RED BLOOD CELL ALLOIMMUNIZATION AND ASSOCIATED RISK FACTORS AMONG TRANSFUSED CANCER PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL, KENYA

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This thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

The piece of work is atribute to my late husband, Dr. Jason Makhumi Wapukha who was a lecturer at Murang'a University, as well as to my children Daniel, Denis, Dorcas, Blssing Anne, Prayer for their unwavering support in getting me this far and my Dr. Precious Wapukha of St. pauls University for her encouragement and moral support during thesis writing God bless them so much.

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ABSTRACT

Alloimmunization is an immune response to foreign antigens after interaction with tissues or cells with genetic disparity, despite the fact that they can save lives. Alloantibodies to one or more RBC antigens can develop as an effect of transfusions of blood. The incidence of alloimmunization in cancer patients presenting at Moi Teaching Hospital has not been assessed, and it is still unknown despite the fact that it is a major causes of transfusion adverse incidences among cancer patients. This study assessed prevalence of red blood cells alloimmunization, association between alloimmunization and transfusion frequency, and association between alloimmunization and age among cancer patients at Moi Teaching and Referral Hospital. It also assessed the association between alloimmunization and gender. Cross-sectional study design was used in investigation and focused on multi-transfused cancer patients treated at Moi Teaching and Referral Hospital Kenya. The study included 162-person sample size based on Fisher's exact test formulae and a consecutive sampling technique was applied. The gel-based antibody screening and identification was performed with "ID-Diacell I-IIIII®" panel cells. Frequency, mean, median, and dispersion of descriptive statistics were shown and the association between alloimmunization with relation to transfusions, age and gender were established by spearman's analysis correlation. The threshold for statistical significance was set at $P \leq 0.05$. Moreover, 95% degree of significance was used for statistical testing. The outcomes were displayed using tables and charts. Participation was voluntary, confidentiality was upheld, and informed agreement was obtained in writing. In the present investigation, the findings highlighted the necessity of meticulously screening for alloantibodies in cancer patients, particularly in those who have received multiple transfusions, in order to significantly improve the safety of blood transfusions in these patients. This study established prevalence of 6.2% alloimmunization among cancer patients with a higher rate in female (8.8%) compared to male (3.7%) patients. Anti-E and anti-K alloantibodies were the most frequent alloantibodies. No correlation was found between frequency of transfusions and alloimmunization ($P= 0.753$). There was moderate correlation between alloimmunization and age ($P= 0.159$), Nonetheless, there was a significant positive correlation with gender ($P= 0.01$). This study proposes that in order to limit the volume of blood removed by alloantibodies following transfusion, cancer patients receiving blood should be tested for alloantibodies and given equal antigen-negative blood units.

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LIST OF ABBREVIATIONS

AHG	:	Anti-Human Globulin
CMML	:	Chronic Myelomonocytic Leukemia
DAT	:	Direct Anti-Globulin Test
DHTR	:	Delayed Haemolytic Transfusion Reactions
FDA	:	Food and Drug Administration
HDN	:	Haemolytic Disease of New-born
HLA	:	Human Leucocyte Antigen
HPA	:	Human Platelet Antigen
HTR	:	Haemolytic Transfusion Reaction
IAT	:	Indirect anti-Globulin Test
MAC		Membrane Attack Complex
MDS	:	Myelodysplastic Syndrome
MDSs	:	Myelodysplastic Syndromes
MHC	:	Major Histocompatibility Complex
MML	:	Myelomonocytic leukemia
MTRH	:	Moi Teaching and Referral Hospital
PRA	:	High Panel-Reactive Antibodies
RBC	:	Red blood cell
RBCs	:	Red blood cells
SCD	:	Sickle Cell Disease
SPSS	:	Statistical Package for Social Sciences

LIST OF ACRONYMS

Anti	:	Antibody
IgG	:	Immunoglobulin gamma
IgM	:	Immunoglobulin Mμ
IV	:	Intra- venous
Rh	:	Rhesus
X-MATCH	:	Cross match

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Alloimmunization is an immune reaction to foreign antigens after interaction with tissues or cells with diverse genetic make-up (Garraud *et al.,* 2019). Red blood cells alloimmunization occur naturally during pregnancy, but it frequently happens as a side effect of blood transfusions and/or organ transplants (Tangvarasittichai, 2017). Immune system response is influenced by recipient-specific genetic and acquired variables as well as the immunogenicity of the antigen (Tangvarasittichai, 2017). Alloantibodies are immunological products that are only formed in reaction to foreign RBC antigens (Tormey & Hendrickson, 2019). They are produced as a result of interaction with non-self, but yet from the same species, red blood cell antigens (Arthur *et al.*, 2017). The clinically relevant alloantibodies are often of immunoglobulin G (IgG) origin, reacting at 37°C in vitro phase (Wang *et al.*, 2021). Alloimmunization is related with blood group systems such as Rh, Kell, Duffy (anti-Fy^a and -Fy^b), Kidd (anti-Jk^a and -Jk^b), and MNS (anti-M, -S, and -s) (Tormey & Hendrickson, 2019). Alloimmunization causes transfusion delays, reduced in vivo donor blood survival, and haemolytic transfusion reactions, some of which may be deadly (Thein *et al*., 2020). Alloimmunization and transfusion frequency, Nearly 50% of cancer patients develop IDA, which is a particularly prevalent reason for anaemia necessitating blood transfusions (Madeddu et al., 2018). Blood transfusion saves live but they also carry the danger of red blood cell alloimmunization (Tormey & Hendrickson, 2019)

Age and alloimmunization, the patient's age has frequently come to light as the primary risk factor among other dangers of RBC alloimmunization in recipients with illness dependent on blood transfusions (Franchini et al., 2019). Age at the beginning of transfusion has been identified in the literature as a significant risk factor with the older patients being more vulnerable to alloimmunization due to longer period of blood transfusion (De Santos et al., 2017).

Another risk factor for alloimmunization is the patient's gender; alloantibodies are more common in women (El Kababi *et al.,* 2019). A larger danger of RBC alloimmunization exists in females this is according to a study by (Oud *et al.*, 2022), Some studies demonstrating a link between the number of prior births and the extent of alloimmunization as a result of higher allogeneic exposure

(Seielstad *et al.*, 2018). Occurrence of alloimmunization in multiple transfusions of sickle cell disease (SCD) patients have been carried out in Kenya, Africa, plus elsewhere (Mangare *et al*., 2015), (Tebuka *et al.*, 2020).

Some studies on occurrence of RBC alloimmunization in cancer patients have been conducted in Kenya with the recent being in 2015 at Kenyatta National Hospital, Kenya (Mangare *et al.*, 2015), association of alloimmunization with factors such as number of transfusions, age and gender has limited documented data is still unclear. In Kenya, most recipients are not screened for antibodies and identified prior to transfusion; blood is merely screened for ABO blood type and Rhesus D antigens, and not for minor blood group antigens like anti E or anti K antigens. As a result, individuals who need blood transfusions may possess alloantibodies which could lead to transfusion responses if the antibody titer following compatibility testing is too low to be detected, resulting in transfusion reactions. These facts motivated the present study to be undertaken at Moi Teaching and Referral Hospital, Kenya, to assess the prevalence of red blood cells alloimmunization and association between alloimmunization and risk variables such as transfusion frequency, age and gender among multi-transfused cancer patients.

1.2 Statement of the Problem

According to estimates, anaemia affects cancer patients at various stages of therapy at a rate between 39 and 68%, and in certain cases, it can be so severe that blood transfusions are necessary Approximately 30,000 cancer patients receive care at the Moi Teaching and Referral Hospital Cancer Center, Kenya each year; 3,600 of these patients receive blood transfusions, and about 144 (4%) of these patients develop blood transfusion reactions for which there is no recognized reason (MTRH, 2020). A potential consequence of RBCs transfusion administration is alloimmunization. Despite alloimmunization being one of the most common reasons of reactions to transfusions among oncology patients, its prevalence and association to factors such as number of transfusions, age and gender among multi-transfused cancer patients presenting at Moi Teaching Hospital had not been evaluated and remained unclear and this created a gap for the study. This investigation assessed the prevalence of red blood cells alloimmunization, association between alloimmunization and transfusion frequency, and associations between alloimmunization and age and gender among cancer patients at Moi Teaching and Referral Hospital Cancer Centre, Kenya.

1.3 Significance of the study

Making sure transfusion safety is a priority in cancer patients cannot be overstated. All cancer patients with several transfusions plus Rhesus Negative women with Rhesus positive children should be screened for alloantibodies. The results of this study highlight the necessity of meticulously screening for alloantibodies in cancer patients, particularly in those men and women who have had several blood transfusions and women who have given birth to male children in order to significantly improve the safety of blood transfusions in these patients.

1.4 Objective of the study

To determine red blood cell alloimmunization and associated risk factors in cancer patients with multiple transfusions at Moi Teaching and Referral hospital, Kenya

1.4.1 Specific Objectives

The specific objectives of the study were:

1. To determine the prevalence of alloimmunization among cancer patients at Moi Teaching and Referral Hospital, Kenya.
2. To establish the correlation between alloimmunization and transfusion frequency among cancer patients at Moi Teaching and Referral Hospital, Kenya
3. To establish the correlation between age and alloimmunization in cancer patients at Moi Teaching and Referral Hospital, Kenya.
4. To determine whether alloimmunization and gender are associated among cancer patients at Moi Teaching and Referral Hospital, Kenya.

1.5 Research Questions

1. Is there prevalence of alloimmunization among cancer patients at Moi teaching and Referral Hospital Kenya?
2. Is there correlation between frequency of transfusions and alloimmunization among cancer patients at Moi Teaching and Referral Hospital Kenya?
3. Is there correlation between age and alloimmunization among cancer patients at Kenya's Moi Teaching and Referral Hospital Kenya?

4. Is there correlation between gender and alloimmunization among cancer patients at Moi Teaching and Referral Hospital Kenya?

1.6 Assumption of the study

The study's findings were unaffected by the cancer patients' therapies at the time the samples were collected and that there are no cross- reactive antibodies from other undetectable infections

1.7 Scope of the study

The purpose of this study was to determine the prevalence of alloimmunization against red blood cells, the correlation between alloimmunization and frequency of transfusions, and the correlation between alloimmunization and age in cancer patients at Moi Teaching and Referral Hospital Cancer Centre, Kenya. To also evaluate the correlation between gender and alloimmunization among cancer patients at Kenya's Moi Teaching and Referral Hospital Cancer Centre. The study's precise goals and the testing protocol of the Moi Teaching and Referral Hospital Laboratory guided the study's scope.

1.8 Limitations of the study

The current study only included one facility, thus it might not be entirely representative of the entire country. Female patients in the study were not asked about their past pregnancies, thus it was not possible to determine whether the identified alloimmunization in them was brought on by pregnancy after blood transfusions.

1.9 Definition of Terms

Antibody: A protein generated by the immune system in reaction to antigens, which are foreign substances.

Antigen: An antigen is a foreign material that causes the body's immune system to react.

Anti-globulin test: A test that identifies modest quantities of antibodies present in patients' plasma/serum before a blood transfusion and is used to identify in-vitro antibody-antigen responses.

Alloantibody: These are immunological antibodies that are only created after being exposed to antigens of foreign RBCs (which are not self-antigenic substances rather are of identical type; they are only responsive with allogeneic cells). For example, they are created in reactions to pregnancy

or transfusion of blood directed against a red blood cell antigen that is absent on the recipient's red blood cells.

Auto-immunization: Means self-antibody production due to malfunction of thymus glands or acquired hemolytic anemia.

Alloimmunization: It is an immunological reaction caused by exposure to foreign antigens via blood transfusion or tissue transplant.

Allorecognition: The capacity of a single organism to discriminate between its own tissues and those of other organisms.

Amino acids: Simple chemical compounds with carboxyl (-COOH) and amino groups ($-NH_2$). They are also organic compounds that combine to form a protein group.

Asymptomatic: There are no symptoms

Cancer: A condition in which uncontrollably dividing aberrant cells can infiltrate surrounding tissues.

Dysplastic: Malformed joints resulting from a genetic condition.

Epitopes: Antigenic determinants that are part of an antigen recognized by the immune system, especially antibodies.

Erythropoietin: A hormone that the kidney secretes after low tissue oxygen levels and which accelerates the synthesis of red blood cells.

Foetus: A baby that is not yet born.

Gender: Physical distinctions between those who are intersex, male, or female. At birth, a person's gender is typically determined by physical factors like the makeup of their chromosomes and genitalia.

Haemolysis: Rupture or destruction of a red blood cell

Haemoglobinopathy: A hereditary condition involving an abnormality in the structure of haemoglobin.

Immunogenicity: The ability of a certain material, such as an antigen or epitope, to elicit an immunological response in a human or animal's body.

Lymph proliferative illness: A group of conditions defined by aberrant lymphocyte proliferation.

Malignancy: Cancerous cells that can spread to other sites in the body.

Myeloproliferative disease: One or more hematologic cell lines developing in the peripheral blood is a symptom of a variety of illnesses.

Myelodysplastic syndrome: A category of diseases caused by faulty or defective blood cells.

Mortality: A state of being mortal or susceptible to death.

Oncology: A branch of medicine focused on preventing, detecting, and treating cancer.

Prevalence: A percentage of a population that, during a specific time period, has (or had) a particular trait, such as a sickness or ailment.

Quality Assurance: A systematic process for the systematic monitoring and evaluation of various aspects of a service to ensure the standard of quality are being met, quality assurance entails safety, quality control, record keeping, External quality assessment and inventory. It ensures accurate test results within reasonable time and period, handling of specimens, the type, quality and amount

Quality control: Measure that are taken to monitor the quality of the test itself. It ensures that the test is working correctly and the tester correctly and can report accurate test results with confidence.

Serum: A liquid part of a clotted blood sample.

Stillborn: A death of a baby after 24 weeks of pregnancy, or the birth of a new born with no indication of life (intrauterine death).

Symptomatic: A state of a symptom or sign, especially of something undesirable.

Thrombocytopenic: A medical condition where there is a decrease in platelets.

Transfusion: The process of injecting someone or something else with donated blood, blood products, or another liquid into their circulatory system..

Transfusion reactions: Events that are harmful when whole blood or one of its components are transfused.

CHAPTER TWO

LITERATURE REVIEW

2.1 Immune Response to Alloantigens

Alloimmunization is an immune reaction to foreign antigens from another person that typically happens during pregnancy or blood transfusions (Handa *et al.*, 2020). These situations involve the presence of foreign cells in the body that have unique antigens or proteins that can trigger an immunological response on the red cell surface (Marshall *et al.*, 2018). White blood cells make antibodies in response to detection of these antigens, which instruct the immune system to eliminate the alien cells that contain foreign antigens (Cohen & Efroni, 2019). This procedure guards the body from hazardous foreign organisms like bacteria (Attiyah, 2022). Alloimmunization, which might have catastrophic consequences, is what happens when this reaction arises in response to another person's blood component (Coleman *et al.*, 2019).

Alloantibody production occur as a result of allorecognition which is the adaptive immune system's activation in response to foreign human leukocyte antigen (HLA) (Grosberg & Plachetzki, 2017). During transplant, transfusion, or pregnancy, the immune system recognizes foreign HLA due to high polymorphism, which causes the germinal center to develop and the production of memory B cells that produce long-lasting alloantibody responses (Singh *et al.*, 2019). Alloantibodies cause graft damage by recognizing antigenic markers presented by the HLA molecule on the grafted allograft and do so in a variety of ways, such as by activating the complement system, which produces the MAC complex and allergic inflammatory substances, by transmitting internal cell signals that induce cytoskeletal reorganisation, expansion, and multiplication of the Graft angiogenesis, and by allowing immune cells to invade the allograft (Kielar *et al.*, 2021). The three unique allorecognition pathways direct, indirect, and semi-direct can all happen separately or simultaneously. Initiating the immune response to alloantigen after transplantation requires the stimulation of the recipient's CD4+ T lymphocytes, which in turn triggers the stimulation of cytotoxic CD8+ T lymphocytes and antibody-producing B cells (Marino et al., 2016).

There are various blood kinds that depend on the markers present on the red blood cells (Cid *et al.*, 2018). These blood groups are categorized using the ABO and Rhesus (Rh) blood group systems. Red blood cells with A and B antigens can be distinguished from those without using the

four blood types that make up the ABO system: A, B, AB, and O (Kumar & Rai, 2020). The Rh system includes approximately fifty antigens that pass through red blood cells' membrane. The D antigen is the one that gets tested the most commonly. Red blood cells that do not have the D antigen are therefore commonly referred to as Rh negative (Rh-), while those that do have D antigen are known as Rh positive (Rh+) (Jacob *et al.*, 2022).

Antibodies are created by the immune system when there are foreign antigens present or any antigens that are absent from one's own red blood cells (Basto & Graça, 2021). The immune system uses antibodies for a variety of crucial purposes, such as neutralizing the intended antigen or designating it for eradication. There are numerous varieties of antibodies, each with a unique structure and purpose. Immunoglobulin M (IgM), the biggest, and Immunoglobulin G (IgG), the smallest, are the most prevalent antibodies in the blood (Nizar *et al.*, 2021). Adults without particular markers on their own red blood cells frequently naturally develop antibodies to cross reactivity with A and B agglutinogens in their plasma. This happens as a result of frequent exposure to antigens produced by bacteria that are comparable to cross reactivity to A and B antigens (Kumar & Rai, 2020). Contrarily, exposure to the Rh antigen is often only experienced during pregnancy or during blood transfusions, which can result in alloimmunization (Mbalibulha *et al.*, 2022).

When the expectant mother's blood type differs from that of her fetus, alloimmunization may happen during pregnancy (Minuk *et al.*, 2020). When blood is transfused, the person receiving it may produce antibodies that are directed against the blood donor's red blood cell antigens (Thein *et al.*, 2020). With each additional blood transfusion, the chance of experiencing alloimmunization reactions rises (Valle Neto *et al.*, 2018). Transfusion reactions can lead to significant clinical problems such the development of clots of blood in different parts of the body, also referred to as disseminated intravascular coagulation, and acute renal failure (Harewood *et al.*, 2023). ABO incompatibility causes the worst responses, while Rh incompatibility is more prevalent (Sonawane *et al.*, 2022). Several screening tests should be conducted to prevent alloimmunization during a transfusion (Gerritsma *et al.*, 2019).

A natural antibody that reacts with foreign tissues from a member of the same species is known as an alloantibody (Hauser *et al.*, 2020). Examples of alloantibodies are Rh- hr (Anti D,C,E,e,c,C^{W}), Kell(Anti K,k,Kp^{a},Kp^{b}), Duffy(Anti Fy^{a},Fy^{b}), Kid (Anti JK^{a},JK^{b}), Lewis (Anti Le^{a},Le^{b}), P, M N

S, S,s(Anti M,N,S,s), Luth (Anti Lu^a,Lu^b) (Routray *et al.*, 2020). An alloantigen is an antigen that is only found in some members of a species (such as those who belong to a specific blood group) and has the ability to cause those members of the species who lack it to produce an alloantibody (Jash *et al.*, 2021). Blood group antigens and histocompatibility antigens are the two main categories of alloantigens (Tran *et al.*, 2019). When an individual is exposed to blood group antigens typically due to pregnancy or transfusion an alloantibody is created as a defense mechanism (Aldarweesh, 2019).

Minor blood groups antibodies such as Lewis blood group system antibodies are typically described as naturally occurring, IgM class fraction antibodies that respond at a lower temperature than 37 °C. They are not regarded as having clinical significance (Gayathri & Gupta, 2020). An early beginning of transfusion process has been seen to enable some patients develop immunological tolerance (Tormey & Hendrickson, 2019).

A positive alloantibody screen is more likely to occur as people get older, according to previous studies. Yet, in routine transfusion practice, age is not usually taken into account (Bhuva & Vachhani, 2017). Red blood cell alloimmunization is assumed to be impacted by disease status; however, other factors (For instance, morphological or genetic variations between the donor and receiver) and the age of the patient also needs to be considered when evaluating these information (Hendrickson, 2020). As people get older, their long-term immunological memory vaccination reflexes and protective responses to diseases eventually decline because of the considerable degrading changes that adaptive immune responses go through (Oud *et al.*, 2022). Some earlier studies have indicated that Red blood cell alloimmunization caused initially by transfusion may also decline with age because of the pathophysiology of RBC alloimmunization's shared features with immune responses to vaccinations (Allali *et al.*, 2017). However, a Dutch study raises questions about the idea that biological aging has a protective effect on the immunization against red blood cells after blood transfusion and suggests that antigen matching be used to prevent transfusion-induced red blood cell alloimmunization, a strategy that should be pursued for both immune-competent young and elderly patients (Oud *et al.*, 2022). An earlier investigation in Malaysia found out that the patient's age and the generation of anti-RBC antibodies are strongly correlated (Abdullah et al., 2023, Desai *et al.*, 2015). In Kenya, there is a scarcity of data about the relationship between the patient's age and red cell alloimmunization.

Another risk factor for alloimmunization is the patient's gender. According to reports, alloantibodies are more common in women (El Kababi *et al.*, 2019), however gender is not usually taken into account while transfusing blood. Prior combined adult research found that women with sickle cell illness had a significantly increased risk (27%) of red blood cell alloantibodies than men (Karafin et al., 2018). In other groups, both women and men had an equal risk of alloimmunization (Karafin *et al.*, 2018). The amount and frequency of transfusions, the underlying sickness of the receiver, the pro-inflammatory state following transfusion, in addition to the immunoregulatory effects on the receiver immune system, all have an effect on the recipient's immune response. (Tormey & Hendrickson, 2019)

2.1.1 Cancer Cells

Cancer is an uncontrolled cell proliferation that has the potential to affect neighboring healthy tissues and spread to far-off places like the bone marrow, leading to illness and death (Gensbittel *et al.*, 2020). The invading of the bone marrow may suppress the bone marrow. Between 30% and 90% of cancer patients are thought to have anaemia at various stages of their illnesses (Kifle et al., 2019). In the world, cancer is the second-most frequent reason for death, accounting for around 1 in 6 fatalities (Mattiuzzi & Lippi, 2019). The National Cancer Taskforce Report, July 2022, states that after cardiovascular and infectious diseases, cancer is the third biggest reason for death in Kenya. From 2012 to 2018, there were an additional 37,000 to 47,887 new cases of cancer per year. The National Cancer Institute of Kenya estimates that during that time, there were 32,987 cancer-related fatalities, an increase of nearly 16% from 28,500 in the previous years.Cancer-related anaemia can develop as a direct side effect of the tumor, owing to the immunological system being more sensitive to the disease, or as a side effect of cancer treatment, including surgery, radiation, and chemotherapy (Madeddu *et al.*, 2018). Cancer can cause or make anemia worse by decreasing the erythroid progenitor cells' ability to respond to erythropoietin, inhibiting the release and synthesis of endogenous erythropoietin, and ultimately impairing erythropoiesis. Cancer can also suppress hematopoiesis by infiltrating the bone marrow or by producing cytokines that sequester iron (Kifle *et al.*, 2019). It is known that tumor cells release cytokines such IL-1, interferon, IL-6, and Tumor Necrosis Factor (TNF), which potentially can lower haemoglobin levels by inhibiting erythropoiesis, hemolysis, and the response of erythroid medullary precursors to erythropoietin (Paulson *et al.*, 2020).

Hepatocellular carcinoma, gastrointestinal tract tumors, bladder tumors, and gynecologic tumors are just a few examples of the types of tumors that might lead to hemorrhage, causing blood loss. Organ damage can make cancer-related anaemia worse (Kifle *et al.*, 2019). In addition to tumor bleeding, poor nutrition, infections and inflammatory diseases are other factors that contribute to anaemia in cancer patients (Bryer & Henry, 2018). For many patients suffering from both acute and long-term disorders, including cancer, blood transfusion is regarded as an unavoidable option that improves tissue perfusion and oxygen delivery (Carson *et al.*, 2017). For the repair of physiological anomalies in acute anaemia or haemorrhage, blood transfusion is frequently employed as a life-saving medication. It is also utilized during various surgical operations as an urgent remedy for hemorrhage caused by surgery (Shander *et al.*, 2020). However, as positive outcomes are often accompanied by a number of negative reactions, the choice to get a blood transfusion ought to be made cautiously as well as after evaluating the dangers and advantages to the receiver (El-Qushayri *et al.*, 2020). Complications from red blood cell transfusion support include red blood cell alloimmunization (Tormey & Hendrickson, 2019).

2.1.2 Alloimmunization In Cancer Patients

An immunocompetent host's immunological reaction to donor or non-self-antigens is known as alloimmunization. Alloimmunization can result in a variety of clinical effects depending on the type of blood cells and antigens involved. Red blood cell alloimmunization is evolution of alloantibodies to counter non-self-antigens (Tormey & Hendrickson, 2019). Individuals who get multiple transfusions are at a greater risk of acquiring alloimmunization. Therefore, prior to choosing red blood cells (RBC) for transfusion, the most crucial assessment for every transfusion is to rule out the existence of clinically substantial alloantibodies in the individual's blood (Mangwana *et al.*, 2019).

Red blood cells have self-antigens expressed on their surface, which establishes their blood grroup specificity. Red blood cell antigen-specific antibodies can either be immune- or naturally-based (Maier *et al.*, 2018). Alloimmunization is sometimes associated with naturally produced antibodies, which first show in the blood around 3 to 6 months of age. Among these are antibodies to the ABO, P, and Le blood group systems, sometimes known as Anti A, Anti B, Anti P, and Anti Le (Tormey & Hendrickson, 2019). There is a very significant danger of alloimmunization to

minor blood group antigens because blood banks typically only supply ABO- and Rh (D)-antigens matched blood (Baine *et al.*, 2018).

Alloimmunization rates are higher in individuals with warm autoantibodies in their serum (Delaney *et al.,* 2020). Warm reactive antibodies are IgG that reacts optimally at 37^0 C (normal human temperature) and have affinity for certain red blood cell antigens such as Duffy, Kell, kid and other minor blood group antigens. The key concern is excluding newly generated alloantibodies in patients who need blood transfusions and have warm autoantibodies (Gadji *et al.,* 2023). It is challenging for patients who already have autoimmune hemolytic anemia to monitor any signs of red blood cells deterioration caused by alloantibodies (Mangwana *et al.*, 2019, Handa *et al.,* 2020). The red blood cell alloimmunization is primarily related with blood group systems as Rh, Kell, Duffy (anti-Fy^a and -Fy^b), Kidd (anti-Jk^a and -Jk^b), and MNS (anti-M, -S, and -s) (Tormey & Hendrickson, 2019). One of the most important post-transfusion problems is alloimmunization, which can cause transfusion delays, reduced in vivo donor blood survival, and haemolytic transfusion reactions, some of which may be deadly (Thein *et al*., 2020).

Prior research has suggested that alloimmunization among cancer patients is a great concern globally and requires intervention (Singhal *et al.,* 2017). These patients undergo bone marrow-suppressive chemotherapy that leads into development of anaemia and thrombocytopenia, and need blood transfusions as a result of their treatment. As a result, they run the risk of being alloimmunized after acquiring alloantibodies (Schiffer *et al.*, 2018). Alloimmunization can significantly complicate transfusion therapy and make blood cross-matching difficult. Alloimmunization against Rhesus antigens has been connected to haemolytic transfusion reactions along with haemolytic disease in newborns (Rai & Raturi, 2020). Red blood cell alloantibodies occur as a result of recipient having been exposed to genetically different red blood cells or tissues and through repeated blood transfusion or transplant from one genetically different individual to another (Aldakheel *et al.*, 2022).

When the foreign antigens enter the body, immune system is triggered to produce antibodies against them which can cause transfusion reaction in alloimmunized patients (Pirenne, 2019). An While some earlier research suggested that the quantity of red blood cell (RBC) units transfused is related to the rise in alloimmunized patients, others did not find this correlation (Hendrickson, 2019).

Earlier studies conducted in the United States and elsewhere have revealed a link between alloimmunization and gender, with female sex being more susceptible than male sex to alloimmunization (Karafin *et al.*, 2018). In Kenya, there are few studies that have examined RBC alloimmunization among cancer patients who have received blood transfusions. The most recent one, conducted in 2015 at Kenyatta National Hospital, only examined the prevalence of alloimmunization without considering its relationship to risk variables. (Mangare *et al.*, 2015). The present study assessed the prevalence of red blood cells alloimmunization, association between alloimmunization and transfusion frequency, and associations between alloimmunization and age and gender among cancer patients at Moi Teaching and Referral Hospital Cancer Centre, Kenya.

Unfortunately, transfusion medicine in developing countries like Kenya, has not given careful regard to the history of the number of transfusions (Weimer *et al.*, 2018). Blood banks normally only issue ABO and Rh (D) antigen matched blood, hence, there is a chance of alloimmunization to rare blood type antigens which blood banks don't match (El Fetouh *et al*., 2020). Investigations into red blood cell alloimmunization are usually conducted only after transfusions, and numerous alloantibodies may not be discovered because no further transfusions are required or because the titer of antibodies lowers over time and achieves a non-detectable level before testing (Prus *et al.*, 2020). Patients who need additional transfusions may experience a secondary immunological response much more quickly if they are exposed to an antigen that has already sensitized them, which could lead to a serious haemolytic reaction (Leal *et al.*, 2022). The majority of fatal haemolytic transfusion events documented to the US Food and Drug Administration (FDA) in the past few years have been associated to abnormal RBC alloantibodies. These antibodies are now considered to be the second primary cause of transfusion-related fatalities in the United States of America (Olaniyi, 2019).

According to the blood bank and transfusion service guidelines, an antibody that reduces red cell survival is referred to as clinically significant red blood cell alloantibody (Flesiopoulou *et al.*, 2020); this primarily targets the Rh [D, C, E, c, and e] and Kell [K] antigens, then the Fy [Fya and Fyb], JK [Jka and Jkb], and MNS [M, N, S, and s] blood groups (Shah *et al.*, 2018). These alloantibodies can lead to haemolytic disorders in fetuses and neonates as well as acute and delayed transfusion responses (Tormey & Hendrickson, 2019). Varying blood type antigens have different effects on the potency of these alloantibodies, with S antigens having the lowest immunogenicity

and the Rh system, K, and JKa antigens having the highest (Fetouh *et al*., 2020). Depending on the oncological diagnosis, the risk of red blood cell alloimmunization in cancer patients receiving immunosuppressive therapy varies (Singhal *et al*., 2017). The likelihood of acquiring alloantibodies is influenced by factors such as the volume and regularity of transfusions, pregnancy, bioavailability of the antigen, immunological response of the receiver, individual ethnicity, and antigenic variations between the donor and the person receiving blood (Fetouh *et al*., 2020) Notwithstanding the existing ABO/Rh D matching regulations, transfused patients continue to develop potentially fatal haemolytic responses each year as a result of booster doses of red blood cell antigen immunization (Belsito *et al.*, 2018). To maximize protection, high-risk individuals can receive red blood cell units that are free from antigen (Floch *et al.*, 2018)

2.2 Prevalence Of Alloimmunization Among Cancer Patients

A blood transfusion is a life-saving treatment for anaemia-related problems as well as for the management of hypoxic symptoms and indications (Obeagu *et al.*, 2020). It exposes the patient to a large number of foreign antigens that are possible Immunogens, which might cause the receiver to acquire antibodies days, weeks, or months after the transfusion (Handa *et al.*, 2020). Multiple cellular actors and precise regulation are involved in the complex and elegant mechanism how the immune system creates antibodies to foreign antigens while maintaining tolerance to self-antigens (Handa *et al.*, 2020). Patients who require repeated transfusions due to conditions including hemoglobinopathies, haematologic diseases, various cancers, organ transplantation, or renal failure may experience up to 60% of alloimmunization (Bhuva & Vachhani, 2017). The immune status of the recipient, the immunomodulatory effects of allogeneic blood transfusions on the receiver's immune system, and the antigenic differences between the recipient and blood donor in red blood cells are at least three major contributing factors in the complex process of alloimmunization (Tangvarasittichai, 2017). Because of the existence of anti-erythrocyte antibodies in individual's plasma, alloimmunization in cancer patients who have received several transfusions is known to have serious negative effects such as difficult in finding compatible blood during cross-match, new born haemolytic illness, acute and delayed transfusion responses that might be fatal, and other conditions. Alloantibodies have been linked to the production of autoantibodies, which can shorten the lifespan of recipients' own red blood cells or transfused red blood cells and perhaps cause hemolysis (Singhal *et al.*, 2017). As a result, these individuals may need many transfusions as well as procedures like splenectomy and/or immune system-suppressing

medications (Singhal *et al.*, 2017). These difficulties must be taken into account when dealing with patients who are likely to require blood transfusions and those who might gain from haematopoietic stem cell transplants (Singhal *et al.*, 2017). Red blood cell alloimmunization risk has previously been reduced by erythropoietin treatment in cancer patients or through phenotypic matching of RBC blood group antigens, such as those for the Rhesus, Kell, Duffy, Kidd, and MNS blood groups (Erhabor *et al.*, 2019). Yet, many healthcare settings, especially in underdeveloped nations, find this to be expensive and unworkable. Previous research done in Egypt found that patients of various racial and ethnic backgrounds had an alloimmunization rate of about 18% (El-Beshlawy *et al.*, 2020). Alloimmunization prevalence rate of roughly 13.7% has been reported in earlier investigations conducted in France (Allali *et al.*, 2017). Also, earlier research revealed that the rates of alloimmunization against red blood cells were 7.4% in Iran, 22% in Saudi Arabia, and 9.2% in Karachi, Pakistan (El-Beshlawy, *et al.*, 2020). In sub-Saharan Africa, the overall alloimmunization rate was reported to be between 6.95% and 7.5% in prior studies (Boateng *et al.*, 2019). A low rate of alloimmunization may be anticipated when the red blood cell antigens of blood donors and recipients are homogeneous (Mahapatra & Panda, 2019). Previous research in Iran found that the majority of the country's regions have significant prevalence of antibodies to the D and E antigens. These earlier investigations also demonstrated the distribution of alloantibodies in alloimmunized transfusion-dependent patients of varied ethnic and racial backgrounds within the Iranian community (Sarihi *et al.*, 2020). In light of the results, approaches for low-cost red blood cell phenotyping and matching for high-risk antigens in donors and chronic transfusion recipients can be developed in a number of nations to lower the rates of alloimmunization (Sarihi *et al.*, 2020).

Additional investigation has revealed that potential organ transplant recipients who have had blood transfusions are more likely to have high panel- reactive antibodies (PRA) values of greater than 80% compared to individuals who have never undergone blood transfusions (Daloul *et al.*, 2021). High panel- reactive antibodies are determined by how many human leukocyte antigen (HLA) antigens, individually or collectively, in a panel, react with the patient's blood (Hussain et al., 2020). These antibodies may also represent how many donors are anticipated to respond with the patient's serum (Daloul *et al.*, 2021). A prior study done in India indicated a 7.5% rate of alloimmunization in patients of solid malignancies (Handa *et al.*, 2020). Blood transfusions in Malaysia are frequently associated with red blood cell alloimmunization, which has been

connected to the volume of donor exposures (Abdullah *et al.*, 2023). Pre-transfusion screening and detection of red blood cell antibodies are not routinely performed in Kenya; instead, pre-transfusion testing is restricted to ABO/Rhesus D group type and crossmatching (Mangare *et al.*, 2015). Although there are some studies of alloimmunization prevalence in multi-transfused Sickle cell disease and cancer patients in Kenya, there is scarcity of data on prevalence of alloimmunization among cancer patients in Kenya. This study therefore was done to assess alloimmunization prevalence among cancer patients at Moi Teaching and Referral Hospital, Kenya.

2.3 Association between Alloimmunization and Transfusion Frequency among Multi-Transfused Cancer Patients

Red blood cell (RBC) antigen alloimmunization is a concern related with blood transfusions, regardless of the fact that they may save lives (Tormey & Hendrickson, 2019). Alloimmunization to red blood cell antigens brought on by genetic variations between the donor and recipient is one of the risks connected to blood transfusions (Allali *et al.*, 2017). Repeated blood transfusions may result in the production of alloantibodies against one or more red blood cell antigens, which might make it more challenging to execute subsequent transfusions (Elkhalifa *et al.*, 2021). Alloantibodies can hinder cross-match testing, which can delay the process of getting compatible blood and occasionally be linked to a delayed type of hemolytic transfusion reaction (Tormey & Hendrickson, 2019). In addition to causing death, red blood cell alloantibodies can also result in renal failure. Failure of the kidneys and kidney transplantation (RRT) have an impact on the immune system's function and may impair RBC alloimmunization (Kurts *et al*., 2017). Several transfusions in sickle cell disease causes alloimmunization resulting into autoantibody formation which induces bystander immune hemolysis leading in destruction of negative red blood cells during immune hemolysis such as delayed hemolytic transfusion reactions (Ackfeld *et al.*, 2022). Patients with multiple red blood cell alloantibodies or antibodies against high-incidence antigens may experience the effects of anemia due to the prolonged time it takes to find compatible red blood cell units for transfusion; some patients may even die if compatible red blood cell units cannot be found (Tormey & Hendrickson, 2019). Finally, red blood cell alloantibodies may harm developing fetuses in addition to being harmful in a transfusion (Aldakheel *et al.*, 2022).

A highly essential assessment for every transfusion is to rule out the existence of clinically significant alloantibodies in the individual's blood before picking red blood cells for transfusion (Mangwana *et al.*, 2019). Theoretically, each transfused unit carries a 1% risk of developing an alloimmunization to RBC antigens; this risk is increased in patients who have had more transfusions (Jariwala *et al.*, 2019). The frequency and volume of transfusions, the immunogenicity of the antigen, and the immunological response of the recipient all contribute to the probability of alloimmunization. It has also been documented how ethnic and antigenic pattern differences between donors and receivers can affect the outcome (Vafaei & Keikhaei, 2017). Periodic red blood cell transfusions are necessary for a number of diseases, including chronic myeloproliferative disease, aplastic anemia, myelodysplastic syndrome, sickle cell disorders, and different malignancies (Weber *et al.*, 2021). Transfusion with antigen matched red blood cells would successfully stop alloimmunization (Sharma *et al.*, 2020). This can be done by typing the patient's ABO, Rhesus, Kell, Kidd, and Duffy systems at the time of the diagnosis or before the initiation of transfusion therapy. Always ensure that blood being transfused matches the ABO, Rhesus, and Kell systems, at the very least (Sharma *et al.*, 2020). Leukocyte filtering during transfusion may also stop alloimmunization brought on by white blood cells (Sharma *et al.*, 2020). Patients with red cell alloimmunization need to be identified, have their antibodies identified, and then get antigen-free blood for additional transfusions (Sachan *et al.*, 2020).

The number of alloimmunized patients, the occurrence of alloimmunization testing (single versus serial), the total number of transfusions, as well as the time between transfusion sessions, the number of alloimmunized patients, and the specificity of the antibodies were all factors in the increase in alloimmunized patients, according to a Malaysian study (mohd noor *et al.*, 2019).

According to earlier research, a history of frequent transfusions affects alloimmunization. According to a 2020 study conducted in Indonesia, 72.1% (72 instances) of participants with a history of prior transfusions experienced transfusion reactions (Rahajeng *et al.*, 2020). Those who have a history of repeated transfusions will have sensitization, which causes the body of the recipient to produce alloantibodies as a result of exposure to HLA (Human Leucocyte Antigen) or HPA (Human Platelet Antigen) through earlier transfusions which leads to alloimmunization (Nwosu *et al.*, 2023). Systemic inflammation is brought on by the production of the cytokine when donor antigens interact with recipient antibodies that have already been sensitized (Nwosu *et al.*,

2023). According to various researches, alloimmunization occurs more or less frequently in individuals who get repeated transfusions. Patients with thalassemia are alloimmunized 4-50% of the time, those with haemato-oncology conditions are alloimmunized 1.9-13% of the time, and those with renal disease are alloimmunized 1.27-13.1% of the time (Das *et al.*, 2022).

The risk of alloimmunization rises with erythrocyte exposure. Erythrocyte alloantibodies were produced in up to 30% of patients who required repeated transfusions. A different study found that 60% of those with a history of chronic transfusion had alloimmunization (Tormey & Hendrickson, 2019). It can be very difficult to get the right blood, which increases the risk of delayed haemolytic transfusion reaction (DHTR), which in some circumstances can be fatal (Tormey & Hendrickson, 2019). Thus, it is highly recommended that donor and recipient erythrocytes be phenotypically matched to prevent sensitization in patients who are dependent on blood transfusions on a regular basis.

A study done in Egypt in 2020 indicated a significant correlation between alloimmunization and how often blood is transfused among thalassemia patients (El Fetouh *et al.*, 2020). However, a research conducted in Tanzania found no connection between alloimmunization and past transfusion history in sickle cell patients (Tebuka *et al.*, 2020). This could be attributable to the homogenicity between donors and recipients. There are some studies of alloimmunization prevalence in multiple-transfused cancer patients in Kenya, but few studies on the relationship between alloimmunization and transfusion frequency among cancer patients has been conducted. The present study therefore was done to assess the correlation between alloimmunization and transfusion frequency among cancer patients at Moi Teaching and Referral Hospital, Kenya.

2.4 Alloimmunization and Its Association with Age among Multi-Transfused Cancer Patients

The patient's age has frequently come to light as the primary risk factor among other risk factors, red blood cells alloimmunization in patients with illnesses dependent on blood transfusions (Franchini *et al.*, 2019). Age at the beginning of transfusion therapy has been identified in the literature as a significant risk factor, with older patients being more vulnerable (De Santo *et al.*, 2017). According to prior authors, patients who started receiving transfusions before they were between two and three years old had an increased rate of alloimmunization due to longer period of blood transfusion (Pessoni *et al.*, 2018). A lower risk of alloimmunization may be caused by a

form of immunotolerance that is produced by the still undeveloped immune system of young patients (Marshall *et al.*, 2018). While early initiation of transfusion therapy after diagnosis may be an appealing tactic to lower the chance of alloimmunization, it may potentially increase the risk of numerous transfusion-related issues for cancer patients (Brand, 2016). Age and alloimmunization have a strong correlation, according to earlier investigations carried out in Brazil (Barbosa *et al.*, 2022).

Aging causes significant changes to the immunological and endocrine systems, which makes people more susceptible to infectious diseases and reduces the effectiveness of immunization. Both the innate immune system as well as the secondary immune system are affected by immunosenescence (Oh *et al.*, 2019). Naive T cell production is reduced as a result of thymus involution, and the spectrum of T cells is constrained by the accumulation of antigen-experienced T cells. More pro-inflammatory cytokines are produced by highly differentiated effector T cells, which coupled with active innate immunity cells help create a structural pro-inflammatory domain as people grow (Goronzy & Weyand, 2017). Thus, it is essential to create specialized vaccinations and adjuvant immunization techniques to maintain healthy aging. As they age, both sexes show diminished capacity to produce adequate immune responses, particularly to novel antigens. It is still unclear how sex variations in immunological aging together with the impact of waning estrogen and progesterone levels on immunosenescence work (Gubbels Bupp *et al.*, 2018).

Age-related changes in immune function and disease susceptibility, women lose their immunological advantage as they age. With relation to hepatitis, meningococcal, or pneumococcal infections in particular, they exhibit higher vulnerability and mortality (Gay *et al.*, 2021). Previous investigations carried out in Greece have shown a high link between age and alloimmunization (Politou *et al.*, 2020). Systematic evaluation study by Franchini and his team show that age poses a serious risk to alloimmunization in addition to other considerations (Franchini *et al.*, 2019). The incidence of alloimmunization in sickle cell disease and cancer patients has been mentioned in earlier research conducted in Kenya (Mangwana *et al.*, 2019), however, there is paucity of data to date on how age relate to red blood cells alloantibodies in cancer patients. This study therefore assessed alloimmunization and its association to age among cancer patients at Moi Teaching and Referral Hospital, Kenya.

2.6 Alloimmunization and its association to gender among multi-transfused cancer patients

One of the most important variables influencing the emergence of alloimmunization is the genetic disparity between red blood cell donors and receivers brought on by racial discrepancies (Yazdanbakhsh *et al*., 2012). Variables such as older age, gender, blood transfusions, use of non-leukoreduced red blood cells, long-term preserved blood products, may all be additional risk factors (Samarah *et al.*, 2018). Women due to exposure to alloantigens during pregnancy, miscarriages, abortions, and births, leukemia, diabetes mellitus, lymphoproliferative diseases, and solid tumors are additional factors that may influence a woman's risk of acquiring alloimmunization (Politou *et al.*, 2020).

It is recommended that preemptive antigen matching for Rh (C, c, E, e) and K be performed cancer patients and women of reproductive age so as to halt alloimmunization and increase transfusion safety by lowering alloantibody formation (Politou *et al.*, 2020). According to previous studies, immune system is strongly influenced by gender, which also affects how different infectious diseases manifest in men and women, how they respond to viral immunizations, and how common autoimmune diseases are the innate, humoral, and cellular immune responses of females to viral infections and vaccination-induced immunity are often higher. Likewise, autoimmune diseases are more likely to affect women and have a higher rate of vaccination-related side effects (Ciarambino *et al.*, 2021). Male and female differences in hormones, genes, and environments may impact immunological responses and the sex-related effects of vaccination. Finding solutions to lessen negative reactions in females and enhance immune responses in males will be made easier with knowledge of the mechanisms underlying sex discrepancy in immune responses (Ciarambino *et al.*, 2021). With the long-term goal of tailoring therapy for men and women, this is required to appropriately protect both sexes against immune-mediated illnesses including alloimmunization and infectious diseases (Fischinger *et al.*, 2019). Even though there is growing evidence that gender-based differences exist in immune responses, susceptibility to infectious diseases, and the prevalence of autoimmune diseases, the majority of immunological studies either fail to disaggregate and analyze data by gender or fail to mention the sex of their subjects. In addition to the underlying variations in sex hormones, the contributions of the X chromosome genes and the influence of aspects of the environment are also linked to the basic disparities in immune systems between men and women (Fischinger *et al.*, 2019).

Females often mount higher in innate immune responses than males, which helps them have a lower burden of microbial diseases. Females also typically mount higher humoral and cell-mediated immune responses than males (Semmes *et al.*, 2021). Nevertheless, the same enhanced immune response that made females more resistant to infections also made them more prone to immunological-mediated illnesses like autoimmune disorders and inflammatory diseases (Angum *et al.*, 2020). Prevalence among cancer and sickle cell disease patients with a history of many transfusions were the subject of earlier Kenyan studies (Mangwana *et al.*, 2019), however, few studies have been done to date on relationship between gender and alloimmunization in cancer patients. This study therefore assessed the alloimmunization and its association to gender among oncology patients at Moi Teaching and Referral Hospital, Kenya.

CHAPTER THREE

RESEARCH METHODS

3.1 Study Site

The current study was conducted at Moi teaching and referral hospital, which is located on Nandi Road in Eldoret, Uasin Gishu County, Kenya. The hospital primarily serves the Western region of the Nation of Kenya. It has a large catchment area to assist people and offers affordable quality health care services to all patients. It has cancer center for oncology patients' diagnosis and management. It has a regional blood transfusion center. The hospital houses Chandaria oncology Centre with an average of 1800-2400 annual cancer patients and with an average of 11500 annual transfusions (MTRH, 2020). Also, the hospital has an ISO 15189 - 2012 certified laboratory that is well-stocked with the hematology and blood transfusion tools needed for the investigation.

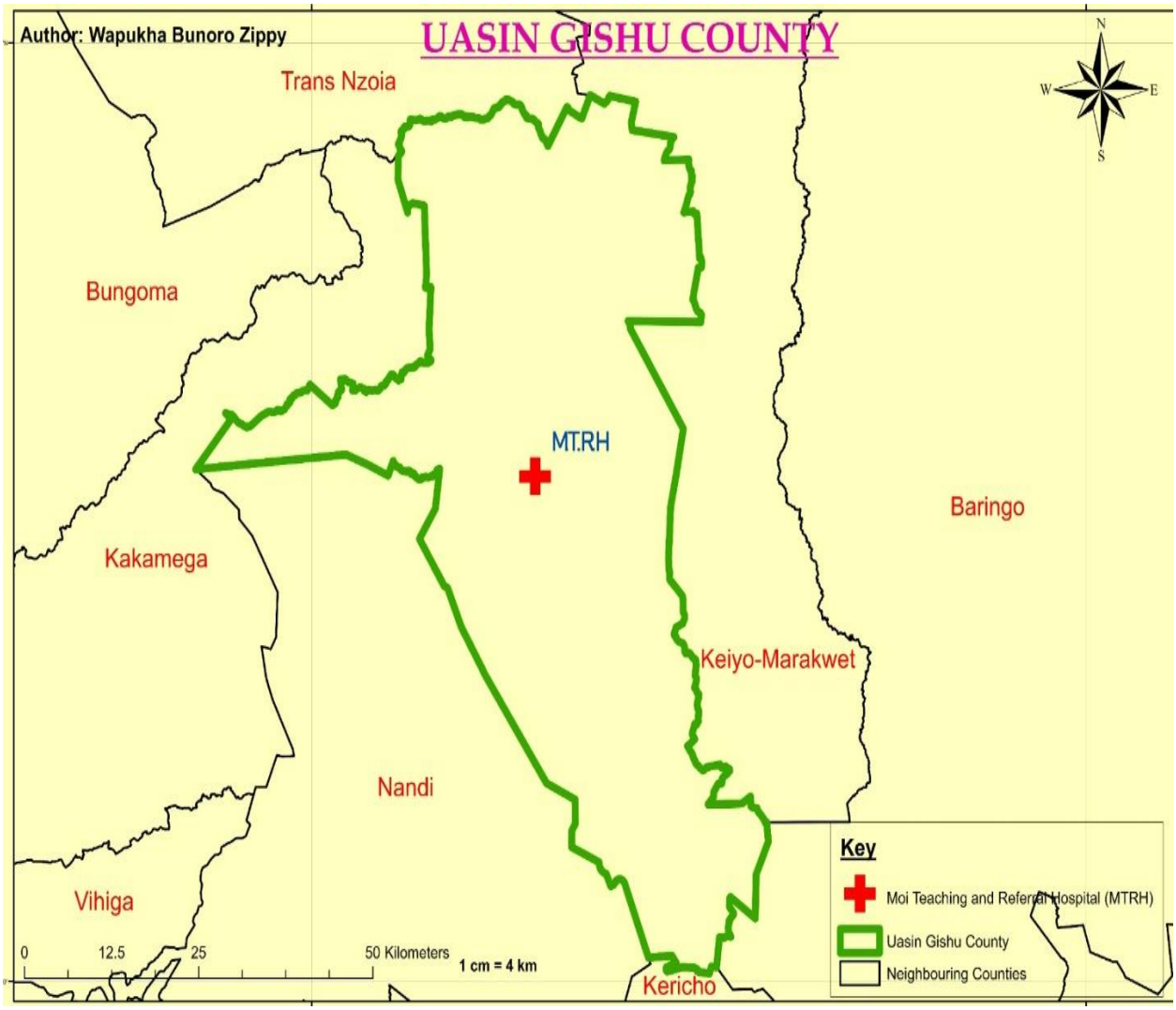


Figure 1: A map showing the location of the Moi Teaching and Referral Hospital

3.2 Study Design

Cross- sectional study design was used in the investigation of the study

3.3 Population of the Study

All transfused cancer patients

3.4 Target Population

The study targeted multi-transfused cancer patients..

3.5 Inclusion Criteria

The study included:

1 Patients who had a history of malignancies and had received more than one blood transfusion.

2 Children below 14 years, permission was obtained from parents or guardians.

3 The current study did not come across pregnant women

3.6 Exclusion Criteria

1. Patients who had received only one transfusion.
2. Those with autoimmune condition, including idiopathic thrombocytopenia, lupus nephritis, and systemic lupus erythematous were excluded from the study.
3. Patients who refused to consent were also not included in the study.

3.7 Sample Size Determination

The sample size of current study was determined by Fishers exact test formulae, because the sample size had a large proportion which had a prevalence of 12% greater than 10% which was suitable for fisher's formulae (Kim, 2016)

$$n = Z^2 pq / d^2$$

Where,

P = True prevalence

Z = Standard variance

q = (1 - p)

d = standard error where

Z = 1.96

The sample size was calculated as follows: approximately 30,000 cancer patients presented annualy at MTRH cancer centre, out of which approximately 3600 were transfused (MTRH, 2020).

3600/30000 x 100 = 12%

Prévalence = 12/100 =0.12

P =0.12

q=1 - p = (1- 0.12) = 0.88

d=0.05

Z=1.96

$$n = Z^2 pq/d^2 \ = (1.96)^2 x0.12x0.88/(0.05)^2 = 162$$

Thus, 162 volunteer patients were chosen as the sample size for the study.

3.8 Sampling Technique

In this investigation, a consecutive sampling method was employed, in which each participant who met the inclusion criteria was chosen one at a time until the sample size was attained.

3.9 Recruitment Process

Patients presenting with malignancy and a background of more than one blood transfusion were recruited into the study. Patient's age, gender, and previous history of the number of transfusions were documented before their samples were taken. Venous blood samples from multi- transfused cancer patients were taken in sample collection test tubes with EDTA anticoagulant for laboratory processing.

3.10 Laboratory Analysis

3.10.1 Sample Preparation

Four millilitres of venous blood were collected into simple Vacutainer® bottles in order to undertake antibody screening and identification. Serum was separated and preserved in the freezer at -20°C if it wasn't immediately analysed. The separated samples were thawed to the proper temperature before to analysis as per the manufacturer's instructions. The findings of the lab tests were recorded using a standard data collection form created for this investigation.

3.10.2 Antibody Screening and Identification

With "ID-Diacell I-IIIII®" panel cells made by Diamed GmbH, Pra Rond 23, 1785 Cressier FR, Switzerland, the gel-based antibody screening and identification was carried out as before (Obi *et al*., 2018). The recipient's serum and group O reagent red blood cells were combined in the upper chamber of the micro-tube containing anti-globulin and low ionic strength saline (LISS) reagent to promote antigen/antibody interaction. At 37 C, they were maintained warm. The controlled

centrifugation idea and the distinct passage of free and agglutinated red blood cells through a dextran-acrylamide gel micro-tube column constitute the fundamental principles of the gel card method. Red blood cells that have been agglutinated adhere to or cover the gel. Non-agglutinated red blood cells as shown in Figure 2 pass through gel particles and form a pellet at the base of the micro-tube. Rh (D,C,E,c,e,Cw), Kell (K,k,Kpa,kpb, Jsa,Jsb), Duffy (Fya, Fyb), Kidd (Jka,Jkb), Lewis (Lea,-Leb), P (P1), MNS (M,N,S,s), and Lutheran (Lua,Lu) were the three panels of reagent red blood cells with the highest antigenic specificity used (Xga). Red blood cell antibodies were recognized using the manufacturer's antigram. An eleven-panel reagent red cell blood panel was used, as before, for the antibody screening with the aforementioned antigenic specificity (Obi *et al.*, 2018).

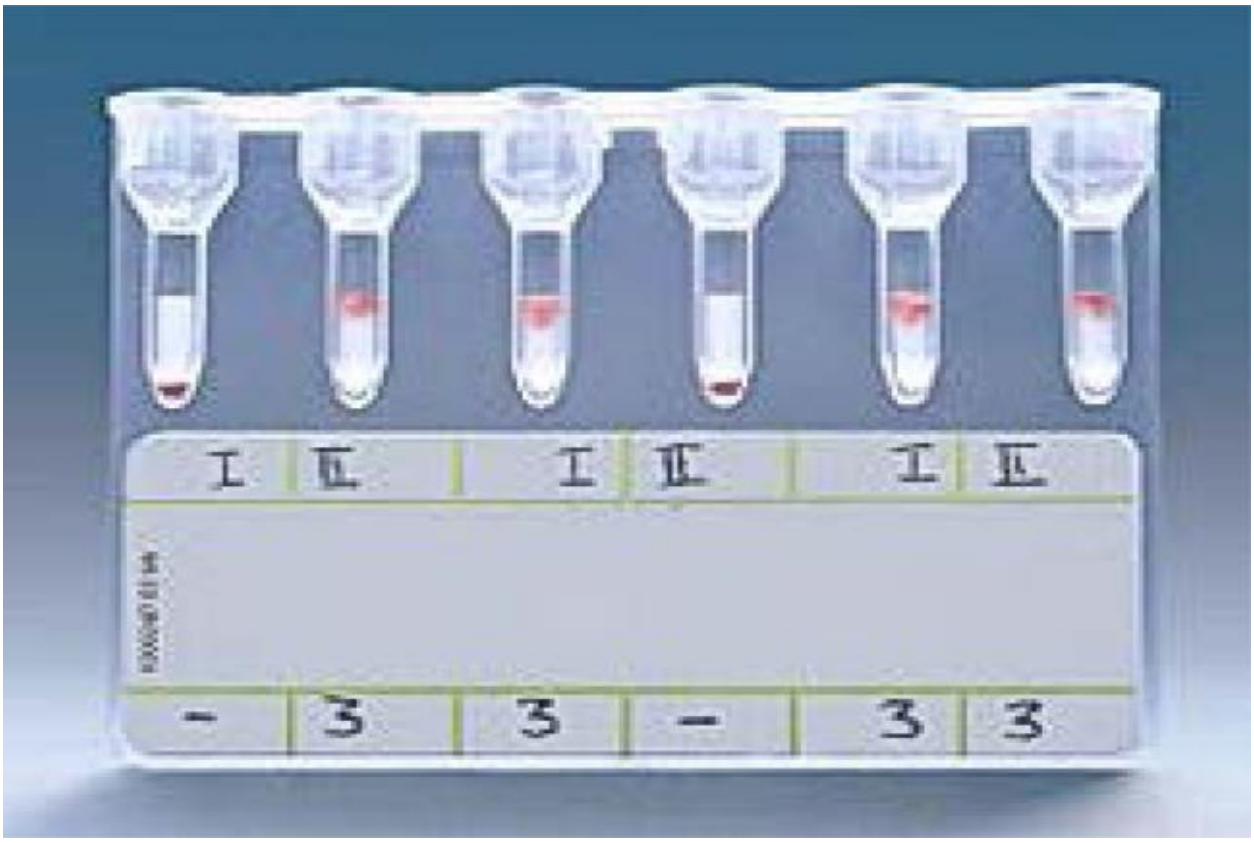


Figure 2: Antibody screening gel card (*source:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6354886/figure/F1/)*

3.11 Quality Control

Only blood samples that met the laboratory sample acceptance requirements were used in order to guarantee the collection of high-quality data. The blood samples were taken by a trained phlebotomist, who made sure that the correct amount of 4 ml was taken. The blood samples were handled with care during storage and transportation. Before testing, stored samples were brought to the required temperature in line with the recommendations of the manufacturer. Throughout the investigation, internal and external quality controls were confirmed and guaranteed. The second person qualified Laboratory technologist checked the accuracy of the results of the first person before they were recorded. Laboratory has signed up for the Human Quality Assessment Services (HUQAS) External Quality Assurance Scheme for Blood Transfusion Science (BTS), Chemistry, which includes the antibody screening test. Additionally, the Kenya Accreditation Services (KENAS) has accredited the Blood Transfusion Science (BTS) scope, enhancing the level of data quality.

3.12 Data Management and Analysis

Microsoft Spread sheets was used to store the data. The Statistical Package for Social Science (SPSS V.24) (IBM Corporation, Chicago, Illinois, United States) was used for the analysis and creation of the graphics. The frequency, mean, median, and dispersion of descriptive statistics were shown. The association between alloimmunization with number of transfusions, age and sex was determined by Spearman's correlation analysis. Statistical significance was established at $P \leq 0.05$. Tables and charts were used to present the results.

3.13 Ethical Considerations

Kisii University provided the letter of support needed to request approval (Appendix III).

The National Commission for Science, Technology, and Innovation (885337) (NACOSTI) granted permission for the study to be conducted (Appendix (V).

The Institutional Research and Ethics Committee (IREC) of the Moi Teaching and Referral Hospital gave its approval (IREC/2016/252) in Appendix (1V).

Each participant's written informed consent was obtained after a brief description of the study's purpose. All sensitive information of the client was omitted from the forms of data collecting tool and only used unique identifier number to replace patients' name. Filled data tools were stored in

a secure location, and password was used to access the computer, participant information was kept under lock and key. Participants who tested positive for alloimmunization were referred to Clinicians and Clinical care management (Appendix 1 and 2)

CHAPTER FOUR

RESULTS

The study determined the prevalence of red blood cells alloimmunization, association between alloimmunization and transfusion frequency, association between alloimmunization and age among cancer patients. The study also determined the association between alloimmunization and gender among cancer patients.

4.1 Description of Data by Gender

A sample size of 162 cancer patients was enrolled. The patients' ages ranged from 1 to 92 years.

Table 4.1: Summary of the study sample distribution

	Frequency and percentage distribution by gender	
Gender	Frequency	Percentage
Males	80	49.4
Females	82	50.6
Total	162	100

The findings from table 4.1 show that out of a total of 162 individuals, 80 are males, accounting for 49.4% of the population, while 82 are females, making up 50.6% of the population. The total percentage adds up to 100%, indicating that all individuals in the sample have been accounted for.

4.2 Prevalence of Red Cells Alloimmunization among Cancer Patients

Red cell alloimmunization was detected in 10 participants (6.2%). The rate of alloimmunization was 3.7 % in male patients and 8.8 % in female patients as shown *table 4.2*

Table 4.1: Distribution of prevalence by gender among multi-transfused cancer patients

	Distribution of prevalence by gender among multi-transfused cancer patients		
	Males (Pa %)	Females (pa %)	Total
Alloimmunized	3(3.7%)	7(8.8%)	10(6.1%)
Non- alloimunized	79(96.3%)	73(91.3%)	152(93.9%)
Total	82(49.3%)	80(59.7%)	162(100)

Table 4.2 shows Alloimmunization Prevalence among the multi-transfused cancer patients, 6.1% of the total population (10 individuals) were found to be alloimmunized. This condition was observed in 3.7% of males and 8.8% of females in the non- alloimmunized patients have the majority of the patients, 93.9% of the total population (152 individuals), were not alloimmunized. Specifically, 96.3% of males and 91.3% of females fell into this category. When considering the entire sample of 162 multi-transfused cancer patients, 49.3% were males, and 59.7% were females. These percentages do not add up to 100% because they represent the distribution of gender within this specific group, and not the entire population.

These findings indicate that a small percentage of multi-transfused cancer patients in this sample were alloimmunized, with a slightly higher prevalence in females compared to males. The majority of patients were not alloimmunized, with a higher percentage of males falling into this category. Gender distribution in the sample is imbalanced, with more females (7) than males (3) among multi-transfused cancer patients.

4.3 Distribution of Different Types of Alloantibodies among Cancer Patients

The frequency distribution of different types of alloantibodies among cancer patients was as shown in *table4.3*

Table 4.2: Distribution of alloantibodies among cancer patients

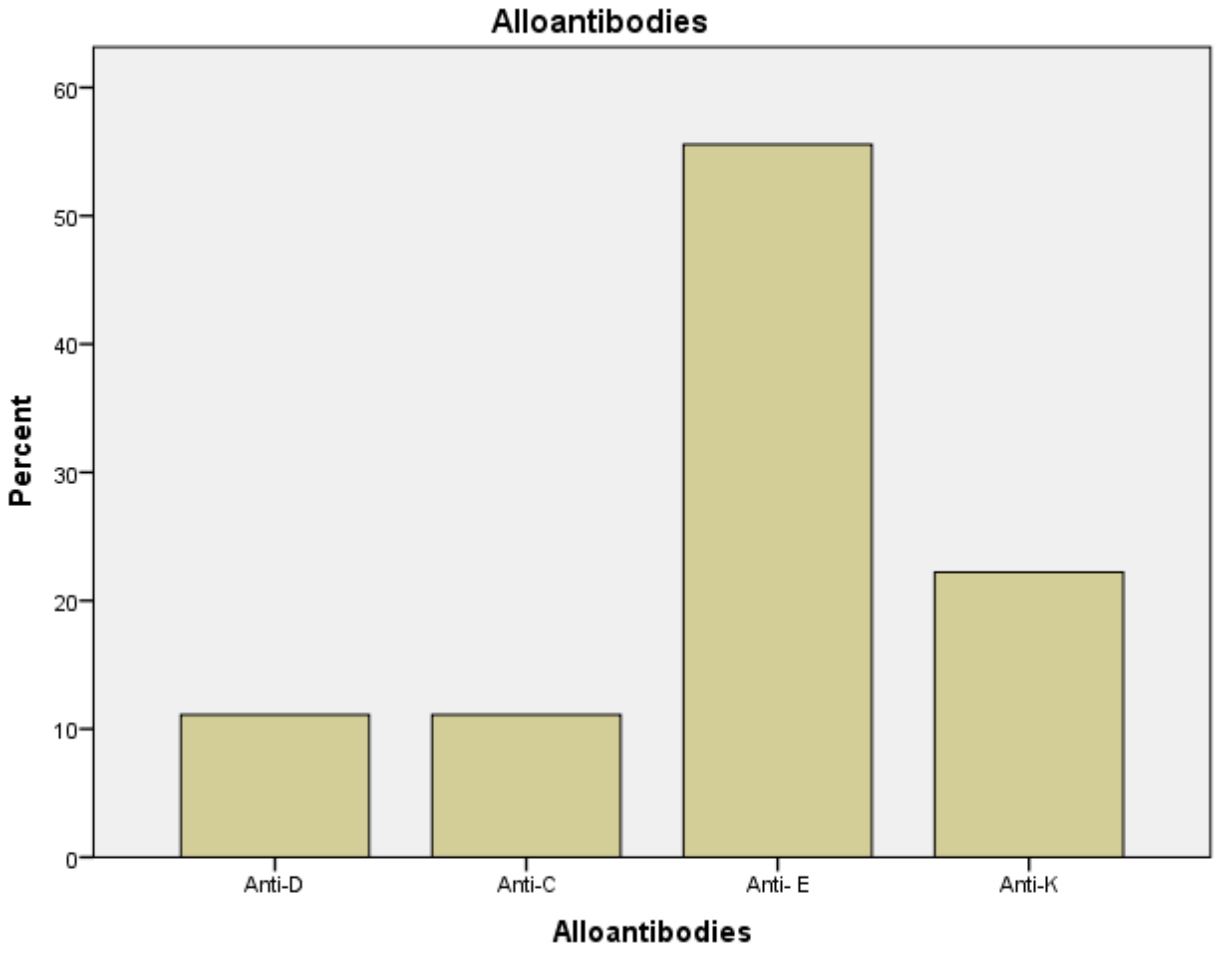
	Frequency and percentage distribution of alloantibodies	
Alloantibodies	Frequency	Percentage
Anti-D	1	11.1
Anti-C	1	11.1
Anti- E	5	56.6
Anti-K	3	21.2
Total	10	100

Table: 4.3 show frequency distribution of alloantibodies where Anti-D alloantibodies were detected in 1 patient, representing 11.1% of the total alloantibodies in the sample. Similarly, Anti-C alloantibodies were found in 1 patient, also making up 11.1% of the total alloantibodies. The most prevalent alloantibody in this sample was Anti-E, with 5 patients (55.6%) testing positive alloantibody among cancer patients. This indicates a higher occurrence of Anti-E alloantibodies in the group. Anti-K alloantibodies were detected in 3 patients, accounting for 22.2% of the total alloantibodies.

These findings suggest that among the detected alloantibodies, Anti-E is the most common, followed by Anti-K, Anti-D, and Anti-C. It's important to note that the percentages provided here are based on the total number of alloantibodies within the sample (10 cases).

4.4 Specificity of Types of Alloantibodies among Multi Transfused Cancer Patients

Figure1: Specificity of types of alloantibodies among alloimmunized cancer patients



Anti- E was the most common alloantibody among alloimmunized cancer patients as shown in figure 1:

4.5 Association between Alloimmunization and Transfusion Frequencies among Multi-Transfused Cancer Patients.

The present study found that there was no statistical significant association between red blood cells alloimmunization and the number of (frequency) of transfusion (*P= 0.753)* as shown in *table 4.5*.

Table 3.4: Association between alloimmunization and transfusion frequencies among multi-transfused cancer patients.

			Alloimmunization	Transfusions
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	-.147
		Sig. (2-tailed)	.	.753
		N	7	7
	Transfusions	Correlation Coefficient	-.147	1.000
		Sig. (2-tailed)	.753	.
		N	7	7

The findings in table 4.4 is the result of a correlation analysis, using Spearman's rank correlation coefficient (Spearman's rho), to assess the relationship between two variables: "Alloimmunization" and “Transfusions." The correlation coefficient between "Alloimmunization" and itself is 1.000 because it's a variable's correlation with itself, which is perfectly correlated with the p-value of 1.000. The correlation coefficient of -0.147 suggests a weak negative correlation between "Alloimmunization" and "Transfusions." However, this correlation is quite weak and close to zero, which indicates that there is little to no linear relationship between these two variables. The p-value of 0.753 is much greater than the conventional significance level of 0.05. Thus there is no statistically significant correlation between "Alloimmunization" and "Transfusions."

Based on these findings, there is no significant linear relationship between alloimmunization and transfusions in the analysed dataset. The correlation is weak and likely not meaningful in practical terms. Other factors or variables may be influencing alloimmunization in this context,

4.6 Association between Alloimmunization and Age among Multi-Transfused Cancer Patients.

The present study found out that there was no statistical significant association between alloimmunization and age among cancer patients (*P= 0.159)* as shown in *table 5*.

Table 4.6: Association between alloimmunization and age among multi-transfused cancer patients

			Alloimmunization	Age
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	.415
		Sig. (2-tailed)	.	.159
		N	13	13
	Age	Correlation Coefficient	.415	1.000
		Sig. (2-tailed)	.159	.
		N	13	13

Table 4.6 show the correlation coefficient between "Alloimmunization" and "Age" is 0.415 with the p-value associated with this correlation coefficient being 0.159. The correlation coefficient of 0.415 suggests a positive correlation between "Alloimmunization" and "Age." This indicates that there is a weak to moderate positive relationship between the two variables. As age increases, there tends to be a slight increase in the likelihood of alloimmunization. The p-value of 0.159 is greater than the conventional significance level of 0.05 but relatively close to it. This suggests that the correlation observed might be due to random chance, especially with a small sample size. Based on these findings, there is a weak to moderate positive correlation between alloimmunization and age. However, this correlation is not statistically significant at the conventional significance level of 0.05,

4.7 Association between Alloimmunization and Gender Among Cancer Patients.

The present study found out that there was statistical significant association between alloimmunization and age among cancer patients (*P= 0.01)*, with female being alloimmunized more than male patients as shown in *table 6*.

Table 4.7: Association between alloimmunization and gender among multi-transfused cancer patients

			Alloimmunization	Gender
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	0.431
		Sig. (2-tailed)	0.01	0.01
		N	13	13
	Gender	Correlation Coefficient	0.431	1.000
		Sig. (2-tailed)	.	.
		N	13	13

**. Correlation is significant at the 0.01 level (2-tailed).

The findings in table 4.5 present the results of a Spearman's rank correlation analysis, examining the relationship between two variables: "Alloimmunization" and "Gender. The correlation coefficient between "Alloimmunization" and "Gender" is 0.431. The p-value associated with this correlation coefficient is 0.01. The correlation coefficient of 0.431 suggests a moderate positive correlation between "Alloimmunization" and "Gender." Thus, there is a tendency for a specific gender to have a higher likelihood of alloimmunization compared to the other gender.

The p-value of 0.01 is less than the conventional significance level of 0.05, indicating that the correlation observed is statistically significant meaning that the observed correlation between "Alloimmunization" and "gender" is unlikely to have occurred by random chance.

Based on these findings, there is a statistically significant moderate positive correlation between alloimmunization and gender. This suggests that there is an association between gender and the likelihood of alloimmunization, with one gender having a higher likelihood than the other in the analyzed dataset.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence and Specificity of Alloantibodies among Multi-Transfused Cancer Patients

The present study has shown that there are incidences of alloimmunization among multiple-transfused cancer patients. A prevalence rate of 6.2%) was noted in this study, a prevalence that was similar to some findings of a study carried out in the department of immune- haematology and blood transfusion in India which had 7% prevalent rate of alloimmunization (Handa *et al.*, 2020). Additionally, the current study also concurred with those of Burkina Faso investigation which illustrated that the prevalence rate of alloimmunization among multi-transfused patients was around 5.9% (Nebie *et al.*, 2022).

The incidence of alloimmunization in cancer patients receiving several transfusions in the current study was however higher than the results of an investigation that was carried out in Macedonia which indicated a prevalence of irregular RBC alloimmunization as 0.32 % in multiple-transfused patient (Makarovska-Bojadzjieva *et al.*, 2017). This prevalence is much lower than what the current study found due study methodology. Macedonia's study used retrospective study whereby data was obtained from patients records. The current study's alloimmunized patients total was very small compared to sample size of 36000 out of which 116 were alloimmunized resulting in low alloimmunization rate compared to the current prospective study of alloimmunization rate of 10% with a sample size of 162 patients, a cross sectional study with small sample size compared to Macedonian study. Macedonian large sample size was attainable because of retrospective study compared to current study that involved laboratory work for generation of results. Likelihood of alloimmunization is affected by the volume and how often transfusions are given, the immunogenicity of the antigen, and the immunological response of the receiver. There have also been reports on the effects of race and antigenic pattern disparities between donors and recipients (Bhuva & Vachhani, 2017). Techniques used to collect, process, and store blood products, characteristics unique to donors, Erythrocyte Antigen-specific parameters, and other elements are examples of possible donor-related variables that could result in alloimmunization (Chemegni *et al.*, 2020). Genetic (MHC/HLA type and polymorphisms of immunoregulatory genes) variables, immunological activation status, phenotype of regulatory immune cell subsets, functional characteristics of immune cells, prior antigen exposures, and other factors are all potential

recipient-related factors (Tumer *et al.*, 2022). It is also believed that the presence of a disease may affect red blood cell alloimmunization (Arthur & Stowell, 2022). The therapeutic relevance of this finding is that antibody detection in transfusion medicine is crucial since it can reveal uncommon or irregular antibodies (Wang *et al.*, 2021).

The frequency of alloimmunization in the current study was lower than the prevalence of the French study, which found out that cancer patients were more likely to be alloimmunized than the general population at a rate of 13.7% (Allali *et al.*, 2017). The sample size of French study was 1000 patients which was a large sample size compared to the current study. The differences in the occurrence rates could be attributed to the fact that the proportion of transfused patients who develop alloimmunization varies depending study groups. The current study had one study group cancer patients compared to French study which studied on sickle cell disease, Thalassemia and cancer patients. The current study recruited patients who had received several transfusions only compared to French study which studied on patients with or without history of transfusion. The current study carried out cross sectional study on alloimmunization and risk variables whereby Laboratory work was performed and results obtained while French study was retrospective study whereby the study used existing patients' records to acquire patients data compared to present data, French data cannot be verified whether the data that was used was verified for accuracy or not. According to Hauser and Karafin, 2020 some patients are responders and respond to repeated transfusions by developing red blood cell alloantibodies, whereas other patients are non-responders (Hauser *et al.*, 2020). The prevalence of alloimmunization in the various populations under investigation also varies according to the transfusion policy. Hauser and Karafin, 2020 in United States study indicated that there is low rate of alloimmunization in nations where donors and recipients are of same ethnic background. Some research studies suggest that the timing and frequency of testing, the sensitivity of the testing processes, and the technical competence of the transfusion laboratory all play a role in the outcome of tests (El-Beshlawy, Salama, El-Masry, El Husseiny, et al., 2020). The sample sizes and methodologies used in the various research may have varied, which could account for these differences in occurrence rates.

5.2 Alloantibody specificity

Regarding specificity of alloantibodies, Ant- E was the most common (56.6%) followed by anti-K (21.2%), then Anti-D (11.1%) and Anti- C (11.1%). Compared to Red blood cell alloantibodies'

specificity varies depending on the studied population (Pessoni *et al.*, 2018). The presence of alloantibodies can have clinical implications, particularly in blood transfusion and pregnancy, where certain antibodies can lead to complications if not managed appropriately. Further investigation and monitoring may be necessary for patients with these alloantibodies.

The current study's findings are congruent with those of Macedonia whereby anti E was most common found in more of 50% of multiple antibody cases. Earlier reports from Japan, which claimed that anti-Rh antigens (E, c) and anti-Lewis (Lea) are the most common (Tormey & Hendrickson, 2019). The research's findings, however, go against those of a Korean study, which showed that anti-Lewis (Lea) sentiment was the most prevalent (Yang et al., 2019). The current findings also contradicted findings from a study done in Iran that indicated that anti-Kell (K) was the most common (Foomani *et al.*, 2023). According to French study findings, duffy blood system (Fy^a and Fy^b) was the most common with 66% followed by Kid blood system at (JK^a and Jk^b) 47% The disparities may be explained by the heterogeneity of the included populations and various screening methodologies: The current study used the gel-based antibody screening and identification " 1D-Diacel 1-11111 R" compared to French study which used Molecular biology for immunological reactions for a variant antigen or phenotype. French retrospective study on antibody specificity data may not be verified whether the data used was verified for accuracy or not compared to the current study that involved laboratory work. Since blood banks normally only supply ABO- and Rh (D)-antigens matched blood, the likelihood of alloimmunization to minor blood group antigens is high at Moi teaching and referral hospital and in Kenyan hospitals that offer blood transfusion services. The current study had prevalence rate of 6.2%, Anti (E, C, D) from Rhesus blood group system and anti K from Kell blood group system were identified. The current study calls for an intervention to reduce alloimmunization prevalence for proper management of cancer patients at Moi teaching and referral hospital and other Kenyan hospitals. Alloimmunization can be reduced by erythropoietin treatment in cancer patients or through phenotypic matching of red blood cell group antigens such as Rhesus, Duffy, Kidd, Kell MNS bloodgroups (Erhabor *et al.*, 2019). Many health care settings in under developed nations find this to be expensive and untenable. Although the majority of alloantibodies are specific for the Rh blood group, extensive antigen matching (C, E, c, e, K) can significantly reduce the danger of red blood cells (RBC) alloimmunization (Mangwana *et al.*, 2019).

5.3 Association between Alloimmunization and Transfusion Frequency

In the current study it was found out that alloimmunization was not statistical associated with the number of transfusions (*P= 0.753)*. This may be because immunogenicity, dosage, and clinical factors including pro-inflammatory conditions all play a role in alloimmunization. Furthermore, some patients fail to respond even after being exposed to large concentrations of red blood cell antigens as demonstrated in a research carried out before in a French university medical center (Allali *et al.*, 2017). The results of the present investigation had similarity with those of a Malaysian study, which showed that there was no appreciable difference in the quantity of blood units transfused between patients who were alloimmunized and those who were not (Abdullah *et al.*, 2023). The results of the current study also agreed with those of a study carried out by (Wafa *et al.*, 2023) which showed lack of a meaningful correlation between the incidence of alloimmunization in individuals with transfusion-dependent thalassemia and the frequency of transfusions. The current study findings also concurred with a meta-analysis study findings done by (Rofinda *et al.*, 2022) which established that erythrocyte antibody is not substantially correlated with alloimmunization on repeated transfusion.

The current study findings however, contradicted with a study done in Brazil which revealed that alloimmunization development is correlated with the frequency of transfusion cycles and blood units received (Pessoni *et al.*, 2018). Reasons for transfusion frequency difference could be, Brazilian study was retrospective study whereby records of all patients who received red blood cells examined by searching in the computer data base from transfuse agency university hospital, whereas the current study used patients recruitment process and cross sectional study in which samples were collected and processed for antibody specificity results this may have been the cause of transfusion frequency disparity. However Brazil retrospective study cannot be verified whether the retrospective data being use was verified for accuracy or not. The difference could have also been due to difference in sample size where the current study had low sample size of 162 compared to Brazilian study which had large sample size of 11,253 patients who had received blood transfusion that was an indication transfusion frequency into which alloantibodies were detected. In the current study, patients' data in patients file and the history of blood transfusions obtained from patients indicated that patients with several transfusions such as 4 transfusion episodes had received 16 units of blood within a year for more than 5 years. In adults and in other patients, a unit or volume of blood was transfused according to age. Some patients were alloimmunized and

others were not. Similarly to those with fewer transfusion episodes within few years or a year on transfusion, some were alloimmunized and others were not. Unit of blood from same donor given consecutively is not considered as multiple transfusions since this blood is from the same patient and has no genetic difference however the preservation and handling of the blood unit may not be verified.

This could imply that repeated blood transfusions may or may not cause the development of alloantibodies against a number of red cell antigens, leading to red blood cell alloimmunization in patients who have had many transfusions or those with few transfusions The results of the current study also differ with those of a study carried out in India, which showed that red cell alloimmunization was correlated with the number of red blood cells received, the duration of blood transfusions, and the frequency of transfusions (Mangwana *et al.*, 2019). The current study results also contradict with the results of a study carried out in Netherland which indicated that Alloimmunization rates rise as the quantity of transfusions increases, although the transfusion course in patients might vary from receiving numerous units at once to receiving them over a longer period of time. The present study results also differ with a study results in Columbia which suggested that reticulocytes in donated red blood cell units have an effect on the quality of transfused blood, are directed to a specific compartment, and could represent an unappreciated risk factor for red blood cell alloimmunization (Thomas *et al.*, 2023). Alloimmunization and transfusion frequency were not related in the current study, this could have been due to donated blood from close relatives or from similar pool gene. Furthermore, some patients fail to respond even after being exposed to large concentrations of red blood cell antigens. There are patients that are responders in their immune system and those that are not responders depending on immune status. Anomalies have been identified in T- cells that undergo functional alterations such as decline in helper and frequency of cytotoxic T-cells precursors with an increase in T- cells that fail to respond to activators, Dormant T- cells that do not respond to APC (antigen presenting cells), thus preventing, T- cells from coordinating with B- lymphocytes to secrete alloantibodies preventing alloimmunization. The same results were observed in an investigation which was carried out in Brazil (Pessoni et al., 2018). This could also be attributed to the likelihood that low titer antibodies may have gone undetected as specified time intervals following blood transfusion were not taken into account prior to antibody screening and antibody detection in the current

investigation and therefore it is important for antibody detection to be done following blood transfusion at predetermined intervals.

5.4 Association Between Alloimmunization And Age

The current study established that there was statistical correlation between alloimmunization and age which was 0.415 with p-value of 0.159 which is greater than 0.05 but closer to it an indication of a weak to moderate positive relationship between the two variables. As age increases there tends to be slight increase in alloimmunization. The current study findings indicated that older age set had an increased alloimmunization than other age sets and this may be due to several transfusions of number of blood units received during their life span exposing them to foreign antigens, leading to alloimmunization. The patient's age has frequently come to light as the primary risk factor among other risk factors of red blood cells alloimmunization in patients with illness dependent on blood transfusions (Franchini *et al.,* 2019). Age at the beginning of transfusion therapy has been identified in the literature as a significant risk factor with the older patients being more vulnerable (De Santos *et al*., 2017). Patients who started receiving transfusions before they were two to three years old had a reduced rate of alloimmunization, according to prior authors (Pessoni *et al.,* 2018). A lower risk of alloimmunization may be caused by a form of immune-tolerance that is produced by still undeveloped immune system of young patients (Marshall *et al*., 2018).The study findings are similar to Greece study which indicated that there is relationship between age and alloimmunization, also similar findings by Palestine study with an indication that there is correlation between alloimmunization and age in which older ages of patients were alloimmunized due to increased number of units of blood transfused to patients.

In the current study, individuals above 40 years had an increase in alloimmunization than the other age group which was similar to Palestinian study data which revealed that the highest rate (55.6) was among patients with older age > 20 years due to several transfusions (Samarah *et al.,* 2018). The current study contradicted with Brazil study which found out that there was no significant relationship between age and alloimmunization (Pessoni *et al.,* 2018). Similar to French study which indicated that there was no significance relationship between age and alloimmunization (Allali *et al.,* 2017). USA study also indicated that there was no relationship between age and alloimmunization (Karafin *et al.,* 2018). The current study conducted on cancer patients while the other studies conducted on Sickle disease and thalassemia patients. The current study found out

that, the number of older patients who were alloimmunized had increased more than the young age. The contradiction of the current study with Brazil, USA, and French study, of no significant relationship between age and transfusion frequency could be due to difference in age of study groups, study methodology where by the current study used cross sectional study while some studies like French study used retrospective study which used patients' records. The current study conducted its study on cancer patients of all age groups while the French studied on sickle cell disease patients and thalassemia and on pediatric patients. However French study and the studies that used retrospective study cannot be verified whether the retrospective data that use was biased or not. The association between a patient's age and red cell alloimmunization has not been reported in Kenya the current investigation found relationship between age and alloimmunization.

5.5 Association Between Alloimmunization And Gender

The current study demonstrated that gender could be associated with alloimmunization. This is comparable to what was seen in a study in Sudan that found that individuals who had received blood transfusions repeatedly may acquire antibodies depending on their gender (Waggiallah *et al.*, 2021). This is explained by the increased exposure to immunizing events in pregnant and/or transfused female cancer patients (Arora *et al.*, 2017). The current study did not come across pregnant women during the study. The current study was similar to an investigation carried out in India that illustrated that the prevalence in women was higher compared to men (Bhuva & Vachhani, 2017). The study findings were also similar to a study conducted in India which found more alloantibodies in female as compared to male donors (Nathani *et al.*, 2021). The research findings also agreed with a research in Greece which indicated a high prevalence in female patients compared to male patients (Poitou *et al.*, 2020) .The current study results also compared with the results of a research carried out at Sains Malaysia university hospital which demonstrated that women had a prominent occurrence of red blood cell alloimmunization in comparison to men, with a men-to-women ratio of 1:1.6 (Abdullah *et al.*, 2023). If Rhogam or anti- D is not given to Rhesus Negative mothers who conceive Rhesus Positive kids within 72 hours of birth, they typically develop alloimmunity against Rhesus Positive newborns (Jeon *et al*., 2020).

This study established that there was a statistically significant positive correlation between alloimmunization and gender (*P= 0.01)*, with many incidences being recorded in female than male patients this could be due to expose to alloantigens throughout pregnancy and childbirth in women

which is biologically plausible (Deshmukh & Way, 2019). According to research, more allogeneic exposure leads to a higher rate of alloimmunization, Red blood cell antibodies are slightly more frequently present in women than in men (Sachan *et al.*, 2020). This is most likely explained by the fact that pregnant female patients have increased exposure to immunizing events (Webb & Delaney, 2018). Before giving an emergency blood transfusion, the blood types of the recipient and donor must be matched to make sure they are compatible. No other match, including one based on race, ethnicity, or gender, exists between the recipient and the blood bag (Muir *et al.*, 2023). Yet, a latest analysis contends that the genders of the donor and receiver should match.

According to the study, a mismatch could occur if a male patient receives blood from a female who has previously given birth to male children (Caram-Deelder *et al.*, 2017). The Study indicated that receiving a transfusion from a donor who is perpetually pregnant, as opposed to a man, was connected to a rise in all-cause deaths amongst male receivers of red blood cell transfusions and not female receivers. Neither male nor female receivers of transfusions from never-pregnant female donors saw an increase in mortality (Caram-Deelder *et al.*, 2017). Researchers from the Netherlands studied hundreds of transfusion patients and found that men under 50 had a 1.5-fold greater chance of dying within three years after receiving red blood cells from a woman who had previously been pregnant (Kuldanek & Silliman, 2018). One of the potential explanations for why males do not respond well to pregnant women's blood is an immune component that women acquire and incorporate in to this immunological element could be influencing the receiver's body to reject the transfused red blood cells, especially in younger males (Caram-Deelder *et al.*, 2017). Some study has argued that mothers who have given birth to boys may have evolved specific antibodies to the Y chromosomes found in their sons' male DNA. This might be as a result of a pregnancy-related immunological response. This may have been the element causing problems with the recipient's blood after the transfusion.

Based on these data, gender must be taken into account as one of the variables in order to prevent alloimmunization and other undesirable outcomes. which is correlated positively with the number of prior pregnancies (Pessoni *et al.*, 2018). According to some studies, the recipient's and donor's genders should be matched (Caram-Deelder *et al.*, 2017). Elsewhere it has been established that H-Y antigens that are in the group of minor histocompatibility complex may lead to alloimmunization in mothers who have delivered male children (Singh *et al.*, 2019). This could be

what contributed to the association between alloimmunization and gender in the current study. Studies have also shown that receiving red blood cells from female donors who have a history of pregnancy is linked to higher mortality rates in male recipients (Edgren *et al.*, 2019).

These results agreed with the results of a study that was done in Brazil that indicated a positive association between alloimmunization and gender (Pessoni *et al.*, 2018). Similar results could have been due to recruitment of similar age and gender. The results of the present study are consistent with those of a study conducted in the USA, which revealed that female patients are more alloimmunized than male patients (Karafin *et al.*, 2018). A larger risk of red blood cell alloimmunization exists in females (Oud *et al.*, 2022), with some research demonstrating a link between the number of prior pregnancies and the rate of alloimmunization as a result of higher allogeneic exposure (Seielstad *et al.*, 2018). Certain circumstances allow for the production of some 'naturally occurring' red blood cell antibodies without prior contact to foreign red blood cells (Pessoni *et al.*, 2018). It is unclear what causes the formation of the majority of these antibodies, however it is frequently hypothesized that environmental or microbial chemicals that share antigenic properties with blood group antigens may act as a trigger (Ewald & Sumner, 2018).

The present study results however contradict with the results of a research done by (Rofinda *et al.*, 2022), which demonstrated no significant association of red blood cell alloimmunization with gender. The current research results also contradicted with the results of a research carried out by (Aldakheel *et al.*, 2022) which found out that there is no association between gender and production of antibodies (Aldakheel *et al.*, 2022). This might have been caused by the methods and setting used in the two investigations, which recruited patients of varied ages and genders. The present study findings did not also agree with a study conducted in Tehran which recorded that Age, gender, previous pregnancies, and splenectomy do not affect the existence of antibodies in patients who have received many transfusions (Shaiegan *et al.*, 2022). The current research results also contradicted with the results of a research carried in Asia that illustrated that alloimmunization is not significantly correlated with gender and splenectomy status of a multi-transfused patient (Dogra *et al.*, 2015). The discrepancies in the results could be credited with different study populations and differences in environment. The current study recommends routine consideration of gender when assessing alloimmunization among multi-transfused cancer patients.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

6.1.1 Prevalence Of Alloimmunization And Alloantibody

The present study identified 10 patients with alloantibodies resulting to 6.2% prevalence rate of red blood cells alloimmunization among multi-transfused cancer patients. The study identified anti E of Rhesus blood group system as the most common antibody, followed by anti K of Kell blood group system at Moi Teaching and referral hospital Kenya.

6.1.2 Alloimmunization And Transfusion Frequency

According to current study, there was no relationship between alloimmunization and transfusion frequency.

6.1.3 Age And Alloimmunization

The current study found out moderate statistical correlation between age and alloimmunization

6.1.4 Gender And Alloimmunization

Compared to male patients, female patients had a greater prevalence rate. Yet, among patients receiving many transfusions for cancer, there was a positive correlation between gender and alloimmunization that was statistically significant.

6.2 Recommendations

6.2.1 Alloimmunization Prevalence And Alloantiodies:

The study recommends routine screening of alloantibodies and the alloimmunized patients to be given corresponding antigen negative blood

Apart from ABO and Rhesus D blood group compatibility testing, additional minor blood group antigens such as anti Rh (E,ec.,), anti Kell (K), anti-Kid (Jka Jkb), should be performed to greatly lower the risk of alloimmunization, according to the study findings, which are strong enough to support this recommendation.

It is important to undertake screening for alloantibodies following blood transfusion at predetermined intervals especially in cancer treatment

The study suggests that cancer patients who need many transfusions should undergo thorough red blood cell phenotyping and routine antibody screening.

6.2.2 Alloimmunization And Transfusion Frequency

Routine antibody screening should be done before blood transfusion.

6.2.3 Age And Alloimmunization

The study recommends age to be considered when assessing alloimmunization in elder cancer patients who could have had several transfusions.

6.2.4 Gender And Alloimmunization

Gender that is most associated with alloimmunization is female.

The study recommends routinely considering female when assessing alloimmunization in patients getting several transfusions for cancer patients.

.Although gender of the patients is not frequently taken into account as important factors during transfusion; they have a substantial impact on the degree to which patients are alloimmunized.

Multi-transfused cancer women should be screened for alloantibodies and be given corresponding antigen negative blood.

Further investigation may be needed to understand the factors contributing to this gender-based difference in alloimmunization risk.

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APPENDICES

APPENDIX I: PARTICIPANT INFORMATION AND CONSENT SHEET (ENGLISH VERSION)

Two sections make up this informed consent form:

1. Informational leaflet (to share information about the study)
2. a document stating consent

PART 1: INFORMATIONAL LEAFLET

Introduction

My name is Ms. Wapukha Bunoro Zippy, and I am a postgraduate student in the Department of Applied Health Sciences, School of Health Science at Kisii University. I am conducting this study to assess the red blood cell alloimmunization among multi-transfused oncology patients at Moi Teaching and referral Hospital, Kenya.

You're invited to take part in this research. Please ask me to pause and explain if there are any terms used in this fact sheet that you do not understand.

Type of Research Intervention:

In order to detect and identify alloantibodies, this study analyses blood. We will take blood samples using the phlebotomy technique as a guide. The laboratory will then screen and identify alloantibodies after receiving the blood. After testing, the blood samples will be thrown away. At the time of sample collection, age, gender and number of transfusions will be recorded.

Participant Selection:

Our goal is to recruit volunteers from multi-transfused oncology patients.

Voluntary Participation:

Your decision to participate in or not engage in this study is completely up to you. Whether you choose to participate or not, there will be no difference in your ability to get treatment or other hospital services.

Procedures and Protocol:

Those that qualify will be asked to participate in the study. Then a consent form will need to be signed by everyone. Children below 15 years will receive consent from their parents or guardians. The incompetent patients will be assisted by relative to fill the form. Each participating individual will have four (4) MLS of venous blood drawn in a plain vacutainer for antibody screen and detection.

Side Effects:

This study has no negative side effects.

Risks:

No dangers are anticipated with this study. At the site of the needle prick, a hematoma can occasionally develop, although this should go away in a few days.

Benefits:

The study has no specific advantages. You and your clinician will be alerted for management if critical results are observed.

Reimbursements:

You won't receive any payment or gifts for taking part in this study.

Confidentiality:

A unique number will be used to identify each participant (names will not be used). The only people who will see the data you provide for this study are the researchers.

Sharing the Results:

In order for other interested parties to benefit from the findings, we shall publish the findings. Your identity will never be made public, though in form of publishing with anonymity and the same data can be used for further research in future and can be shared in scientific word.

Request to participate in the study

Please let us know if you're interested in participating in this study. I kindly ask that you complete the consent form if you are willing to participate in the study.

Right to Refuse:

Your treatment intention won't change if you choose not to take part in the study. All the advantages you would have otherwise remain yours.

Who to Contact:

You can get in touch with the researcher listed below at any time if you have any queries about this study:

WAPUKHA BUNORO ZIPPY

MOB NUMBER: 0725710896

PART II: A DOCUMENT STATING CONSENT

The information that came before has been read to me or I have read it myself. I have the option to ask any questions I may have regarding taking part in the study, and all of my inquiries have received satisfactory responses. I've been informed of my rights, and I willingly agree to take part in this study.

Name of Participant:

Signature of Participant:

Date:

If illiterate:

The witness must be literate (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should also add their thumbprints.

I saw the permission document being correctly read to the prospective participant, who also got the chance to ask any questions. I attest that the person voluntarily gave their consent.

Print Name of Witness:

Signature of Witness:

Date:

Thumb Print of Participant:

APPENDIX II: PARTICIPANT INFORMATION AND CONSENT SHEET (KISWAHILI VERSION)

Sehemu mbili hufanya fomu hii ya idhini iliyo na habari:

1. Kijitabu cha habari (kushiriki habari kuhusu utafiti).
2. Hati inayosema idhini.

SEHEMU YA 1: KIJITABU CHA HABARI

Utangulizi

Jina langu ni Bi Wapukha Bunoro Zippy, na mimi ni mwanafunzi wa kuhitimu katika Idara ya Sayansi ya Afya iliyotumika, Shule ya Sayansi ya Afya katika Chuo Kikuu cha Kisii. Ninafanya utafiti huu kutathmini uchunguzi wa seli nyekundu za damu kati ya wagonjwa wa saratani waliotafsiriwa kwa wagonjwa wengi huko Moi Kufundisha na Hospitali ya rufaa, Kenya.

Umealikwa kushiriki katika utafiti huu. Tafadhali niulize nipumzishe na kuelezea ikiwa kuna maneno yoyote yanayotumika katika karatasi hii ya ukweli ambayo hauelewi.

Aina ya Uingiliaji wa Utafiti:

Ili kugundua na kutambua alloantibodies, utafiti huu unachambua damu. Tutachukua sampuli za damu kwa kutumia mbinu ya phlebotomy kama mwongozo. Maabara basi itaangalia na kutambua alloantibodies baada ya kupokea damu. Baada ya kupima, sampuli za damu zitatupwa mbali. Wakati wa ukusanyaji wa mfano, umri, jinsia na idadi ya uhamishaji itarekodiwa.

Uteuzi wa Mshiriki:

Kusudi letu ni kuajiri watu wa kujitolea kutoka kwa wagonjwa wa saratani waliobadilishwa.

Ushiriki wa Hiari:

Uamuzi wako wa kushiriki au kutoshiriki katika utafiti huu ni juu yako kabisa. Ikiwa unachagua kushiriki au la, hakutakuwa na tofauti katika uwezo wako wa kupata matibabu au huduma zingine za hospitali.

Taratibu na Itifaki:

Wale wanaostahili wataulizwa kushiriki katika utafiti. Halafu fomu ya idhini itahitaji kusainiwa na kila mtu. Kila mtu anayeshiriki atakuwa na nne (4) MLS ya damu yenye sumu inayotolewa kwenye utupu wazi wa skrini ya antibody na kugundua.

Hatari:

Hakuna hatari inayotarajiwa na utafiti huu. Kwenye tovuti ya sindano, hematoma inaweza kukuza mara kwa mara, ingawa hii inapaswa kwenda kwa siku chache.

Faida:

Utafiti hauna faida maalum. Wewe na daktari wako mtaarifiwa kwa usimamizi ikiwa matokeo muhimu yanazingatiwa.

Kulipia:

Hautapokea malipo yoyote au zawadi kwa kushiriki katika utafiti huu.

Usiri:

Nambari ya kipekee itatumika kutambua kila mshiriki (majina hayatatumika). Watu pekee ambao wataona data unayotoa kwa utafiti huu ni watafiti.

Kushiriki Matokeo:

Ili vyama vingine vinavyovutiwa kufaidika na matokeo, tutachapisha matokeo. Kitambulisho chako hakitawahi kutangazwa, ingawa.

Ombi la kushiriki katika utafiti:

Tafadhali tujulishe ikiwa una nia ya kushiriki katika utafiti huu. Ninaomba kwa huruma umalize fomu ya idhini ikiwa uko tayari kushiriki kwenye utafiti.

Haki ya Kukataa:

Kusudi lako la matibabu halitabadilika ikiwa utachagua kutoshiriki katika utafiti. Faida zote ambazo ungebaki zako.

Nani wa Mawasiliano:

Unaweza kuwasiliana na mtafiti aliyeorodheshwa hapo chini wakati wowote ikiwa una maswali yoyote kuhusu utafiti huu:

WAPUKHA BUNORO ZIPPY

MOB NUMBER: 0725710896

SEHEMU YA II: HATI INAYOSEMA IDHINI.

Habari ambayo ilikuja hapo awali imesomwa kwangu au nimeisoma mwenyewe. Nina chaguo la kuuliza maswali yoyote ambayo naweza kuwa nayo kuhusu kushiriki katika utafiti, na maswali yangu yote yamepokea majibu ya kuridhisha. Nimearifiwa juu ya haki zangu, na ninakubali kushiriki katika utafiti huu.

Jina la Mshiriki:

Saini ya Mshiriki:

Tarehe:

Ikiwa hajui kusoma na kuandika:

Shahidi lazima asome (ikiwezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano wowote na timu ya utafiti). Washiriki wasio na kusoma wanapaswa pia kuongeza alama zao za vidole.

Niliona hati ya ruhusa ikisomwa kwa usahihi kwa mshiriki anayetarajiwa, ambaye pia alipata nafasi ya kuuliza maswali yoyote. Ninathibitisha kwamba mtu huyo alitoa idhini yao kwa hiari.

Chapisha Jina la Shahidi:

Saini ya Shahidi:

Tarehe:

Chapisha kidole ya Mshiriki:

APPENDIX III: KISII UNIVERSITY'S LETTER OF PERMISSION FOR THE COLLECTION OF DATA

KISII UNIVERSITY
(ISO 9001:2008 Certified Institution)
ELDORET CAMPUS
OFFICE OF THE DEPUTY DIRECTOR-ACADEMIC AFFAIRS
Phone: 020-2610479 P. O. Box 408- 40200
Email:eldoretcampus@kisiiuniversity.ac.ke **ELDORET-KENYA**

24TH AUGUST , 2016

TO WHOM IT MAY CONCERN

Dear Sir / Madam.

RE: RESEARCH DATA COLLECTION PERMIT.

WAPUKHA BUNORO ZIPPY MHS12/40016/14

The above named is a bonafide student of Kisii university- Eldoret Campus pursuing a **Masters Of Science in Haematology /Blood Transfusion** in **the faculty of Health Sciences.**

She is working on her research entitled "***Occurrence Of Transfusion Related Alloimmunization Among Multi-Transfused Oncology Patients At Moi Teaching And Referal Hospital ,Kenya .***" in partial fulfilment for the requirement of the Award of Masters in **MSc. Haematology/Blood Transfusion.**

We are kindly requesting your office to provide her with the permit to proceed to the field for data collection and completion of her research.

Please do not hesitate to call the undersigned for any verification.

Any assistance extended to her will be highly appreciated.

Yours faithfully,

Charles O. Ongoyo (0720986205)
DEPUTY DIRECTOR – ACADEMIC AFFAIRS

APPENDIX IV: APPROVAL FROM MTRH INSTUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2016/251
Approval Number: 0001920

13th July, 2021

Wapukha Bunoro Zippy,

Kisii University,

P.O. Box 408-40200,

KISII -KENYA.

Dear Mrs. Wapukha,

RE: CONTINUING APPROVAL

The Institutional Research and Ethics Committee has reviewed your request for continuing approval for your study titled:-

"Occurance of Transfusion Related Alloimmunization among Multi-Transfused Oncology Patients at Moi Teaching and Referral Hospital Kenya

Your request has been granted Approval with effect from 13th July, 2021. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 12th July, 2022. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
13 JUL 2021
APPROVED
P. O. Box 4606 - 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc:	CEO	-	MTRH	Dean	-	SPH	Dean	-	SOM
	Principal	-	CHS	Dean	-	SOD	Dean	-	SON

APPENDIX V: NACOSTI RESEARCH LICENCE

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **885337**

Date of Issue: **02/November/2021**

RESEARCH LICENSE

This is to Certify that Ms.. ZIPPY BUNORO WAPUKHA of Kisii University, has been licensed to conduct research in Uasin-Gishu on the topic: Occurance of Transfusion Related Alloimunization among Multi-Transfused Oncology Patients at Moi Teaching and Refferal Hospital for the period ending : 02/November/2022.

License No: **NACOSTI/P/21/11340**

885337

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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