att: Mr. Likam

Supervisors:

1. Dr. Scholastica Mathenge
   C/o Department of Medical Lab. Sciences
   Kenyatta University

2. Dr. Nelson Menza
   C/o Department of Medical Lab. Science
   Kenyatta University

2. Prof. Jessie N. Githanga
   University of Nairobi, Human Pathology Department
   C/o Department of Medical Lab. Science
   Kenyatta University

RM/nn

Appendix XII: KNH-UoN Ethical Approval

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

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

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

Ref: KNH-ERC/A/48

10th February 2021

Njenga K. John
PhD Candidate
Reg. No.P97/27900/2019
Dept. of Medical Laboratory Sciences
School of Medicine
Kenyatta University

KENYATTA NATIONAL HOSPITAL APPROVED 10 FEB 2021 KNH/UoN-ERC P. O. BOX 20723 - 00202 NAIROBI

Dear John

RESEARCH PROPOSAL – TOTAL BLOOD VOLUME, IRON STATUS AND HEMATOLOGICAL PROFILES OF WHOLE BLOOD DONORS AT KENYATTA NATIONAL HOSPITAL, NAIROBI COUNTY, KENYA (P548/10/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 10th February 2021 – 9th February 2022.

This approval is subject to compliance with the following requirements:

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

Appendix XIII: NACOSTI Approval

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **618511**

Date of Issue: **16/June/2021**

RESEARCH LICENSE

This is to Certify that Mr.. John K. Njenga of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: Total Blood Volume, Iron Status and Hematological profiles of Whole Blood Donors at Kenyatta National Hospital, Nairobi County, Kenya for the period ending : 16/June/2022.

License No: **NACOSTI/P/21/11112**

618511

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.

Appendix XIV: Material Transfer Agreement

KU/MTA FORM

KENYATTA UNIVERSITY

MATERIAL TRANSFER AGREEMENT FORM

SECTION A: ***Terms and conditions***

1. MATERIALS transferred will be used for teaching purposes only.
2. MATERIALS collected from human subjects will not be used in any diagnostic purposes or clinical trials without written consent from the PROVIDER
3. All MATERIALS will not be transferred to other institutions/centers without written consent from the PROVIDER
4. All publications involving human MATERIALS should acknowledge the source
5. After analysis, all MATERIALS associated with human subjects should be disposed of according to regulatory guidelines with written consent from the PROVIDER.

SECTION B:

A brief explanation of intended use(s), storage duration of the material(s) and disposal plan of remnants

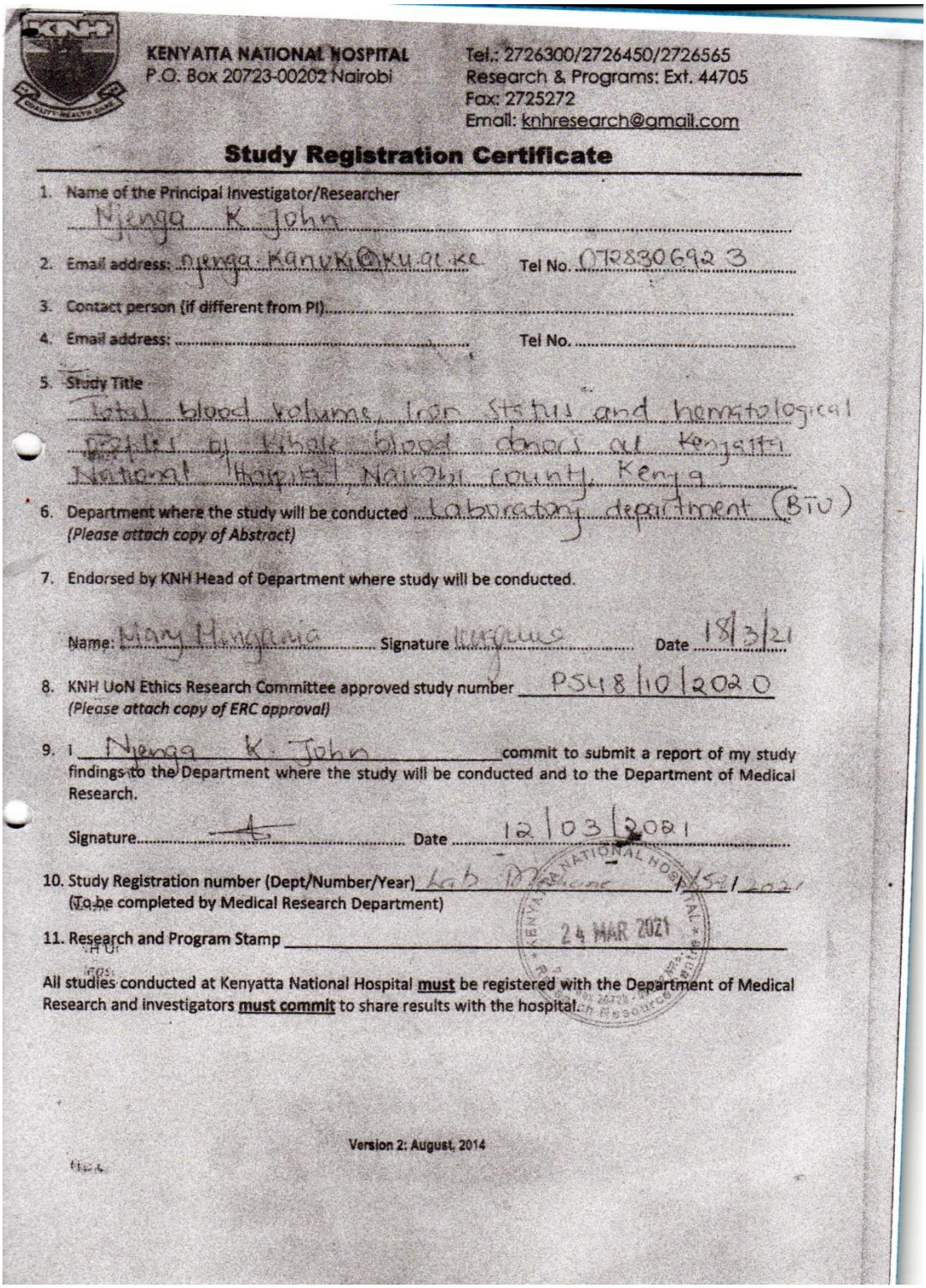
-**Material:** 6-8 ml of whole blood sample

-**Services:** transfer and performance of biological analyses/testing and storage as per the protocol/ appendices

-**Use of Materials**: RECIPIENT will only use MATERIALS received to perform the services and observe all applicable laws. RECIPIENT will not use the MATERIALS for testing in humans or for any commercial purposes. "Commercial purposes" shall mean the sale, lease, license, or other transfer of the Biological Material or modifications to a third party.

-**Restriction on Distribution**: RECIPIENT will not distribute the MATERIAL(S) to a third party. All procedures will be performed at Kenyatta University Health Centre.

Appendix XV: Study Registration at Kenyatta National Hospital



KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

Study Registration Certificate

1. Name of the Principal Investigator/Researcher
   Njenga K John
2. Email address: njenga.kanuki@ku.ac.ke Tel No. 0728830693
3. Contact person (if different from PI)
4. Email address: Tel No.
5. Study Title
   Total blood volume, iron status and hematological profiles of whole blood donors at Kenyatta National Hospital Nairobi county, Kenya
6. Department where the study will be conducted Laboratory department (BTU)
   (Please attach copy of Abstract)
7. Endorsed by KNH Head of Department where study will be conducted.
   Name: Mary Mungania Signature [signature] Date 18/3/21
8. KNH UoN Ethics Research Committee approved study number P548/10/2020
   (Please attach copy of ERC approval)
9. I Njenga K. John commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Medical Research.
   Signature [signature] Date 12/03/2021
10. Study Registration number (Dept/Number/Year) Lab [illegible]/5[illegible]/2021
    (To be completed by Medical Research Department)
11. Research and Program Stamp KENYATTA NATIONAL HOSPITAL 24 MAR 2021

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Medical Research and investigators **must commit** to share results with the hospital.

Version 2: August, 2014