PREVALENCE OF SELECTED RED BLOOD CELL ABNORMALITIES IN DONATED BLOOD AT THE REGIONAL BLOOD TRANSFUSION CENTRE – MOMBASA, KENYA

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Prevalence of Selected Red Blood Cell Abnormalities in Donated Blood at The Regional Blood Transfusion Centre – Mombasa, Kenya

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DECLARATION

I declare that this is my original work and has not been presented to any other University or Institution.

Signature..... Date.....

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DEDICATION

I dedicate this work to my beloved Sylvia Achieng Nyarambe, my mother Matabel Wigina you are the pillar I lean on and my strength in adversity. And to my sons Wigina and Gambo and my daughters Wisiko and Wankio you give me a reason to smile.

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LIST OF ABBREVIATIONS

CBC	Complete Blood Count
EDTA	Ethylene Diamine Tetra Acetic Acid
fL	Femtolitres
G6PD	Glucose-6-Phosphate Dehydrogenase
G6PDd	Glucose-6-Phosphate Dehydrogenase deficiency
g/dL	Grams/decilitre
GPI	Glycophosphatidylinositol
Hb	Haemoglobin
HbF	Foetal Haemoglobin
HbSE	Haemoglobin S E
HPFH	Hereditary Persistence of Foetal Haemoglobin
HPP	Hereditary Pyropoikilocytosis
HS	Hereditary Spherocytosis
ICSH	International Council for Standardization in Haematology
KNBTS	Kenya National Blood Transfusion Service
MCV	Mean Cell Volume
NO	Nitric Oxide

OFT	Osmotic Fragility Test
PKd	Pyruvate Kinase deficiency
PNH	Paroxysmal Nocturnal Haemoglobinuria
RBC	Red blood cells
RBTC	Regional Blood Transfusion Centre
RDW	Red Cell Distribution Width
RMPs	Red Cell Microparticles
SCD	Sickle Cell Disease
TTIs	Transfusion Transmissible Infections
WHA	World Health Assembly
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Blood transfusion: This is the clinical use of blood and/or blood products as a therapeutic agent in the treatment of conditions associated with blood

Chronic Transfused patient: These are patients whoare periodically transfused to sustain life due to a lifelong ailment affecting blood cell survival.

Enzyme deficiency: Conditions in which there is reduced activity or complete lack of red cell enzymes important in its metabolic pathways

Haemoglobin defects: Hereditary defects of the red blood cell component

Haemolysis: The breakdown of red blood cells in which red cell membrane is breached resulting in the release of cytoplasmic contents into the surrounding medium

Hereditary disorder: Conditions affecting the red blood cell membrane or activity that may have been genetically acquired.

Hyperchromasia: This is the increased staining capacity of the red blood cells owing to the presence of defects in its membrane or cellular composition

Membrane permeability: The physiological state of the cell that allows materials to be transported in and out across the membrane

Red cell membrane defects: The pathologic or otherwise variations on the red blood cell membrane that affects its lifespan.

Sickle cell disease: a defect in the composition of the haemoglobin molecules of the red blood cell that makes these molecules band up under oxidative stress causing a deformation of the cell membrane

Spectrin and ankyrin: Two of the integral proteins found in the red blood cells

Thalassemia: This is a genetic defect arising from deletion of part or all of the genes that are responsible for the formation of globin chains in the haemoglobin molecule.

ABSTRACT

Blood transfusion is an important clinical intervention during surgery and in the treatment of severe tissue hypoxia. During transfusion, components of blood including red blood cells, Platelets or plasma are directly administered into the recipient to alleviate conditions such as anaemia and haemostatic deficiencies. Effective blood transfusions will positively affect prognosis. The efficacy of a red blood cell unit depends on the amount of blood delivered, the quality of cells and the life span of a given unit. These parameters are assayed during blood donation except for the red cell abnormalities such as sickle cell, Glucose 6-Phosphate dehydrogenase deficiency and other parameters that have an impact on the quality of red cells delivered and their life span. This cross-sectional study was done at the regional blood transfusion centre, Mombasa and the Technical University of Mombasa. Consecutive blood samples were analyzed for selected red cell parameters. The objective of this study was to determine the occurrence of red cell abnormalities in donor blood. Six hundred and seventy-six samples were analyzed. A significance level of $p<0.05$ was set for all statistics. The study found that 31.07% of the donor samples had abnormal values. There was a significant variation (t - 0.03, CI 95%) in the total red blood cell count. A significant Pearson's positive correlation was realized between the osmotic fragility and haemoglobin concentration ($r= .195$, and $p< 0.001$). The study also found that Hb values were not the same across the G6PD conditions ($P = 0.033$, CI 95%). The study concludes that significant proportion of donated red cells had an abnormality. These results point towards RBC indices or morphological Red cell abnormality. The study recommends that hospital blood banks should screen for red blood cell abnormalities before transfusion. Further studies should be done to guide in the development of strategies that will filter out these abnormalities and investigate the effect of these abnormalities to the recipient.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Blood transfusion continues to save lives since Landsteiner and his colleagues discovered blood group antigens and antibodies (Klein & Anstee, 2008). Blood transfusion has therefore become an integral part of clinical practice in most parts of the world (Janatpour & Holland, 2007). The World Health Organization (WHO) issues guidelines for the practice of donation, storage, distribution and clinical use of blood and blood products which are adhered to by member states. These guidelines that were adopted by the World Health Assembly (WHA) are incorporated in blood bank practices in member states. The WHO recommends that each country formulates specific guidelines to govern blood banking and transfusion service. (Force, 2004; WHO, 2010, 2012). *et al*

The most common factors assessed during donor selection are blood group serology and the presence or absence of transfusion transmissible infections (Milkins *et al*., 2014; Yazdanbakhsh, Ware, & Noizat-Pirenne, 2012). This is primarily in order to minimize transfusion transmissible infections and avoid adverse events occasioned by immunological and non-immunological agents (Pham *et al*., 2009; Woodfield, Perkins, & Johnson, 2007). Other factors assayed are the levels of haemoglobin and the general well-being of the donor. These group of tests help to ascertain the donors fitness and ability to donate without any adverse effects to themselves(Carson, Carless, & Hebert, 2012).

The presence of red blood cell abnormalities and minor red cell membrane defects is not routinely assayed in many countries. This is despite evidence that these red cell membrane defects may encourage the formation of storage lesions and thus may adversely affect

transfused patients (D'Alessandro et al., 2015a; Renzaho, Husser, & Polonsky, 2014; Tzounakas, Kriebardis, Georgatzakou, et al., 2016). The provision of safe blood for transfusion is the primary goal of blood bank centralization, yet it is also one of the greatest challenges facing the clinical use of blood and blood products. In this respect, and to minimize adverse effects, the presence of red cell anomalies should be ascertained. This has been proposed for routine practice in countries with advanced health care facilities and more so when transfusing neonates or persons having transfusion dependent conditions (Chassé *et al*., 2016; Ho, Sibbald, & Chin-Yee, 2003).

Studies have shown that these defects and especially those intrinsic to the red blood cell may be the cause of mild to severe red cell haemolysis (Howes, Battle, Satyagraha, Baird, & Hay, 2013). The haemolysis may have clinically significant adverse effects to the health of the transfused patient and may be fatal (Han, Serrano, & Devine, 2010; Klein & Anstee, 2008). The deleterious effects of these red cell anomalies to the transfused patients are more prominent during massive transfusion and exchange transfusion or neonatal transfusion (Renzaho *et al*., 2014).

Red cell membrane defects are common causes of haemolysis in most parts of the World (Barcellini *et al*., 2011). These are attributable to a host of abnormal environmental and hereditary causes (Surgenor, 2013). These red blood cell abnormalities range from haemoglobin defects, abnormalities on the cell membrane, defective enzymatic activity and storage changes during component processing (Hoffbrand, Catovsky, Tuddenham, & Green, 2011). Haemoglobin defects result in abnormalities such as thalassemia and sickle cell disease and traits. These diseases and traits are common in Africa (Frederic B Piel, Hay, Gupta, Weatherall, & Williams, 2013) and in Asia while enzyme and membrane permeability defects occur to a great extent in the European and Australasian regions (Colah *et al*., 2010; Da Costa *et al*., 2013). The existence of sickle cell and thalassemia traits in the community suggests that individuals with these conditions may be recruited donate blood (Horowitz & Confer, 2005; Tsaras, Owusu-Ansah, Boateng, & Amoateng-Adjepong, 2009). Such blood however contains unstable β-chains which may be haemolysed in the peripheral blood (Hoffbrand *et al*., 2011; Kudale, Sethi, Dhaliwal, &

Kher, 2014). In Kenya another condition associated with haemolysis of red cells is known as High Persistence of Foetal Haemoglobin (HPFH) (Wilcox *et al*., 2009). This has been associated with 6 types of mutations on the β-chain resulting in Hb Black and Hb Kenya among others (Hoffbrand *et al*., 2011). During transfusion with blood from sickle cell trait or from a thalassemia traits and other abnormalities, blood flow disorders and optical disturbances may occur (Li *et al*., 2018; Lu, Wood, & Higgins, 2016)

Red cell membrane anomalies resulting from enzyme defects include Glucose-6-Phosphate Dehydrogenase deficiency (G6PDd) and Pyruvate Kinase deficiency (PKd) among others (Howes *et al*., 2012). These have been reported to have clinical effects on exchange transfusion patients and individuals with chronic haemolysis and haemorrhagic disorders (Brunskill, Wilkinson, Doree, Trivella, & Stanworth, 2015; Renzaho *et al*., 2014).

During storage, changes occurring in the red blood cell may affect the quality of the blood to be transfused. These changes include the formation of red cell storage lesions (Kim-shapiro, Lee, & Gladwin, 2012). The lesions occur due to the decrease in intra-erythrocytic energy sources, making the cells less deformable and fragile leading to accelerated haemolysis and release of red cell microparticles (RMPs) (Kim-Shapiro, Lee, & Gladwin, 2011). The presence of red cell defects increases the likelihood of accelerated lesion formation. Upon transfusion, haemolysed cells and RMPs consume Nitric oxide (NO) causing a decrease in NO bioavailability. Resulting in substantial changes in the rheological properties of the transfused blood (Adams, Bellairs, Bird, & Oguntibeju, 2015; D'Alessandro *et al*., 2015a). Other defects include citrate poisoning and potassium ion leakage (Hess, 2010). The changes may cause clinically significant adverse effects to the transfused patient (Renzaho *et al*., 2014). The effects and severity depend on the patient condition and the storage time of the transfused unit (Papay *et al*., 2012).

This study determined the presence of selected red cell anomalies in donated blood at the Regional Blood Transfusion Centre (RBTC) Mombasa.

1.2 Statement of the Problem

One of the major concerns for the use of blood and blood products is the existence of adverse reactions during and after transfusion. These reactions maybe caused by factors either inherent in the recipient or intrinsic to the donor blood. Currently, blood grouping, antibody testing, cross-matching and infectious disease assays are routinely performed to certify blood safety. However, adverse effects associated with red cell defects are not assayed. These defects have been demonstrated to pose risks to the transfused patients that may include haemolysis and ineffective transfusion. In addition, donors with sickle cell trait may suffer from vascular occlusion and kidney failure as a result of increased oxygen tension (D'Alessandro *et al*., 2015b; Kücükakin *et al*., 2011).

Clinically, the desired efficacy of a single transfusion results in an increase of 1g/dL of haemoglobin (Hb). This is sometimes not achieved owing to factors inherent to the transfused donor cells. The unprecedented destruction of donor cells may affect the desired reversal of tissue hypoxia leading to prolonged hospital stay and multiple transfusions. This ultimately leads to a strained local and national health budget and more specifically a strain to both the individual and the community. Adverse clinical conditions lead to increased morbidity and mortality and therefore loss of man hours and an increased national health care burden. Thus there is need to investigate these abnormalities in red cells of donated blood.

1.3 Justification

The provision of safe blood for transfusion is one of the main mandates of the National Blood Transfusion Service (NBTS). It is necessary that the system for provision of safe blood is re-evaluated especially in resource poor settings to capture current trends and practices. With increasing evidence of risk factors associated with transfusion of blood, there is need to increase the awareness and safety levels of all transfused blood. The presence of haemolysis and RMPs due to either aging or insidious unapparent disease

contributes greatly to these risk factors. Such processes may have deleterious effects on neonates and transfusion dependent patients.

The transfusion of blood with abnormalities increases the likelihood of developing cardiovascular conditions and may also lead to ineffective transfusion (Aubron, Nichol, Cooper, & Bellomo, 2013; Carson *et al*., 2012). This in turn leads to longer hospitalization and an increase in the economic burden to the individual and community. This burden is estimated at between 2.5% - 7.0% for general illness (Russell, 2004) and upwards of 10% of yearly income for sickle cell anaemia patients (Adegoke, Abioye-Kuteyi, & Orji, 2014) in developing countries. These maybe reduced significantly when blood without the abnormalities is used for transfusion. To ensure good patient care, blood banks need to adopt dynamism and employ assays that will maximize clinical efficiency by the provision of safe red blood cells. The findings of this study will contribute to the update of blood transfusion guidelines.

1.4 Research Questions

1. Are red cell indices of donated blood within the normal ranges?
2. Are there red cell membrane abnormalities in donated blood?
3. Are there unstable haemoglobins in donated blood?
4. Do donor cells exhibit normal G6PD enzyme activity?

1.5 Objectives

1.5.1 General objective

To determine the point prevalence of selected red blood cell abnormalities in donated blood at the RBTC Mombasa.

1.5.2 Specific objectives

i. To determine the Red cell indices of donated blood

ii. To determine the Osmotic fragility of donated red blood cells

iii. To determine the Hb lysis patterns of donated blood

iv. To determine G-6-PD activity of donated Red blood cells

CHAPTER TWO

LITERATURE REVIEW

2.1 Abnormalities in Red Cells

The occurrence of abnormalities on the red blood cell causing its deformity was first documented as a mythical phenomenon in Africa (Onwubalili, 1983) and demonstrated as elongate red blood cells in anaemic patients (Herrick, 2001). Other abnormalities have been detected on the red cell membrane by various workers in the 20th and 21st century. These include the discovery of spherocytosis as a cause of haemolysis and haemolytic anaemia and ankyrin deficiency as a major cause of red cell the deformity (Eber *et al*., 1996; Hassoun & Palek, 1996). Other abnormalities have been demonstrated to result from defects in the activities of enzymes associated with red blood cell for metabolism. These include the discovery of the contribution of G6PD deficiency (G6PDd) and Pyruvate Kinase Deficiency (PKD) in adult red cell haemolysis (Ernest Beutler, 2008; Keitt, 1966). Red cell abnormalities often occur due to genetic variation mutation or defect. These genetic conditions often have carrier or minor states that may present with normal blood pictures and clinical histories (Bitan *et al*., 2016). However they may affect clinical stability of the subjects when exposed to some natural trigger such as pollen, some proteins or drugs (Mohandas & Gallagher, 2008; Salomao *et al*., 2008). Transfusion dependent patients have a compromised immunity, and a low oxygen stress threshold. This renders abnormalities in donated red cells more hazardous to their health than in normal subjects (Armstrong, 2008). Such hazards may include haemolysis and jaundice, transfusion reactions and ineffective transfusion regimes (Lasalle-Williams *et al*., 2011; Tsaras *et al*., 2009). Transfusion of stored blood has been linked to increased occurrence of deep vein thrombosis (Offner, Moore, Biffl, Johnson, & Silliman, 2002; Spinella *et al*., 2009) and increased rate of infection during the post operation period (Cholette *et al*., 2015) Especially vulnerable groups include neonates and patients with transfusion dependent chronic conditions.

Red blood cell abnormalities associated with hemoglobin defects include thalassemia traits and sickle cell traits. Microcytic Thalassemic red cells may contaminate blood products such as platelet concentrates (Rosline *et al*., 2006). This may sometimes lead to blood group mismatch and transfusion reactions. The risks for blood donation in sickle cell trait individuals are more pronounced on the donor. There is increased risk of sudden death due to vascular occlusion, microembolism and kidney failure (Tsaras *et al*., 2009). These conditions are more pronounced in highly stressful environments such as during exercise or even upon blood donation (Tripette *et al*., 2013). The effects of enzyme deficient red cell cells has been documented in recent studies. A review by Renzaho *et al* (2014) concluded that there was a decrease in effective therapy rate and therefore longer hospital stay when G6PDd blood was transfused to neonates (Renzaho *et al*., 2014).

Apart from intrinsic defects, storage changes have been identified as an extrinsic cause of red blood cell haemolysis leading to adverse effects in transfused patients (Hod *et al*., 2011; Karon, van Buskirk, Jaben, Hoyer, & Thomas, 2012). During transfusion the haemolysed blood may cause haemoglobinemia, and jaundice which may have deleterious effect especially in neonates.

2.2 Haemoglobin defects

2.2.1 Sickle Cell and Thalassemia traits

Haemoglobin defects resulting in trait or carrier conditions are a common phenomenon in the malaria endemic zones of Africa, Asia, parts of the Mediterranean and among the African Americans (Makani, Williams, & Marsh, 2007; Frédéric B Piel *et al*., 2010; Weatherall, Williams, Allen, & O'Donnell, 2010). The Haemoglobin concentrations and red cell life span of these individuals is within the normal. Sickle cell trait is estimated to affects up to 10% of African Americans, up to 30% of West Africans and more than 10% of the Kenyan population (Hoffbrand *et al*., 2011; Frederic B Piel *et al*., 2013). A study in western Kenya revealed a prevalence of 17% for Sickle cell trait in children (Suchdev, Ruth, Earley, Macharia, & Williams, 2014) while another study in Nairobi recorded a 15%

prevalence of sickle cell traits (Etyang et al., 2018). Normally, the condition presents with mild clinical effects. However, severe symptoms may be seen high oxidative stress conditions that include, athletics, during pregnancy or in high altitude mountain climbing among others. Other asymptomatic sickle cell trait conditions include HbSE (Hoffbrand *et al*., 2011). The half cycle of transfused sickle cell trait cells in circulation has been estimated at (Belcher, Nath, & Vercellotti, 2013)

The occurrence of thalassemia traits in Kenya has been documented in studies carried out in the coastal and western regions of the country, a prevalence 35% of alpha thalassemia carriers was found among children in western Kenya (Suchdev *et al*., 2014). The survival of transfused red blood cells is greatly affected by abnormalities that may be present.

2.2.2 Hereditary Persistence of Foetal Haemoglobin

The maturity of red blood cells from embryonic to adult stages occurs with a change in the various haemoglobins occurring during the stages of development. Initially, embryonic haemoglobins such as Gower I and Gower II are seen in the primitive red cells occurring in the very early stages of foetal life. Later, haemoglobin F (HbF) which has a greater affinity to oxygen is produced (Kaushansky, 2016). This is due to the competition for oxygen with maternal cells. In normal circumstances this foetal haemoglobin is replaced by the adult haemoglobin (Hb A_1). This activity occurs during the later stages of gestation and early in neonatal life (Hoffbrand *et al*., 2011). However, instances exist where these foetal haemoglobin persists in circulation as a result of some defect in the formation of normal adult haemoglobin. In these cases, the persistence occurs when there are defects affecting the quantity of adult haemoglobin being produced (E Beutler *et al*., 2006; Mmbando *et al*., 2015).

2.2.3 Haemoglobin Kenya

A rare occurrence in the population having a mutation in the δγ-region of the beta chain. This phenomenon presents with a range of conditions including asymptomatic carrier

states with normal haemoglobin to severe cases of anaemia(Hoffbrand *et al*., 2011; Wilcox *et al*., 2009). Information on Hb-Kenya is scant and the effects of transfusion of patients with Hb Kenya has not been documented

2.3 Enzyme Defects

2.3.1 Pyruvate Kinase Deficiency

Red cell pyruvate kinase deficiency (PKD) is a condition of the red blood cell characterized by non-spherocytic, haemolytic anaemia without evidence of immune mediated lysis, or red cell membrane disorder, haemoglobin abnormality or even G-6-PD deficiency (Grace *et al*., 2015). In the absence of family history, the diagnosis of the disorder can be can be complicated by the existence of many variable phenotypes. In a study in Mozambique however it was shown that PKD indeed is present in sub-Saharan Africa with a frequency of up to 6.5% as heterozygous carriers (Machado *et al*., 2012). A correlation between the reduction in PK activity and malaria was also reported (Machado *et al*., 2012). Zweiten *et al* (2015) showed that there was a relationship between PKD and Hereditary Spherocytosis attributable to a decreased PK activity. The phenotypic expression of band 3 protein deficiency which results in spherocytosis has been linked to reduced PK activity (Zwieten *et al*., 2015). The increased fragility of PKD deficient cells will lead to ineffective transfusion as has been shown in studies of red cell conditions exhibiting spherocytosis (Suchdev *et al*., 2014).

2.3.2 Glucose-6-Phosphate dehydrogenase deficiency

This is the most common genetically determined red cell enzyme deficiency in the world (Hoffbrand *et al*., 2011). It is a clinically silent red cell anomaly affecting people of all groups. Many variants of G6PDd have been isolated and it is estimated that 400 million people are affected worldwide (Hoffbrand *et al*., 2011). A study in Yemen found that among healthy male donors, 7.2% had G6PDd. This shows the potential danger of transfusion therapy and especially in patients who have oxidative stress (Al-nood, Bazara,

& Al-absi, 2012). Another study of the Italian population suggested that there was a 1.1% prevalence of G6PDd in the population (Maffi *et al*., 2014). Several studies that have shown the importance of screening for G6PDd especially in neonatal transfusion (2014). In Eritrea, a recent study found that G6PDd variants B and A were common in the population (Tseghereda, Murega, Kimang'a, Hagos, & Yishak, 2018). Although majorly silent, the adverse clinical symptoms may be triggered by a range of foods and drugs (Howes *et al*., 2012). Enzyme deficiencies have been implicated in hemolytic symptoms after transfusion and are viewed as potentially fatal to patients transfused with such blood (Grace *et al*., 2015; Machado *et al*., 2012).In Kenya the estimated prevalence for G6PDd is in the range of 10% to 13% (Howes *et al*., 2012)

2.4 Membrane abnormalities

2.4.1 Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Red cell membrane disorders occur as a result of defects in the membrane proteins on the RBC plasma membrane. These defects occur in the vertical proteins, integral proteins and the band proteins of the red blood cell as well as the glycophorins (Barcellini *et al*., 2011). Hereditary elliptocytosis (HE) and pyropoikilocytosis (HPP) occur as a result of mutations in Glycophorin C, α-spectrin and protein 4.1. It has been suggested that the presence of these mutations may cause a reduction in *Plasmodium falciparum* clinical manifestation (Barcellini *et al*., 2011; Jeremiah, Jeremiah, & Emelike, 2010). Patients are normally asymptomatic in the two conditions. A prevalence rate of 2% has been recorded in highly endemic malarial regions of West Africa (Barcellini *et al*., 2011).

2.4.2 Hereditary Spherocytosis

Hereditary spherocytosis (HS) is a common condition that affects the general population. Often there are asymptomatic cases that may not be prominent in routine haematological analysis. About 1 in 650 patients with hyperchromasia may have HS (Almizraq, Tchir, Holovati, & Jason, 2013). This condition is typically as a result of membrane disorders of

genetic origin (Da Costa *et al*., 2013). Historically, the condition progresses with age due to the increased destruction of the older red cell subpopulations by the spleen (Hassoun & Palek, 1996).

2.4.3 Paroxysmal Nocturnal Haemoglobinuria (PNH)

This is a condition that affects worldwide populations with an estimated incidence of up to 1.3/1000000 (Risitano, 2012). This is a clonal haematopoietic disorder characterized by three major clinical manifestations. These include haemolytic anaemia, bone marrow failure and formation of thrombus (Brodsky, 2014; Risitano, 2012). The condition results from a mutation of the phosphatidylinositol glycan-A gene and therefore leads to a deficiency in the glycophosphatidylinositol (GPI) anchor protein. The effect of this is an interference with the activity of complement regulation factors CD55 and CD59 making cells more prone to complement lytic activity (Hoffbrand *et al*., 2011). PNH conditions may be categorized into three main subgroups. These include classic PNH characterized by haemolysis without involvement of other bone marrow disorder, i.e. haemolysis without cytopenia, PNH with underlying bone marrow disorder such as aplastic anaemia or myelodysplastic syndrome and subclinical PNH characterized by presence of PNH cells without any clinical or laboratory signs. However it is recognized that this classification may not fit all known subtypes of PNH (Risitano, 2012). PNH cells are prone to lysis when the complement activation pathways are enhanced. This characteristic is utilized in Ham's test to diagnose PNH (Risitano, 2012).

Blood banks operate on the well-known attribute of storage before dispatch (Greer *et al*., 2010; Janatpour & Holland, 2007). This gives room for laboratory testing to rule out TTIs and blood group serology (Kubio *et al*., 2012; Schubert & Devine, 2010; Wilkinson *et al*., 2012). However despite the good intentions of the practice, it is emerging that blood and blood products may be associated with haemolysis and red cell membrane deformations that may affect the clinical efficacy of transfusion therapy (Antonelou *et al*., 2012; Gevi, D'Alessandro, Rinalducci, & Zolla, 2012; Karon *et al*., 2012; Koch *et al*., 2008). The

knowledge of the occurrence of these abnormalities is an essential tool towards mitigating any effects that may be associated with these anomalies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was based at the Regional Blood Transfusion Centre – Mombasa, Kenya. The centre is located along the main Mombasa Malindi highway within the precincts of the Coast Provincial general hospital. Laboratory analysis was done at the Technical university of Mombasa, Department of Medical Sciences Main Laboratory.

The Mombasa RBTC is the main centre offering blood bank services to the coastal region. The blood bank's annual collection is estimated at 12000units (KNBTS & ICF Macro, 2010). .

3.2 Study design

This was a cross-sectional observational study conducted during the months of July to November 2017. Blood donor samples were analyzed for the presence or absence of selected abnormalities.

3.3 Study Population

Samples were obtained from donated blood obtained during blood campaigns by the RBTC in the months of July to November 2017.

3.3.1 Inclusion Criteria

Donor units that were collected by the Mombasa RBTC during the months of July to November 2017.

3.3.2 Exclusion Criteria

1. Donor units that had less than 420 millilitres of blood
2. Blood units showing signs of deterioration including haemolysis, cloudiness or clots

3.4 Sample Size Determination

Sample size was determined using a prevalence rate of 50.4% based on values obtained in a research on pattern of deferrals in south of Nigeria (Ekwere, Ino-Ekanem, Motilewa, & Ibanga, 2014). The dichotomous variables (Hulley, Cummings, Browner, Grady, & Newman, 2007) method as shown below.

$$N = 4Z_{\propto}^{2}P(1-P) \div W^{2}$$

For 99% confidence interval (Z_{α}=2.58) P = 50.4%, W = 0.1

$$N = 4 \times (2.58)^{2} \times 0.504(1-0.496) \div 0.1^{2}$$

$$N = 676.33$$

$$N = 676$$

Where:

Z_{α} = the standard normal deviate for a two sided α

P = the expected proportion who have Red Cell abnormalities

W = the total desired width of the confidence interval

3.5 Sampling Method

Convenient consecutive sampling was used to achieve the desired sample size

3.5.1 Sample collection

Samples were collected on various days during the blood donor campaigns in Mombasa County (**Figure 0-1**).

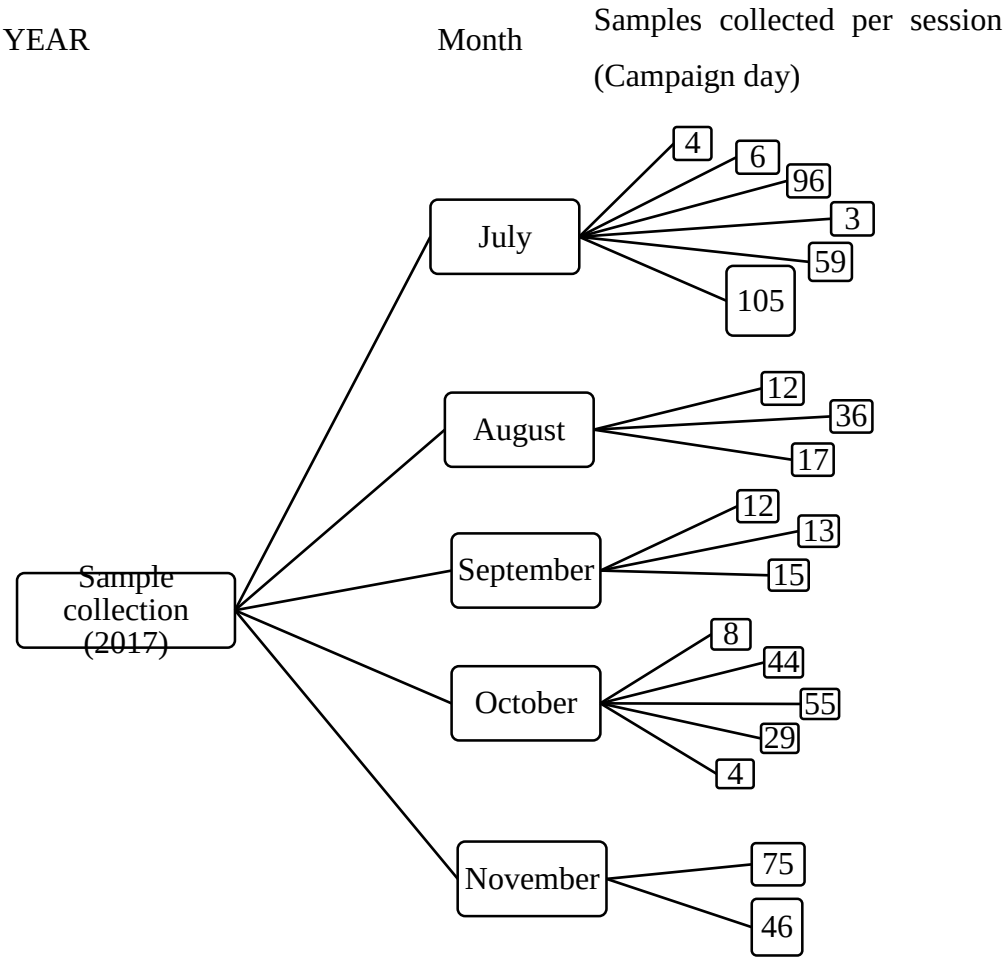


Figure 0-1: Sample collection schedule

3.6 Laboratory Procedures

Four millilitres of blood was collected in EDTA vacutainers directly from the blood bag tubing. The blood was then analyzed for the set parameters. Blood cell count and osmotic fragility testing were done within 6-8hours of collection. Samples were refrigerated at $4^0C \pm 2^0C$ to maintain red cell viability. All other assays were done within 24hours of collection.

3.6.1 Total Red Cell counts

Red cell Count parameters including Total red cell count, haemoglobin concentration, Mean cell volume and packed cell volume were analyzed using the Medonic M 20M (Boule Medical AB, Sweden) haematology analyzer.(Appendix 3).

3.6.2 Red cell lysis

Red cell lysis was determined by the use of increasingly hypotonic saline solution method (Appendix 4). The haemolysis observed was detected using the Genesys™ 10S Vis UV spectrophotometer at 540nm. The values obtained were expressed as a percentage of the 100% lysis from a reference tube containing deionized water (Bain, Bates, Laffan, & Lewis, 2017; Parpart, Lorenz, Parpart, Gregg, & Chase, 1947).

3.6.3Glucose-6-Phosphate dehydrogenase deficiency

Sodium nitrite was used to convert hemoglobin to hemiglobin which was then converted to methaemoglobin by addition of methylene blue. Samples were incubated with methylene blue for 90 minutes to stimulate the pentose pathway. Samples that were G6PD deficient were not able to reduce methaemoglobin, were therefore lysed and retained the colour of the reagent (appendix 5).

3.6.4 Haemoglobin stability test

The presence of unstable haemoglobin was assayed using the Isopropanol solubility test. Freshly prepared haemolysates (appendix 6) were incubated with isopropanol in tris-isopropanol buffer Flocculation indicated the presence of unstable haemoglobins

3.7 Quality Control

3.7.1 Pre-analytical Quality Control

Proper sample labelling was ensured during collection. Sufficient anticoagulation of all samples was ensured by adequate mixing of blood samples. The use of blood bank collection standard operating procedures ensured uniformity in blood collection. All blood samples were stored at 4^0C $\pm 2^0$C in the blood bank refrigerators to maintain red cell viability. Biohazard precautions were observed during the study to minimize as far as possible risks of infection by using protective wear.

3.7.2 Analytical Quality Control

All procedure plans, SOPs and manufacturer instructions for the Medonic M 20M GP and Genesys equipment were followed. Quality control specimen for TRBCs, PCV and Hb concentration were obtained from the RBTC laboratories these were incorporated in all batch sample runs. Normal and abnormal tubes were set for each sample in the methaemoglobin reduction test. Osmotic fragility and isopropanol lysis tests were done in duplicate to minimize errors.

3.7.3 Post Analytical Quality Control

Recording of results was done in a clear and concise manner giving all relevant details. The reports were verified and duly signed. Interpretation of results was correlated with normal range values as set by the International Council for Standardization in

Haematology (ICSH). Two technicians counterchecked entries carefully to minimize transcription errors.

3.8 Data Management

3.8.1 Data collection

Data was recorded in excel spread sheets for management and manipulation. This data was then transferred into excel spreadsheets. Data cleaning was done using Microsoft office excel software.

3.8.2 Data analysis

Data was analysed using the SPSS and Microsoft excel. Descriptive statistics was done to determine means and standard deviations in the population. A multivariate approach was used to determine the statistical significance of the abnormalities found. Chi square distribution was used to determine the significance of associations between various observations. ANOVA distribution was used to predict within-group statistical significance of continuous variables. p- Values of less than 0.05 were considered significant.

3.8.3 Data presentation

Data was presented in tables, graphs and charts to illustrate the results obtained during the study.

3.9 Ethical Review

Consent was inferred after the donors signed the donor health assessment questionnaire. All information obtained was treated with confidentiality in conformity to the Kenya National Blood Transfusion Service (KNBTS) guidelines on donor records. Prior permission to use the samples was obtained from the director of KNBTS. The study was

approved under reference number ERC/MSc/009/2017 by the Pwani University Ethical Review Board (PU-ERB).

CHAPTER FOUR

RESULTS

4.1 Donor characteristics

A total of 676 donor units were assayed during the study period. Out of these, 660 (98%) were obtained from male donors while 16 (2%) were from female donors as shown in **Table 0-1** below.

Table 0-1: Showing the percentage of males and females in the study.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	16	2.4	2.4	2.4
	M	660	97.6	97.6	100.0
	Total	676	100.0	100.0	

4.2 Overall abnormality

The study found that 31.06% of the donor units had at least one abnormal parameter while 58.73% had none of the abnormalities according to the parameters assayed. Donor cells that had reduced G6PD activity but all other parameters within normal were 10.21%. (Figure 0-1). Internal quality control was done to check for accuracy of results (Appendix 4 & 5).

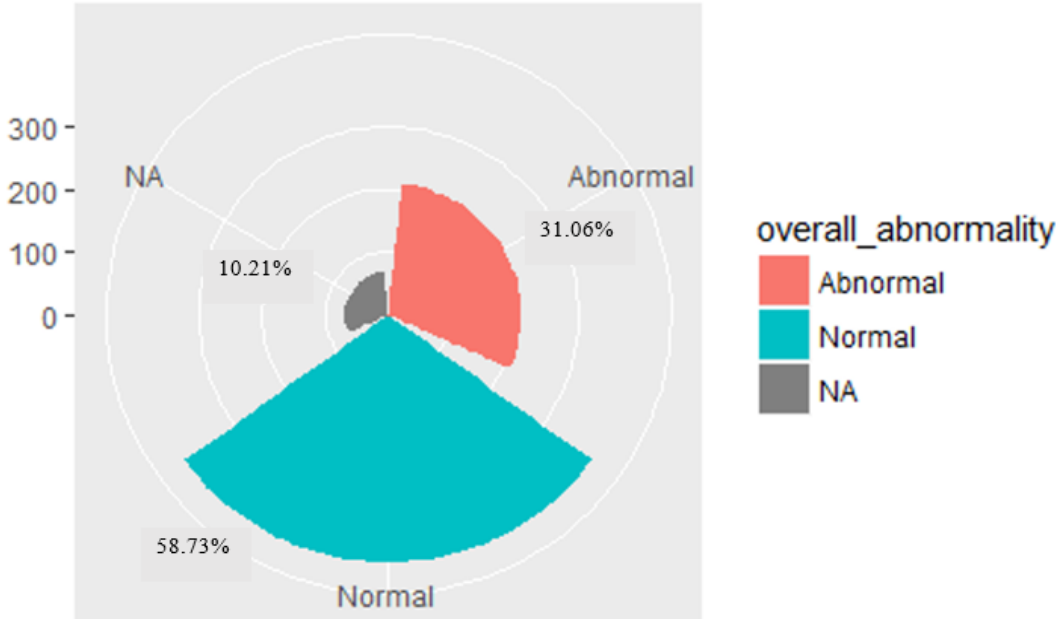


Figure 0-1: Percentage abnormality observed in the donor red blood cells assayed

4.3 The proportion of donors having specific abnormalities independently

4.3.1 Packed Cell Volume

Packed cell volume for the Red blood cells assayed ranged from 34% to 50%. 3% of the donor units (Figure 0-2) were found to have haematocrit levels below the normal reference range (appendix 2).

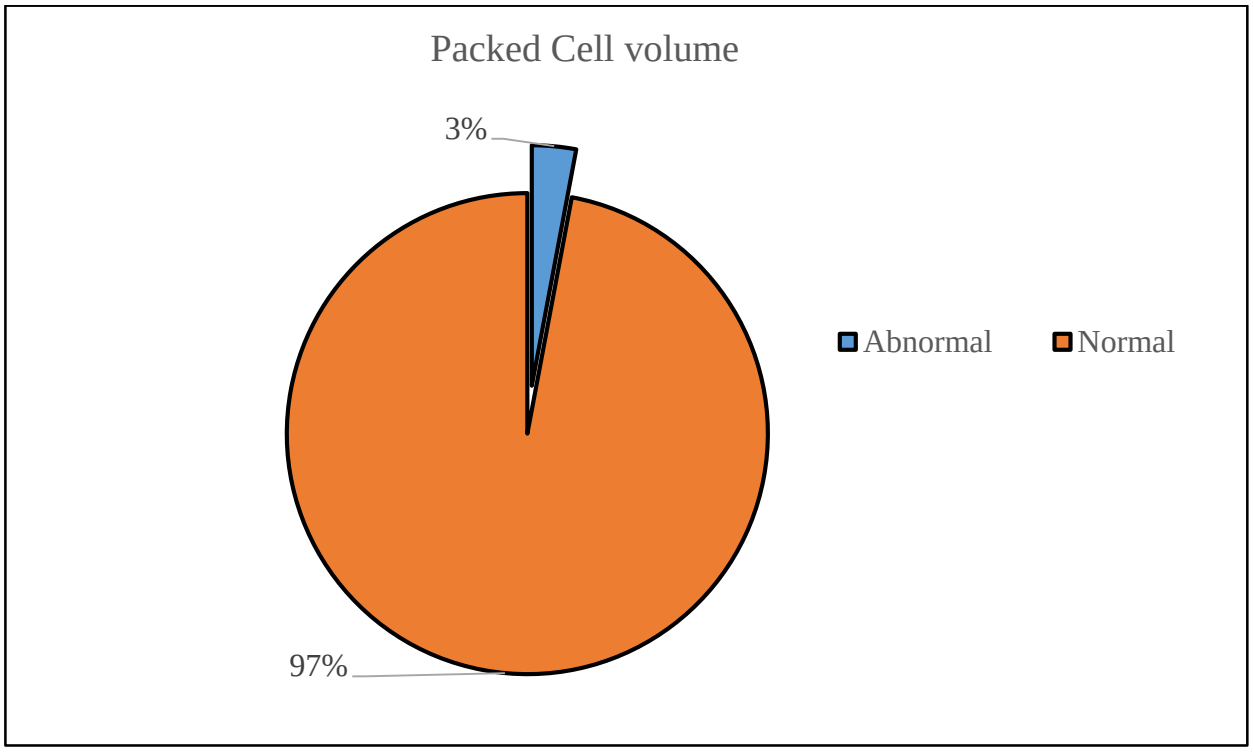


Figure 0-2: Proportion of donor red cells with normal and abnormal haematocrit values

4.3.2 Haemoglobin concentration

There was a statistically significant abnormal proportion of donors having decreased haemoglobin concentrations (Figure 0-3). Ten percent (10%) of the donors had abnormal haemoglobin concentration at 0.07 (CI 95%, t > 0.05). Hb levels greater than 18g/dL or less than 12g/dL were considered to be abnormal reference (appendix 2).

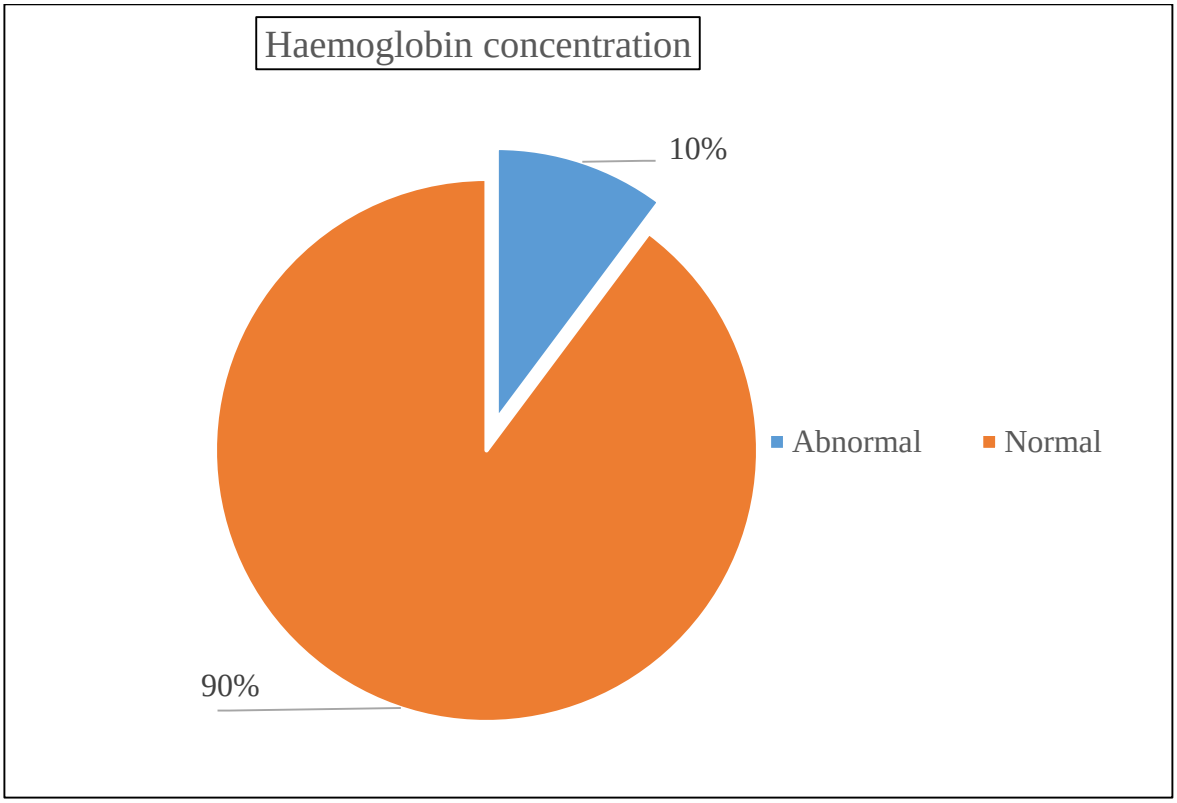
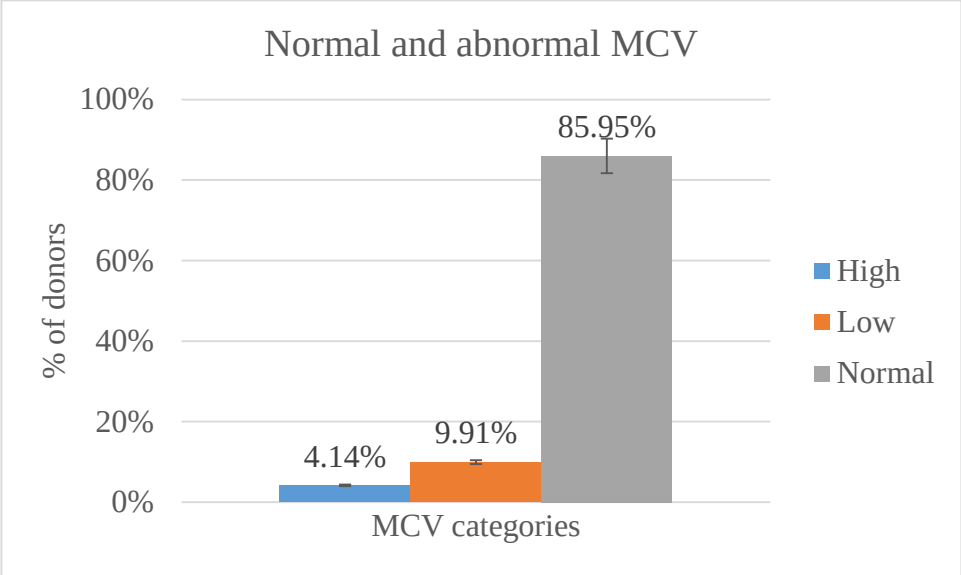


Figure 0-3: Proportion of donors with abnormal haemoglobin

4.3.3 Mean Cell Volume

MCV assayed and recorded as Normal (80fl – 100fl) Low MCV (<80fl) High MCV (>101fl) (Appendix 2). A high proportion of donor blood had low MCV (9.91%) as compared to those with high MCV (4.14%). A cumulative 14.05% (Figure 0-4) of the donor red cells had MCV values outside of the normal reference range (Appendix 2).

Figure 0-4: Donor red cell Mean cell volume.



4.3.4 Total Red Blood Cell Count

The normal TRBC count in the range of 3.8 – 6.4 x 10^{12} cells/l was considered to be within the normal reference range (Appendix 2). There was no donor blood having TRBC above 6.4cells x 10^{12} Cells/l. More than 4.4% of the donors had TRBC count below the normal range (Figure 0-5)

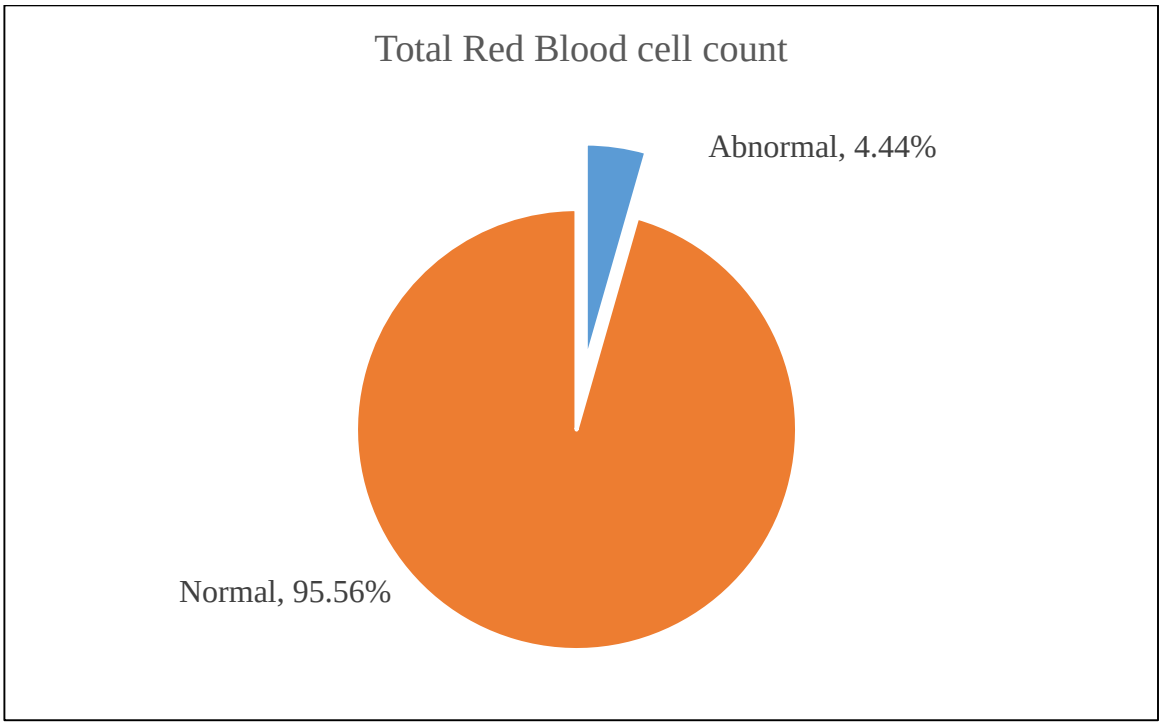


Figure 0-5: Proportion of donors with normal and abnormal TRBC counts

4.4 Red cell membrane defect assays

4.4.1 Glucose 6-phosphate Dehydrogenase Deficiency

The study found that. 9.61% of the donors had G6PD deficiency while 13.17% had reduced enzyme activity and are therefore likely to be heterozygous for the G6PD deficiency gene. Cumulatively 22.78% of the donor red cells did not have the colour of the normal reference tube (Appendix 6). This therefore showed that the G6PD activity was abnormal (Figure 0-6).

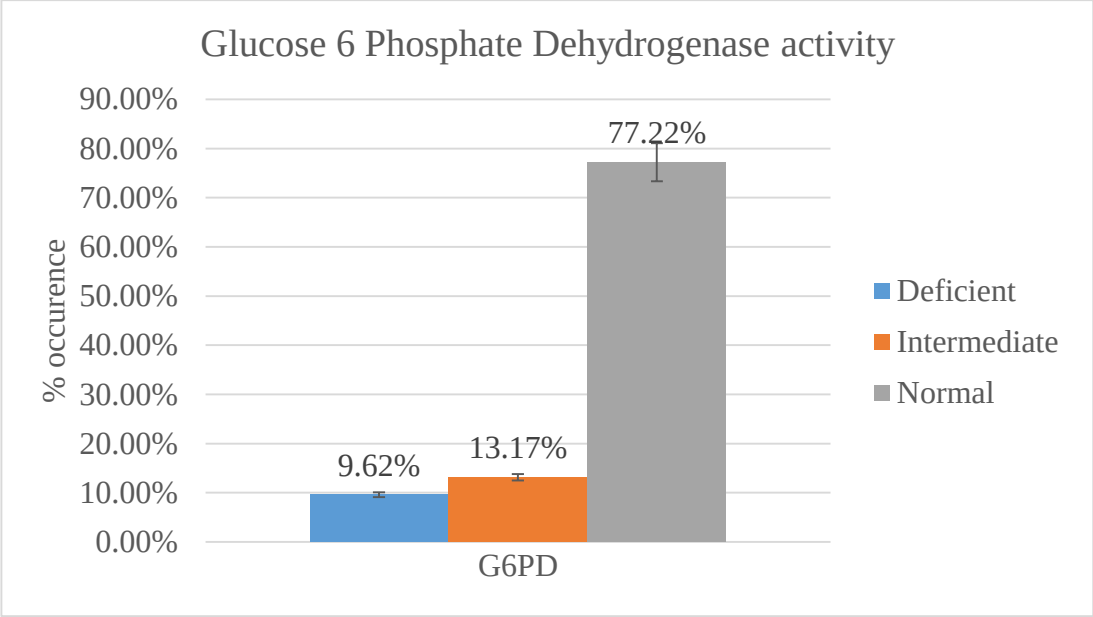


Figure 0-6: Proportion of donor blood with abnormal G6PD activity.

4.4.2 Osmotic Fragility

Donor red cells that exhibited an increased resistant to lysis were 7% while those that were more fragile than normal 3.7%. Of the red cells showing increased resistance, 0.3% were highly resistant (Figure 0-7).

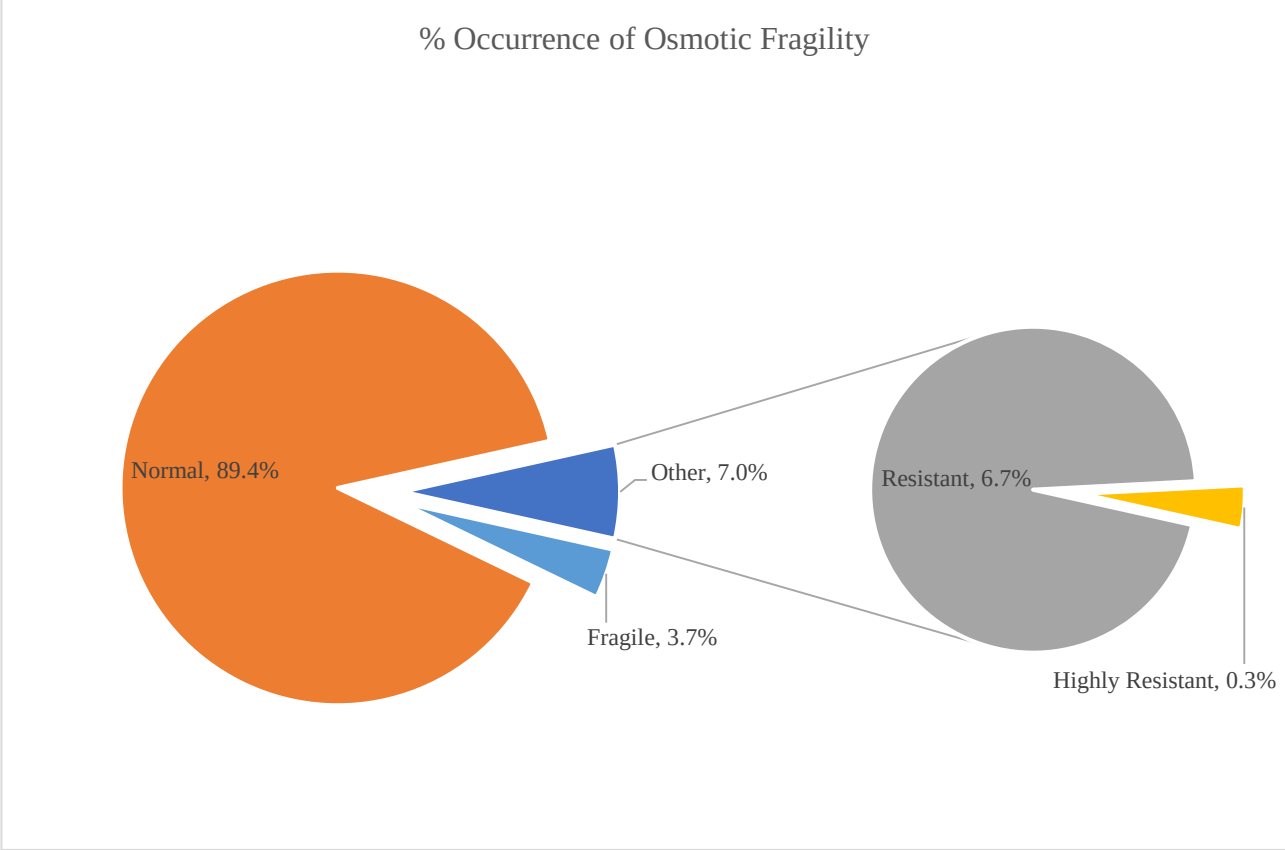


Figure 0-7: Osmotic fragility of donated red cells

4.5 Haemoglobin defect assays

4.5.1 Haemoglobin stability

There were no unstable haemoglobins observed amongst the donor units assayed. No flocculation was observed earlier than 30 minutes in any of the units. Twenty percent of the donor cell Haemoglobin showed flocculation at 30minutes while the rest of the Hb samples flocculated at between 40 and 70minutes.

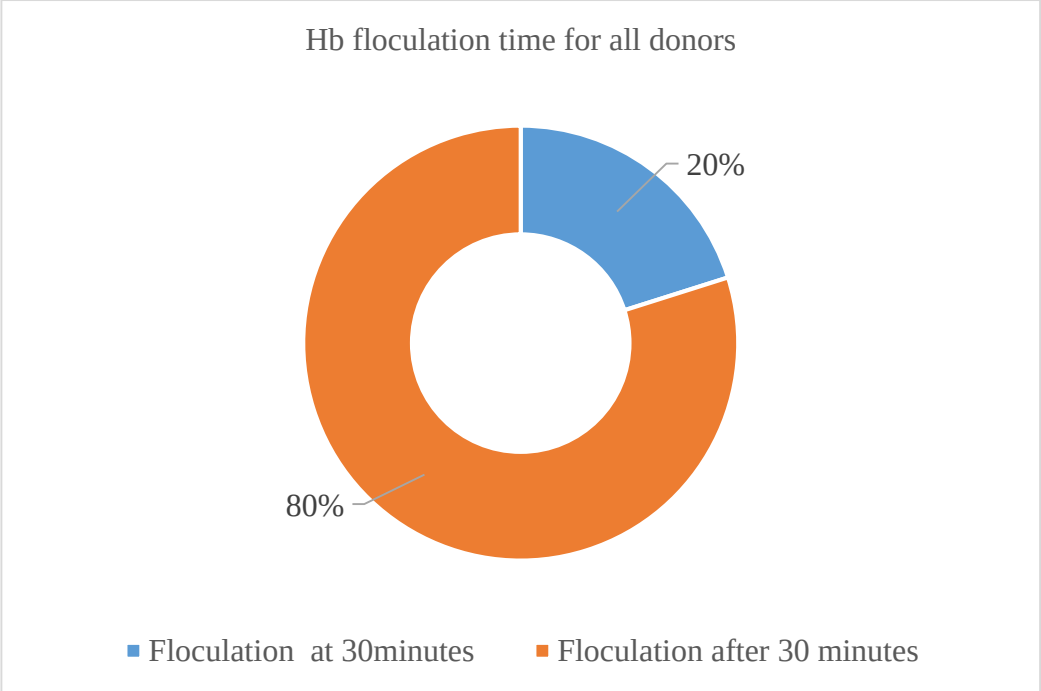


Figure 0-8: Hb stability test by isopropanol lysis for donor cells at the RBTC Mombasa

4.6 Statistical significance of red cell values

4.6.1 Descriptive statistics for donor red cell values

There was a statistically significant variation in Total red blood cell count (CI 99%, p – 0.01). (Table 0-2). Other red cell values did not return statistically significant variations

Table 0-2: Statistical analysis of red cell values in donor blood.

	Hb g/dL	PCV %	TRBC $x10^6$/µlitre	MCV
Mean	13.3712	39.8797	4.6194	84.8302
Standard Error	0.0397	0.1374	0.0194	0.3268
Median	13.5000	39.4000	4.6100	83.8942
Mode	13.5000	38.8000	4.6100	80.2966
Standard Deviation	1.0317	3.5732	0.5042	8.4969
Sample Variance	1.0644	12.7680	0.2542	72.1973
Confidence Level (99.0%)	0.0779	0.2698	0.0381	0.6417

4.6.2 Mean Cell Volume in relation to Osmotic fragility

The Pearson's correlation coefficient was run by SPSS and returned a significant positive correlation between osmotic fragility and Mean cell volume (r=0.195, p>0.01).

Table 0-3: Statistical relationship between Osmotic fragility and Mean Cell volume

		Mean_cell_volume	Osmotic_fragility
Mean_cell_volume	Pearson Correlation	1	.195**
	Sig. (2-tailed)		.001
	Sum of Squares and Cross-products	49385.824	380.994
	Covariance	73.164	.564
	N	676	676
Osmotic_fragility	Pearson Correlation	.195**	1
	Sig. (2-tailed)	.001	
	Sum of Squares and Cross-products	380.994	77.148
	Covariance	.564	.114
	N	676	676

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

CHAPTER FIVE

DISCUSSION

5.1 Overall abnormality

The study assayed 676 samples from units of blood collected at the Regional Blood Transfusion centre. Of the total donors, 16 (2.4 %) units were from female donors while 660 (97.6%) were from male donors. This has been observed in Nigeria (Felix, Ogodo, & Ngozi, 2017) and other developing countries. This may be due to perceptions that only men are likely to donate and low Hb levels in women of child bearing age resulting from iron lack anaemia. The overall proportion of red cell abnormalities was 31.07% while 10.21% could not be categorized as abnormal as these only had reduced G6PD activity with all other parameters as normal. Studies have shown that blood parameters not meeting the criteria for donation find their way into the blood bank (Al-nood *et al*., 2012; Rosline *et al*., 2006; Tailor, Patel, Pandya, & Mangukiya, 2017). These studies range from whole blood parameters to those that affect only some aspects of the blood components.

5.2 Selected Red Cell and haemoglobin values

The current study was designed to selectively assay red blood cell indices that included haemoglobin estimation, Packed cell volume, total red cell counts, mean cell volume. When considering the haemoglobin levels, the national guidelines for Hb levels at donation is set at 12.5g/dL it was observed that 10.21% of the donors had haemoglobin levels lower than the normal. A similar study by Rajab *et al*.,(2005), they found that in Western Kenya, 16.5% of the donors had low Hb levels. As compared to 3.4% of donors in Nairobi region. The lower Hb concentration may be attributed to low altitude and high malaria endemicity in both Western Kenya (Kisumu) and Coastal Kenya (Mombasa) against the relatively malaria free Nairobi region.

Out of the total, 12.4% of the donors had abnormal Mean cell volume this is as compared to the WHO classification of anaemia by MCV which categorizes normocytic cells as

those with MCV within 80-100fL±2, microcytic cells as those having MCV lower than 80fL±2 and macrocytic cells as those with MCV greater than 100fL±2. In the study, it was observed that microcytosis occurred in 9.91% (>80fL±2) of the donors while 4% were macrocytic (<102fL). These findings correspond to findings by Rajab *et al.*, for microcytosis in Kisumu (12.4%) and macrocytosis in Nairobi (4.1%). The variation in total red blood cell count was statistically significant with a p-Value of 0.038 (CI 95%, p -0.05) where about 4.4% of the donors had counts lower than the normal reference range. These variations may have occurred as a result of inadequate screening of blood donors. Other red blood cell parameters assayed did not have clinically significant variations at $p<0.05$.

5.3 Osmotic fragility of donated red blood cells

Osmotic fragility test is valuable in assessing the red cell response to lysis and osmotic stress. The study found that 10.6% of the donors had abnormal osmotic fragility patterns. Seven percent (7%) of these exhibited high resistance to lysis while 3.6% were abnormally fragile. In a Norwegian study, about 0.9% of blood donor red cells were found to be osmotically more fragile than normal while in Germany, a prevalence of 1.1% for osmotically fragile cells was observed (Godal & Heistø, 1981) these studies show that abnormal cell variations occur in much lower frequencies in the two countries than in Kenya. Abnormal lysis patterns of RBCs occurs due to microcytosis and macrocytosis. Owing to the variations in both MCV and G6PD activity osmotically resistant cells will tend towards microcytosis while osmotically fragile cells are likely to be macrocytes. These macrocytes may have been as a result of G6PD deficiency states that lead to spherocytosis and increased fragility. Osmotically fragile cells have been shown to alter transfusion outcomes as a result of the donor variation effect (Tzounakas, Kriebardis, Papassideri, & Antonelou, 2016). This effect is related to the increased formation of storage lesions and RMPs.

5.4 Haemoglobin lysis patterns of donated blood

Unstable haemoglobin occurs in individuals having HbS, HbH and other abnormal molecules. These haemoglobins are inherently prone to abnormal lysis. This lysis may be demonstrated as a precipitate when these unstable proteins are mixed with isopropanol. Lysis may occur within 5 minutes for homozygous individuals or 30 minutes in the hemizygous persons. The study found that 20% of donor cells lysed after 30minutes but before 40minutes showing heterozygosity for unstable haemoglobins. A study in western Kenya found that 17% and 38% of school going children were sickle cell trait and α-thalassemia minor traits respectively (Suchdev *et al*., 2014). Unstable haemoglobins are characterized by the presence of variable proportions of abnormal Hb proteins.

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5.5 G-6-PD activity in donated blood

The point prevalence of 22.8% for all forms of G6PDd found amongst the donors' assayed is higher than that of some parts of Mozambique with as high as 16.8% (Galatas *et al*., 2017) The study found that 13.17% of the donors had reduced G6PD activity and are most likely heterozygotes. This compares with the 16.4% proportion of heterozygotes found in the Tanzanian population (Mwakasungula *et al*., 2014) Studies worldwide have shown that the G-6-PD deficiency offers either partial or full resistance to P. falciparum infection and has therefore been found in polymorphic proportions in malaria endemic areas (Howes *et al*., 2012). These studies show that the condition is predominant in Asia and sub-Saharan Africa (Howes *et al*., 2013) A study in Yemen found that among healthy male donors, 7.2% had G6PDd in the capital city of Sanaa (Al-nood *et al*., 2012). In Kenya the estimated prevalence for G6PDd is in the range of 10% to 13% (Howes *et al*., 2012). The current study compares favourably with the results from Nigeria by Akanni *et al*., who found a prevalence of 19.5% in blood donors in Osogbo, Nigeria (2010) and Nguetse *et al*., who reported that 73% of the study subjects in selected African countries had normal G6PD activity (2016). However these findings do not correspond with estimates by

Howes *et al.,* which indicate that prevalence of G6PDd in Kenya is about 13% (2012). The association of G6PDd with health conditions is a major area of study today. Akanni *et al*., have associated neonatal jaundice to the deficiency showing 47% of the jaundiced neonates in the study were G6PD deficient (2010). It has also been found that altered G6PD activity may play a critical role in severe pulmonary hypertension (Chettimada *et al*., 2014). Results from the current study show that that the presence of G6PD deficiency is widespread in the general population.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Red cell abnormalities were found to be present in the donated red blood cells. In this study, 31.05% of the donor cells exhibited one or more of the assayed abnormalities. There was a statistically significant variation in the Total red blood cell counts and osmotic fragility. The study found that there were donors having osmotically fragile and resistant red cells which points towards red cell membrane abnormalities. A marginal association between osmotic fragility and mean cell haemoglobin. These findings help to point out that there may be gaps in the screening and testing methods at the blood transfusion centre.

Abnormal haemoglobins were not found to be present in donor blood. However there was likelihood of having traits in the donor population. Glucose 6 phosphate dehydrogenase deficiency is present among donors at the regional blood transfusion centre, Mombasa. The screening of G6PDd in donated blood using the methaemoglobin reduction method was able to yield results that point to the existence of homozygous and heterozygous G6PDd states in the population.

The relationship between the parameters tested yielded marginal positive correlations between haemoglobin concentration and G6PD deficiency categories donors. This study was not able to conclusively determine the relationship between G6PDd and haemoglobin concentration.

6.2 Recommendations

The study recommends that

1. The blood donation centres should strengthen capacity by assaying for red blood cell parameters and using more sensitive methods in determining donor blood Hb content.
2. The detection of G6PDd in donor blood should be incorporated as a screening method at the blood bank and blood labelled as either G6PDd deficient or non-deficient.
3. The hospital blood banks should determine the osmotic fragility of donor blood before transfusion. This may be limited to blood given to patients with chronic blood needs i.e. transfusion dependent patients such as sicklers.
4. Further study should be done to assess the correlation between these abnormalities and their impact on the patient upon transfusion.

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APPENDICES

Appendix I Information Sheet

S/N	Donor No.	Gender	ABO blood group	RhD type
1				
2				
3				
4				
5				
6				

Results entry Sheet

RBC indices and Osmotic Fragility

S/N	Donor No.	Hb g/dL	PCV	MCV	% fragility
1					
2					
3					
4					
5					
6					

Red Cell defects

S/N	Donor No.	G6PDd	Hb g/dL	PCV	MCV	% fragility
1						
2						
3						
4						
5						
6						

Appendix II: Complete Blood Count

Medonic M-20 GP haematology analyzer

The Medonic M 20M GP (boule Medical AB, Sweden) incorporates proven electronic impedance with high-resolution flow cytometry. An online diagnostic program continuously monitors vacuum, pressure and voltages, helping to prevent system failures by automatically notifying the operator when corrective action is required. Specimens with immature granulocytes, variant lymphocytes, blasts and other immature or abnormal cells are flagged for review.

Procedure

1. Whole blood from the EDTA tube will be introduced into the analyzer sample probe.
2. The analyzer will then aspirate 250µl of sample
3. The analyzer will then perform a three part differential
4. A 20 parameter output will be available for printing
5. This will be printed and filed
6. Control specimen will be run in parallel with the sample tests
7. The analytical quality control incorporates levy-Jennings plot and sample mean bar plots which will be used to plot and monitor MCV, MCH and MCHC

Interpretation

The results will be interpreted as either normal or abnormal according to the ICSH guidelines and in line with the manufacturers normal range values critical value reporting though unexpected will be done according to the ICSH guidelines (Keng *et al*., 2016).

Appendix III: Red cell normal values for adults expressed as a mean ±2SD (95% range)

Normal reference values for red cell parameters in the general population (Bain *et al*., 2017)

Red blood cell count	
Men	$5.0 \pm 0.5 \times 10^{12}$/l
Women	$4.3 \pm 0.5 \times 10^{12}$/l
Haemoglobin	
Men	15.0 ± 2.0 g/dL
Women	13.5 ± 1.5 g/dL
Packed cell volume (PCV) or Haematocrit (Hct)	
Men	0.45 ± 0.05 (l/l)
Women	0.41 ± 0.05 (l/l)
Mean cell volume (MCV)	
Men and women	92 ± 9 fl
Red cell distribution width (RDW)	
As coefficient of variation (CV)	12.8 ±1.2%
As standard deviation (SD)	42.5 ± 3.5 fl
Red cell diameter (mean values)	
Dry films	6.7–7.7 mm
Red cell density	1092–1100 g/l
Reticulocyte count	50–100 × 10^9/l (0.5–2.5%)

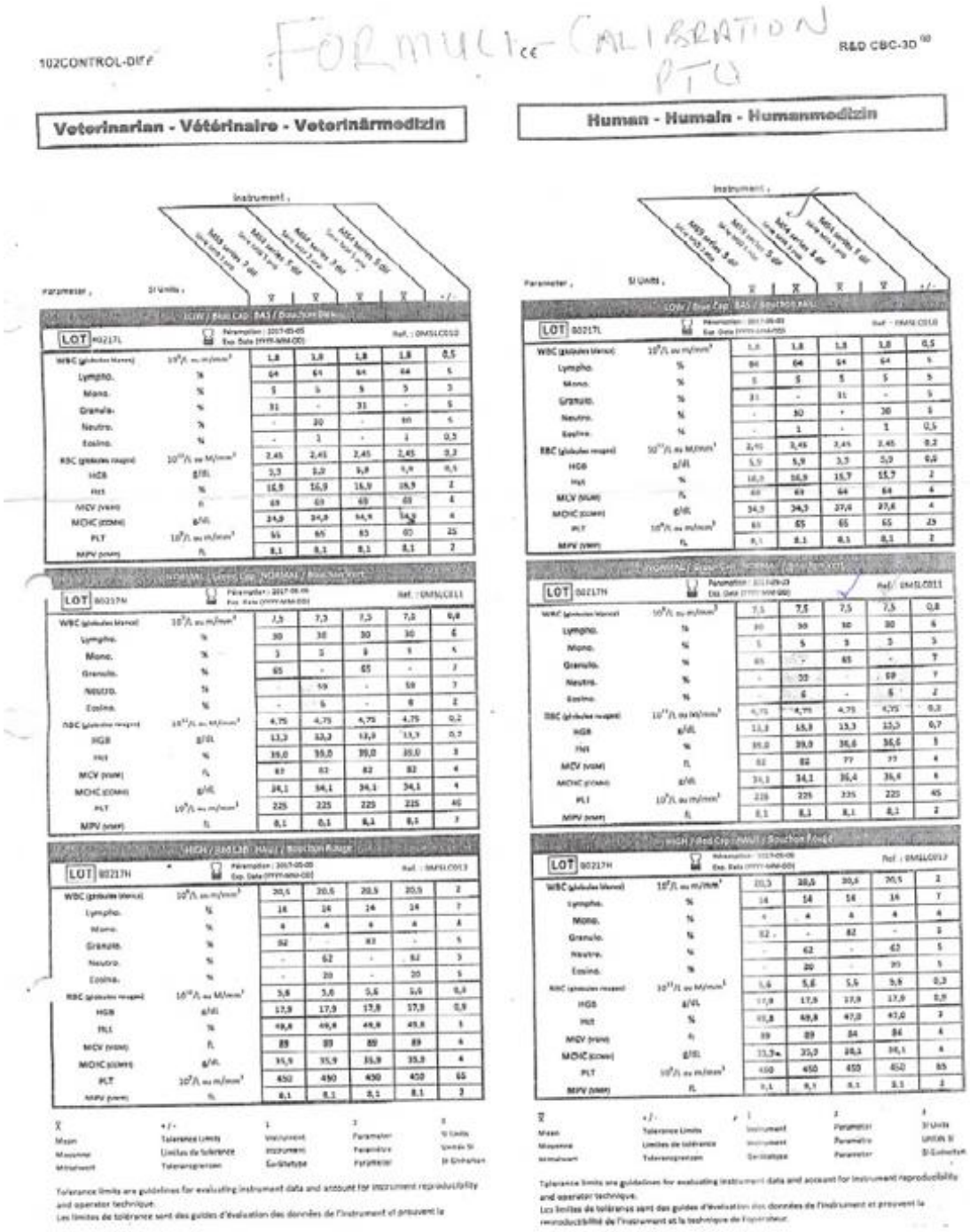
N/B for 99% of normal values 3 SD is used. This factors for physiological changes that may be present during testing. The current study used the 3SD points to determine normal and abnormal values.

Appendix IV: Internal quality control report Medonic M20

Internal quality control parameters done during the study

RBC parameter	July 2017	December 2017
RBC	$4.72*10^{12}$cells/ltr	$4.78*10^{12}$cells/ltr
MCV	76.5fl	76.9fl
MCH	27.9pg	28.2pg
MCHC	36.5g/dl	35.9g/dl
Hct	36.1%	35.8%
Hb	13.2g/dl	13.8g/dl

Appendix V: Interpretation of IQC (Medonic M20) calibration readings



Appendix VI: Red Cell Lysis (Osmotic fragility test)

Principle

This test is based on the increasingly hypotonic saline method (Bain *et al*., 2017; Parpart *et al*., 1947) when small amounts of blood are mixed with increasingly hypotonic solutions the red cells are progressively lysed depending on their ability to resist lysis. The fraction of cells lysed is determined colorimetrically at room temperature.

Reagents

Buffered Sodium chloride solution

Stock solution

1. NaCl 90g
2. NA_2HPO_4 13.65g
3. $NaH_2PO_4.2H_2O$ 2.34g
4. Adjusted to 1000mls with distilled H_2O

Stock solution will be stored at 4^0C

Working solutions (Hypotonic)

1. Five mls of stock solution will be diluted with water to make 50mls of working solution (10g/ml)
2. Hypotonic solutions of 9.0, 7.5, 7.0. 6.5, 6.0, 5.75, 5.5, 5.25, 5.0, 4.75, 4.5, 4.25, 4.0, 3.5, 3.0, 2.0, and 1.0 will be made.

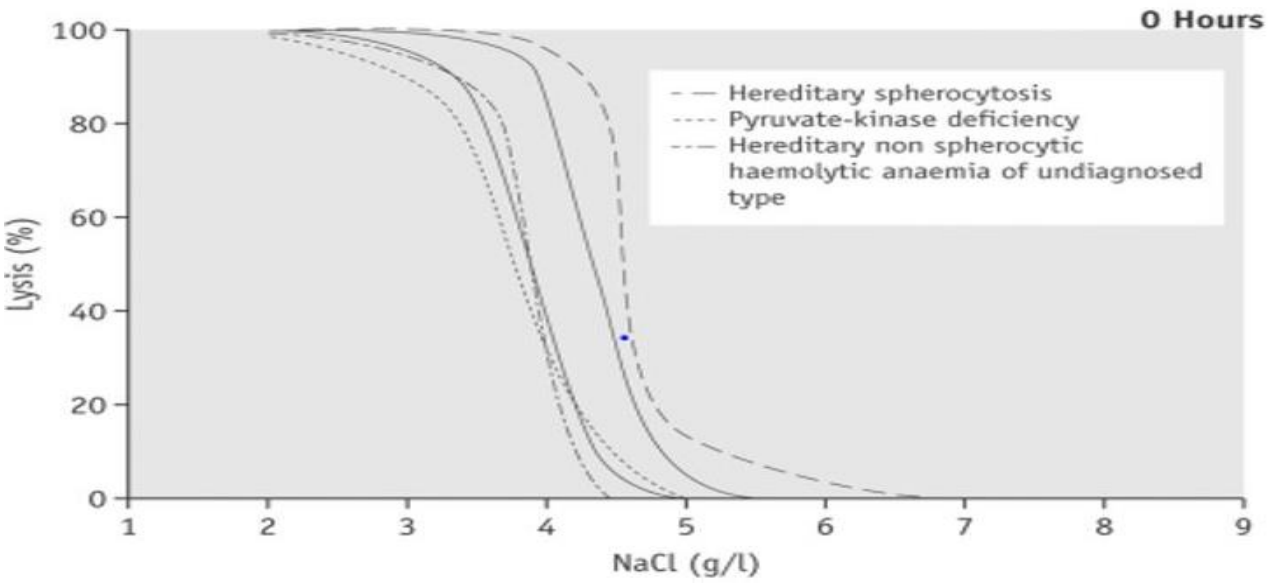
Technique

1. Normal saline (NS) concentrations will be prepared using Normal saline and distilled water

2. 18 tubes of concentrations between 0.90% normal saline to 0.00% with graduations of 0.05% Normal saline will be prepared
3. 50microlitres of blood will be delivered into 5mililitres of increasingly hypotonic solutions in 12x75mm tubes.
4. The suspensions of blood and saline will be mixed and left at room temperature for 30minutes.
5. The solutions will then be mixed again and centrifuged at 1000 revolutions per minute for 5 minutes.
6. The supernatant will then be separated and any haemolysis present analysed by the Genesys™ 10S Vis UV spectrophotometer. at 540nm
7. The values will then be recorded
8. The values read will be expressed as a percentage of the 100% lysis from tube 1 which will contain water.
9. Control samples will be run in parallel with each batch of prepared sample

Interpretation

Osmotic fragility test is based on the ability of cells to resist lysis. Older cells will be more fragile while younger cells will be more resistant. A sigmoid curve depicts normal blood cells while a shift to the right indicates increased resistance. A shift to the left indicates decreased resistance. In red cell membrane abnormalities, there will be increased lysis against the normal blood osmotic fragility.


0 Hours
100
80
60
40
20
0
Lysis (%)
1
2
3
4
5
6
7
8
9
NaCl (g/l)
Hereditary spherocytosis
Pyruvate-kinase deficiency
Hereditary non spherocytic haemolytic anaemia of undiagnosed type

Appendix VII: Methaemoglobin Reduction Test

Principle

When sodium nitrite is added to blood, it will convert the Hb to Haemiglobin (Hi). The addition of methylene blue allows for stimulation of the pentose phosphate pathway leading to reduction of the haemiglobin and thus normal lysis. In G-6-PD deficiency, the blockage of the pentose phosphate pathway prevents the reduction of haemiglobin.

Reagents

1. Sodium nitrite 180mmols/l
2. Dextrose 280mmols/l
3. Methylene blue 0.4mmols/l (dissolve 50mg of methylthionine chloride in 1l of H_2O

Working solution

1. Dextrose 5g and 1.25g of $NaNO_2$ will be dissolved in 100mls of H_2O
2. Equal volumes of the methylene reagent and dextrose/$NaNO_2$ will be mixed

Technique

1. EDTA Anticoagulated blood will be used
2. One mL of blood will be added to 0.1mL of combined reagent and mixed by inversion
3. Control tubes
    a. Normal reference, 1mL of blood without reagents
    b. Deficient reference 1mL of blood 0.5 mL of $NaNO_2$ reagent without methylene blue
4. All tubes are stoppered and mixed by inversion

5. Tubes will then be incubated at 37^0C for 90 minutes
6. After incubation, 0.1mL from each tube will be pipetted into separate tubes
7. 10mL of H_2O will be added and colour change compared between the three tubes

Interpretation

Normal blood will have the colour of the normal reference tube (clear red) G6PD deficient blood will have the colour of the deficient reference tube (brown) intermediate reactions will show the presence of heterozygote G6PD conditions

Appendix VIII: Haemoglobin Stability test

Isopropanol Test for Unstable Haemoglobins

Principle: The presence of isopropanol makes the buffer less polar, weakening the haemoglobin hydrophobic bindings that facilitate its denaturation and precipitation

Haemolysate preparation

1. Wash 1 ml of fresh blood in EDTA twice in 0.9% NaCl
2. Lyse the packed cells by the addition of one or two volumes of distilled water
3. Mix gently and wait few minutes
4. Prepare a fresh normal sample in the same conditions as a control.

Isopropanol reagent

a. Tris-HCl 0.1 M, pH 7.4:
    i. 1.21 g tris-hydroxymetyl-amino-methane
    ii. 100 ml distilled H_2O
    Bring to pH 7.4 with 4 M HCl
b. Tris-isopropanol buffer:
    i. Tris-HCl 0.1 M, pH 7.4 solution: 83 ml
    ii. Isopropanol: 17 ml
    Keep stoppered at room temperature

Procedure

1. Place 2mls of tris-iso-propanol buffer into a stoppered bijou bottle
2. Add 0.2 ml of freshly prepared haemolysate and mix by inversion
3. Place in a water bath at 37^0C
4. Check for any precipitation at 5, 20 and 30 minutes

Interpretation: Flocculent precipitate indicates the presence of an unstable haemoglobin. The control solution should remain clear.

Appendix IX: Ethical approval

NACOSTI ACCREDITED

ERC/MSc/009/2017

Pwani UNIVERSITY

ETHICS REVIEW COMMITTEE

ACCREDITTED BY THE NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION (NACOSTI, KENYA)

CERTIFICATE OF ETHICAL APPROVAL

THIS IS TO CERTIFY THAT THE PROPOSAL SUBMITTED BY:

RONALD N. WIGINA

REFERENCE NO:
ERC/MSc/009/2017

ENTITLED:
Selected red blood cell abnormalities in donated blood at the Regional Blood Transfusion Centre – Mombasa, Kenya

TO BE UNDERTAKEN AT:
MOMBASA COUNTY, KENYA

FOR THE PROPOSED PERIOD OF RESEARCH
HAS BEEN **APPROVED** BY THE ETHICS REVIEW COMMITTEE
AT ITS SITTING HELD AT PWANI UNIVERSITY, KENYA
ON THE **7TH JUNE 2017**

CHAIRMAN SECRETARY LAY MEMBER

PTO

Pwani UNIVERSITY
Ethics Review Committee,
Pwani University, www.pu.ac.ke, email: r.thomas@pwaniuniversity.ac.ke, tell: 0719 182218.
The ERC, Giving Integrity to Research for Sustainable Development

NOTICE:

This decision is subject to the information available at the time of APPROVAL. The Committee may on its own motion and/or by application by a Party, review its decision on the grounds of discovery of new and important information which was not reasonably within its knowledge at the time of decision or on account of mistake or error apparent on the face of the record, or for any other sufficient reason, provided the researcher shall be given prior opportunity to be heard.

Appendix X: Letter of no objection from the KNBTS

MINISTRY OF HEALTH

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When replying please quote:

NATIONAL BLOOD TRANSFUSION SERVICE - HQS
LOCATION: KENYATTA NATIONAL HOSPITAL NPHLS GROUNDS
P.O.BOX 29804-00202
NAIROBI

Ref No. MOH/KNBTS/EXT COMM.

12th June, 2017

The Secretary,
Ethical Review Committee,
Pwani University,
P. O. Box 195-80108,
MOMBASA

RE: STUDY ON SELECTED RED BLOOD CELL ABNORMALITIES IN DONATED BLOOD AT RBTC MOMBASA

This is to let you know that Kenya National Blood Transfusion Service (KNBTS) has no objection for Mr. Ronald Nyarembe Wagina, a master of medical laboratory science student at JKUAT to obtain samples of donated blood from RBTC Mombasa for the above study.

It is noted that the samples will be collected between June and August 2017.

By a copy of this letter, Mr. Hamisi Kithi, Head of RBTC Mombasa is notified to allow him access the remnants of blood grouping samples.

MOduor

Dr. Margaret Oduor

Head, KNBTS

Kenya National Blood Transfusion Service

Appendix XI: Publication

The Occurrence of Glucose 6 Phosphate Dehydrogenase Deficiency amongst Blood Donors at the Regional Blood Transfusion Centre-Mombasa, Kenya

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Abstract

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect that is estimated to affect 400 million people worldwide. This deficiency is associated with hemolytic disorders that may manifest depending on the molecular variant present, exposure to hemolytic triggers such as consumption of foods including fava beans and exposure to drugs including dapsone and primaquine. This disorder has been found to be more prevalent in malaria endemic zones of Asia, Africa and South America. This study determined the occurrence of G6PD deficiency among the donors at the Regional Blood Transfusion Centre-Mombasa, and whether any correlation existed between the occurrence of G6PD deficiency and either ABO blood type or haemoglobin concentration. Methaemoglobin reduction test was used to check for the presence of G6PDd among the blood donors, anti A and anti B sera were used to determine the blood types. Haemoglobin concentration was estimated using haematology analyzer. Multivariate analysis was done to establish the point prevalence of G6PDd in the donor population, the relationship between G6PD and ABO blood types and the correlation between G6PDd and haemoglobin concentration. Out of the 676 donors 9.6% were deficient of G6PD activity while 13.17% had red cells exhibiting partial activity. The point prevalence for all forms of G6PDd was found to be 22.79%. Blood group AB donors were least likely to exhibit G6PD deficiency compared to the rest of the ABO blood types. The association between G6PDd and haemoglobin concentration was inconclusive. The current findings indicate that G6PD deficiency exists among healthy donors without manifestation of clinical symptoms. G6PDd screening as part of donor blood testing regime would therefore allow for the discriminate use of G6PDd blood in transfusion dependent patients and neonates. It would also aid in establishing risk in the use of drugs associated with triggering clinical manifestations.

Key Words: *Transfusion, Glucose-6-Phosphate dehydrogenase, deficiency, blood donors.*

Introduction

Red blood cells are an important part of the body's metabolic processes. With a lifespan of 120 days, the non-nucleated red cells perform their metabolic functions and are also able to perform their specialized core function of oxygen transport and delivery to the body tissues [1]. The transfer of oxygen across cell membranes and its utilization are dependent on the cytochrome P24 group of molecules, the Embden Meyerhof glycolysis pathway and the hexose monophosphate shunt. The free oxygen radicals that are produced during these processes are harmful to cells and tissues [2]. To mitigate the effects of these free radicals, various enzymes and metabolic intermediates are involved in these glycolytic pathways. These include the 2-3 diphosphoglycerate, pyruvate kinase and glucose 6 phosphate dehydrogenase (G6PD) enzymes [3]. Glucose-6-phosphate dehydrogenase plays an important role in all cells particularly the red blood cells (RBC) [4]. It protects the cells from potential damage by reactive oxygen species (ROS) [5]. G6PD reduces nicotinamide adenosine dinucleotide phosphate (NADP) to nicotinamide adenoside dinucleotide phosphate hydrogenase (NADPH). The NADPH then reduces oxidised glutathione (GSSG) to reduced glutathione (GSH). The reduced glutathione is then able to bind to the ROS and protect the cells from oxidative stress [6]. The lack of mitochondria in the RBCs make the action of G6PD vital in the protection against oxidants [5]. A Glucose 6 Phosphate Dehydrogenase deficiency (G6PDd) would therefore be potentially hazardous to Red Blood Cells (RBCs) in particular due to their role in oxygen