Sudan University of Sciences and Technology

College of Graduate Studies

Hematological Changes in Stored Whole Blood in Central Blood Bank – Khartoum

التغيرات الدموية في الدم الكامل المحفوظ في بنك الدم المركزي الخرطوم

A Thesis Submitted for partial fulfillment of the requirements of M.Sc. Degree in Medical laboratory sciences (Hematology and immunohematology)

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الاية

بسم الله الرحمن الرحيم

قال تعالي (اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الإِنسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الإِنسَانَ مَا لَمْ يَعْلَمْ (5))

صدق الله العظيم

سورة العلق الآية (1-5)

Dedication

:I dedicate this work to

My dear brother, my beloved mother

My sisters and teachers who prayed for my prosperity in … education, and encouraging me in my life

My friends and love …. With them I enjoyed my

Life and I am nothing without them

Acknowledgement

First and foremost, praise to ALLAH, who gives me the strength to complete this work

I am indebted to my wonderful supervisor *Abdalla M. Abdalla*

His offered help , patients from when blood collected , lab staff under supervision of them that where done .People provide computer assistance , statistical whole gave there advice and time to analyse data and every one who help to complete this work

Abstract

Blood transfusion is the process of referring blood or blood based product from one person into circulatory system of another to treat a certain medical condition. The blood bank is the section of the laboratory that processes and distributes blood products under the supervisions of medical director certified in transfusion medicine.

This study was conducted to determine the effect of storage over varying periods on some hematological parameters.

The study includes 100 blood samples donated to central blood bank in Khartoum State. All samples collected from donors who met the specifications required to donate blood. The Complete Blood Count (CBC) was performed on it immediately at the time of collection, and this was considered as control, then CBC performed at day 7, day 14, day 21 and day 28. The results of each week were compared with the control.

Complete Blood Count (CBC) was measured by automated cell count (Sysmex KY21N) for each day of collection, changes of various hematological properties in stored blood was compared with control (Day 0).

In the first (day 7) and second weeks (day 14) there were insignificant changes in all hematological parameters except for platelets, there was a significant decrease (mean of plt in first week =2.086 and p value =.005) compared to the control (mean of plt in control = 3.286), while in third (day 21) and fourth weeks (day 28) all changes that occurred with a significant decrease, except for the MCV, which was significantly increased (mean in day 28 =90.725, p value =.001 and mean of control 87.846)

The study concluded that all hematological parameters that evaluated are affected by storage, but platelets are more affected which have a significant decrease in Day 7.

المستخلص

نقل الدم هو عملية إحالة الدم أو نقل منتج دموي من شخص إلى شخص اخر لمعالجة حالات طبية معينة. بنك الدم هو قسم المختبر الذي يعالج ويوزع منتج الدم تحت إشراف مدير طبي معتمد في طب نقل الدم.

أجريت هذه الدراسة لتحديد تأثير فترات التخزين المتفاوتة على بعض المعلمات الدموية وتشمل الدراسة ١٠٠ عينه من دم متبرعين تم قبولها في بنك الدم المركزي بولاية الخرطوم حسب اشتراطات نقل الدم. تم جمع ثلاثة ملليلتر من عينة الدم الوريدي بواسطة محقنة بلاستيكية يمكن التخلص منها من دماء جديدة في وقت التجميع كعينة ضبط (اليوم 0). ثم اخذت نفس الكمية من الدم المخزن (350 مل من الدم + 49 مل من.مانع التجلط CPDA-1 على فترات مختلفة (اليوم ٧، اليوم ١٤ ، اليوم ٢١ واليوم ٢٨).

تم قياس صورة الدم الكاملة (CBC) بجهاز عد الخلايا الآلي (Sysmex) لكل يوم من أيام الجمع، التغييرات في خصائص الدم المختلفة في الدم المخزن تمت مقارنتها مع عينة الضبط (اليوم0).

في الأسبوعين الأول والثاني ، كانت هناك تغييرات طفيفة في جميع معاملات الدم باستثناء الصفائح الدموية حيث اظهرت الدراسة انخفاض معنوي (متوسط الصفائح الدموية في الأسبوع الأول = 2.086القيمة الاحتمالية 005.) مقارنةً بالكنترول (متوسط plt في العينة الضابطة = 3.286) ، بينما في الأسبوعين الثالث (اليوم21) والرابع (اليوم 28) ، اظهرت الدراسة انخفاضا كبيرا في كل القياسات ، باستثناء متوسط حجم الكرات (MCV)، حيث حدث زيادة ذات دلالة احصائية (المتوسط في الأسبوع الرابع = 90.725 ، ومتوسط الكنترول 87.846 مستوى المعنوية 001.)

خلصت الدراسة الى أن كل المقاييس الدموية تتأثر بالتخزين بعد مرور أسبوعين، لكن الصفائح الدموية اكثر تأثراً حيث انها أعطت نتائج انخفاض منذ اليوم السابع من التخزين

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List of abbreviations

CBC	Complete Blood Count
Hb	Hemoglobin
RBCs	Red Blood cells
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
WBCs	White Blood Cells
PMN	Polymorph Nuclear
PLTs	Platelets
CPDA	Citrate Phosphate Dextrose Adnine
SAGM	Saline Adenine Galcose and Mannitol
ACD	Acid Citrate Dextrose formula A'
CP2D	Citrate Phosphate Double Dextrose

CHAPTER I

Introduction

Blood is always considered essential for life, is a mixture of cells and watery liquid, called plasma that the cells float in. It also contains other things like nutrients (such as sugar), hormones, clotting agents and waste products to be flushed out of the body. There are three kinds of cells in the blood; red blood cells, white blood cells and platelets. (Dern D.J etal, 1999; Mc. Crosson L. etal, 2002)

A blood bank place where blood is collected from donors separated into different types, stored, and / or prepared for transfusion to the recipient. A blood bank may be a separate free – standing facility or part of a larger laboratory in a hospital. (Lave EM. etal, 2002)

The blood transfusion was first attempted in 1422 great strides have been achieved in the field of blood donation, the discovery and recognition of the standard blood groups in 1901, the addition of dextrose to the storage medium in 1914, the importance of refrigeration of stored blood in 1937, and the discovery of the Rh factor in 1940. (Swon H.etal, 1962; Rassmusem M. G etal, 1961)

Blood is collected into a plastic bag for blood donation. One blood unit consisted of 450 ml of blood mixed with anticoagulant; these include citrate –phosphate dextrose (CPD), acid – citrate dextrose (ACD), with adenine to prolong red cell storage. (Lave EM. etal, 2002; Hebert P. C. etal, 2000).

The indications of fresh blood transfusion in case of anemia, leukemia, thrombocytopenia, sever liver diseases, burns, hemodialysis, hemolytic disease of new born and treatment of coagulation disorders, usually the specimen for collection is tested for hepatitis B and C, Human Immune Virus (HIV), AIDS, malaria and other infectious diseases, the only blood that tests negative for these are given to patients.(Mc. Crosson L. etal, 2002; Cohle S. D. etal, 1981).

Each unit of whole blood normally is separated into several components, red blood cells may be stored under refrigeration for a maximum of 42 days, or they may be frozen for up to 10 years. Red blood cells are used to treat anemia, while the platelets are important in the control of bleeding and are generally used in patients with leukemia and other forms of cancer, the platelets are stored at room temperature and may be kept for a maximum of five days, while the fresh frozen plasma used to control bleeding due to low levels of some clotting

factors is kept in a frozen state for usually up to one year.(Mc. Crosson L. etal, 2002; The stationary Office UK 2000; Tvedten H. W. etal, 1996)

While the granulocytes are sometimes used to fight infections, although their efficacy is not well – established, they must be transfused with 24 hours of donation. (Heaton W. A. etal, 1999; Tvedten H. W. etal, 1996).

Whole blood may be preserved for up to 21 days, without losing its usefulness in blood transfusions an anticoagulant is added to prevent clotting blood plasma, the fluid portion of the blood, may be frozen and / or dried and stored indefinitely.(Hebert P.C. etal, 2000). During storage a number of biochemical and hematological changes occur (generally known as storage lesion), which can affect the efficacy of blood transfusion. A variety of changes have been identified in red blood cells during red blood cell (RBC) preservation. They are collectively termed as storage lesion and include extensive biochemical and biomechanical changes. Over time the glucose in stored blood is consumed, levels of 2, 3-diphosphoglycerate (DPG) and ATP decrease, leading to reduced structural integrity of cells. Thus red cells become less deformable and more fragile as they age. This fragility leads to the release of cell free hemoglobin and formation of micro particles, sub-micron hemoglobin containing vesicles and additional haemolysis that effect on RBCs indices.

The changes that occur in platelet under blood bank storage conditions are collectively known as platelet storage lesions (PSL) the platelets are affected by hydrolytic enzymes released by leucocytes during storage which affects platelet membrane and may lead to their destruction. (Saran P. K. etal, 2003).

This study aimed to evaluate the changes that may occur during storage of blood considering fixed storage conditions.

CHAPTER II

Literature Review

2.1Complete blood count (CBC)

A complete blood count (CBC), also known full blood count (FBC), or full blood exam (FBE), is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC.

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions Procedure. A phlebotomist collects the sample through venipuncture, drawing the blood into a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting. The sample is then transported to a laboratory. Sometimes the sample is drawn off a finger prick using a Pasteur pipette for immediate processing by an automated counter. A complete blood count (CBC) is a blood test used to evaluate overall health and detect a wide range of disorders, including anemia, infection and leukemia. (Oluyomb R. etal, 2003)**.** Complete blood count test measures several components and features of your blood, including:

2.1.1 Hemoglobin

Also spelled hemoglobin and abbreviated Hb or Hgb, is the iron-Containing oxygen-transport metallic protein in the red blood cells of all vertebrates.

Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism. (Maten A. etal, 1993).

Hemoglobin has an oxygen-binding capacity of 1.34 mL O2 per gram (Dominguezde etal, 1981). This increases the total blood oxygen capacity seventy-fold compared to

dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules. (Bain B. Seed etal, 1984).

Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobin's may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leg hemoglobin, is used to scavenge oxygen away from anaerobic systems, such as the nitrogen fixing nodules of leguminous plants, before the oxygen can poison the system. (Biagioli M. etal, 2009).

2.1.2 Hematocrit

Hematocrit is a blood test that measures the percentage of the volume of whole blood that is made up of red blood cells. This measurement depends on the number of red blood cells and the size of red blood cells. The hematocrit is almost always ordered as part of a complete blood count (CBC).

This test may be ordered to investigate anemia, diet deficiency, leukemia and other medical condition (Biagioli M. etal, 2009).

2.1.3 Red blood cell

Erythropoiesis is the development process by which new erythrocytes are produced, it lasts about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a healthy adult. (In the embryo, the liver is the main site of red blood cell production.) The production can be stimulated by the hormone erythropoietin (EPO). (EPO), synthesized by the kidney. Just before and after leaving the bone marrow, the developing cells are known as reticulocytes, these comprise about 1% of circulating red blood cells.

Red blood cells (RBCs), also called erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O2) to the body tissues via blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries. The cytoplasm of erythrocytes is rich in hemoglobin, an iron containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. In

humans, mature red blood cells are flexible and oval biconcave disks.

They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second in human adults, quarter of the cells in the human body are red blood cells. Red blood cells are also known as, red cells. Red blood corpuscles (an archaic term), hematids, erythroid cells or erythrocytes (from Greek *erythros* for "red" and *kytos* for "hollow vessel", with *-cyte* translated as "cell" in modern usage).

The functional lifetime of an erythrocyte is about 100–120 days, during which time the erythrocytes are continually moved by the blood flow push (in arteries), pull (in veins) and a combination of the two as they squeeze through micro vessels such as capillaries. (Wheater P. R. etal, 2002)

Increase in red blood cells known as polycythemia, result from excess erythropoietin. (e.g., hemolytic anemia, etc.). Decrease in red blood cells known as anemia result from: Acute or chronic bleeding, RBC destruction, nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency), bone marrow or damage, kidney failure and chronic inflammatory disease.

2.1.4 Red cell indices

2.1.4.1 Mean Corpuscular Volume (MCV)

Decrease Mean Corpuscular Volume Indicates RBCs are smaller than normal (microcytic), for example in anemia caused by iron deficiency anemia or thalassemias. Increase Indicates RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 or folate deficiency.

2.1.4.2 Mean Corpuscular Hemoglobin (MCH)

Mean Corpuscular Hemoglobin Mirrors MCV results; small red cells would have a lower value. Mirrors MCV results, macrocytic RBCs are large so tend to have a higher MCH.

2.1.4.3 Mean Corpuscular Hemoglobin Concentration (MCHC)

Mean Corpuscular Hemoglobin Concentration may be low when MCV is low; decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and thalassemia. Increased MCHC values (hyperchromia) are seen in conditions where the hemoglobin is more concentrated inside the red cells, such as autoimmune hemolytic anemia, in burn patients, and hereditary spherocytosis, a rare congenital disorder.

2.1.5 White blood cells (WBCs)

Also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All leukocytes are produced and derived from a multipotential cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system

Five different and diverse types of leukocytes exist. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. (LA Fleur Brooks etal, 2008)

The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count is usually between 4 and 11 × 109/L. In the US this is usually expressed as 4,000–11,000 white blood cells per microliter of blood. They make up approximately 1% of the total blood volume in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis result from lymphoma or other cancer that spread to the bone marrow and disease of immune.

The name "white blood cell" derives from the physical appearance of a blood sample after centrifugation. White cells are found in the buff, a thin, typically white layer of nucleated cells between the precipitated red blood cells and the blood plasma. The scientific term leukocyte directly reflects its description. It is derived from the Greek word leuko- meaning "white" and kytos meaning "hollow vessel", with cyte translated as "cell" in modern use. The buffy coat may sometimes be green if there are large amounts of neutrophils in the sample, due to the hem containing enzyme myeloperoxidase.

2.1.5.1 Neutrophil

Neutrophils are the most abundant white blood cell, constituting 60- 70% of the circulating leukocytes. (Bain B. etal, 1984)

They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus. They 10 are commonly referred to as polymorph nuclear (PMN) leukocytes, although, in the technical sense, PMN refers to all granulocytes. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands. (Bain B. etal, 1984; Saladin K. etal, 2012)

This gives the neutrophils the appearance of having multiple nuclei, hence the Name

polymorph nuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac when stained. Neutrophils are active in phagocytizing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytized a few pathogens. (Bain B. etal, 1984; Saladin K. etal, 2012; Wheater P. R. etal, 2002)

Neutrophils are the most common cell type seen in the early stages of acute inflammation. The life span of a circulating human neutrophil is about 5-4days. (Kenneth. R etal, 2007)

2.1.5.2 Eosinophil

Eosinophils compose about 2-4% of the WBC total. This count fluctuates throughout the day, seasonally, and during menstruation. It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts. They primarily deal with parasitic infections. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. They secrete chemicals that destroy these large parasites, such as hook worms and tape worms that are too big for any one WBC to phagocytize. In general, their nucleus isbi-lobed. The lobes are connected by a thin strand. The cytoplasm is full 11 of granules that assume a characteristic pink-orange color with eosin staining. (Pilly. J etal, 2016)

2.1.5.3 Basophil

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study. They can be recognized by several coarse, dark violet granules, giving them a blue hue.

The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules that hide it. They excrete two chemicals that aid in the body's defenses, histamine and heparin. Histamine is responsible for widening blood vessels and increasing the flow of blood to injured tissue. It also makes blood vessels more permeable so neutrophils and clotting proteins can get into connective tissue more easily. Heparin is an anticoagulant that inhibits blood clotting and promotes the

movement of white blood cells into an area. Basophils can also release chemical signals that attract eosinophils and neutrophils to an infection site. (Pilly. J etal, 2016)

2.1.5.4 Lymphocyte

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes Include: B cells make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction, and T-cells which include CD4+ helper T cells: T cells displaying co-receptor CD4 are known as CD4+ T cells. These cells have T-cell receptors and CD4 molecules that, in combination, bind antigenic peptides presented on major histocompatibility complex (MHC) class II molecules on antigen-presenting cells. Helper T cells make cytokines and perform other functions that help coordinate the immune response. In HIV infection, these T cells are the main index to identify the individual's immune system integrity.CD8+ cytotoxic T cells, T cells displaying co-receptor CD8 are known as CD8+ T cells. Nearly all nucleated cells display MHCI. γ δ T cells possess an alternative T cell receptor (different from the αβ TCR found on conventional CD4+ and CD8+ T cells). Found in tissue more commonly than in blood, γδ T cells share characteristics of helper T cells, cytotoxic T cells, and natural killer cells. Natural killer cells are able to kill cells of the body that do not display MHC class I molecules, or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A). Decreased expression of MHC class I and up-regulation of MIC-A can happen when cells are infected by a virus or become cancerous. (Kenneth. R etal, 2007)

2.1.5.5 Monocyte

Monocytes, the largest type of WBCs, share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an extra role, they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and become tissue macrophages, which remove dead cell debris as well as attacking microorganisms. Neither dead cell debris nor attacking microorganisms can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life.

They have the kidney shaped nucleus and are typically a granulated. They also

possess abundant cytoplasm. Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages.

2.1.5.6 Blood platelet

Also called thrombocytes, are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injury.

Platelets have no cell nucleus, they are fragments of cytoplasm which are derived from the megakaryocytes of the bone marrow, and then enter the circulation. These inactivated platelets are biconvex discoid(lens-shaped) structures 2–3 μm in greatest diameter. Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells. On a stained blood smear, platelets appear as dark purple spots, about 20% the diameter of red blood cells. The main function of platelets is to contribute to hemostasis, disorder of platelet function is a thrombocytopathy. Low platelet concentration is thrombocytopenia and is due to either decreased production or increased destruction.

Examples for causes of thrombocytopenia are viral infection (mononucleosis, measles, hepatitis), mountain spotted fever autoimmune disorders, sepsis, myelodysplastic, radiation therapy and proliferative disorder as essential thrombocythaemia. (Osman. M .M etal, 2013)

Elevated platelet concentration is thrombocytosis and is either congenital, reactive (to cytokines), or due to unregulated production one of the myeloproliferative neoplasm's or certain other myeloid neoplasm's. (Osman. M .M etal, 2013)

2.2 Blood bank

2.2.1 background

Blood banking refers to the process of collecting, separating, and storing blood. The first U.S. blood bank was established in 1936. Today, blood banks collect blood and separate it into its various components so they can be used most effectively according to the needs of the patient.

The hospital blood bank is responsible for management of the hospital's blood stock. This includes maintaining an inventory for each blood group, ensuring an average age of blood at time of issue, and monitoring the amount of blood that becomes out dated or is not used for other reasons.

The term "blood bank" typically refers to a division of a hospital where the storage of blood product occurs and where proper testing is performed (to reduce the risk of

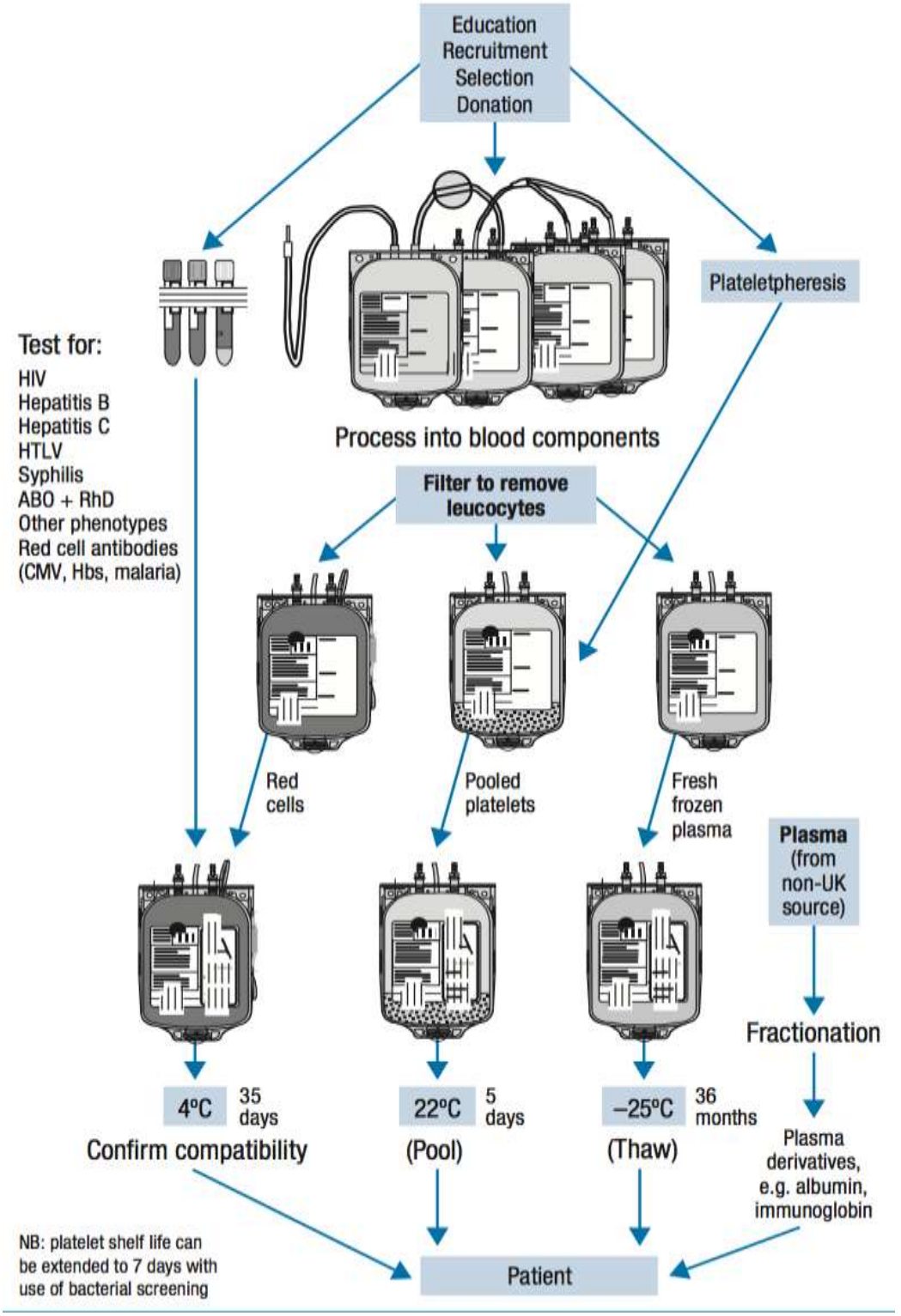
transfusion related adverse events). (Wikipedia etal, 2013).

2.2.2 Blood components

These are classified as blood components prepared in the blood transfusion centre (red cells, platelets, fresh frozen plasma and cryoprecipitate) or plasma derivatives manufactured from pooled plasma donations in plasma fractionation centres (such as albumin, coagulation factors and immunoglobulins). Plasma derivatives are covered by the medicines act and like any other drug must be prescribed by a licensed practitioner. Since 1999, as a vCJD risk-reduction measure, all plasma derivatives used in the UK are manufactured using donations from countries with a low risk of vCJD.

Whole blood is now rarely used for transfusion. Blood component therapy makes clinical sense as most patients require a specific element of blood, such as red cells or platelets, and the dose can then be optimized. Each component is stored under ideal conditions (e.g. red cells must be refrigerated, platelets must not) and the use of precious blood donations becomes more efficient.

The process of producing blood components and plasma derivatives is summarized in Figure (2.1.). (Kawthalkar SM. etal, (2013))


Education
Recruitment
Selection
Donation
Plateletpheresis
Test for:
HIV
Hepatitis B
Hepatitis C
HTLV
Syphilis
ABO + RhD
Other phenotypes
Red cell antibodies
(CMV, Hbs, malaria)
Process into blood components
Filter to remove leucocytes
Red cells
Pooled platelets
Fresh frozen plasma
Plasma (from non-UK source)
Fractionation
4°C
35 days
22°C
5 days
–25°C
36 months
Confirm compatibility
(Pool)
(Thaw)
Plasma derivatives, e.g. albumin, immunoglobin
NB: platelet shelf life can be extended to 7 days with use of bacterial screening
Patient

2.2.1.1 Red cells

These are used to restore oxygen carrying capacity in patients with anemia or blood loss where alternative treatments are ineffective or inappropriate. They must be ABO compatible with the recipient.

Red cells in additive solution: In red cells in additive solution (Table 2.1) the majority of plasma is removed and replaced by 100 mL saline, adenine, glucose and mannitol additive solution (SAG-M).

Table 2.1 Red cells in additive solution

Volume (mL)	220–340
Hematocrit (L/L)	0.5–0.7
Hemoglobin content (g)	>40 (in more than 75% of units tested)
Residual plasma (mL)	5–30
Storage temperature	2–6°C
Shelf life	Up to 35 days from donation

Irradiated red cells: Irradiated red cells are indicated for patients at risk of transfusion-associated graft-versus-host disease (TA-GvHD). The component must be irradiated by gamma or X-rays within 14 days of donation and it then has a shelf life of 14 days from irradiation.

Washed red cells: Indicated for patients with recurrent or severe allergic or febrile reactions to red cells, and severely IgA-deficient patients with anti-IgA antibodies for whom red cells from an IgA-deficient donor are not available. They are produced either manually (24-hour shelf life) or by a closed, automated system in which the red cells are sequentially washed to remove most of the plasma (<0.5 g residual plasma per unit) and then resuspended in 100 mL SAG-M (shelf life 14 days from washing). (Kawthalkar SM. etal, (2013))

2.2.1.2 Platelets

Platelet transfusion is indicated for the treatment or prevention of bleeding in patients with a low platelet count (thrombocytopenia) or platelet dysfunction. An adult therapeutic dose (ATD) of platelets is $>240\times10^9$ per transfusion.

Platelets have ABO antigens on their surface and may have reduced survival if transfused to an ABO-incompatible recipient, although this is not usually clinically significant. They are usual0ly only available in groups O, A or B, with only a small

number of group AB platelets produced.

Anti-A or anti-B antibodies in the plasma of platelet components may rarely cause haemolysis of the recipient's red cells, especially in babies and small children. Group O platelets should ideally only be given to group O recipients. Selection of platelets for patients of other ABO groups is summarised in Table 2.2. RhD negative platelet concentrates should be given to RhD negative patients where possible, especially to RhD negative women of child-bearing potential. When RhD-incompatible platelets have to be given, administration of anti-D immunoglobulin may prevent immunisation. Platelets are produced in two ways see (Tables 2.2 and 2.3)

Whole blood donations are centrifuged and the buffy coats (between the red cell and plasma layers) from four donations are pooled in the plasma of one of the donors (male, to reduce the risk of transfusion-related acute lung injury (TRALI).

An ATD of platelets is obtained from a single donor by apheresis (donors may give two or three ATDs at a single session).

The UK Blood Services aim to provide more than 80% of platelet doses by apheresis to reduce the exposure of patients to multiple donors (a vCJD risk-reduction measure). Platelets are stored in temperature-controlled incubators (20–24°C) with constant agitation (refrigerated platelets are rapidly removed from the circulation). The recent introduction of automated bacterial screening has allowed some Blood Services to extend the shelf life from 5 to 7 days after donation.

Table 2.2 Platelets from pooled buffy coats

Number of donors per pack	4
Mean volume (mL)	300
Mean platelets (×109 per unit)	308 (range 165–500)
Anticoagulant	CPD
Storage	20–24°C with agitation
Shelf life	5 days (7 days if bacterial screening)

Table 2.3 Platelets from apheresis donation

Number of donors per pack	1
Mean volume (mL)	199
Mean platelets ($\times 10^9$ per unit)	280 (range 165–510)
Anticoagulant	Acid citrate dextrose
Storage	20–24°C with agitation
Shelf life	5 days (7 days if bacterial screening)

Irradiated platelets: Platelets may be irradiated to prevent TA-GvHD in susceptible patients. They retain their normal shelf life.

Platelets in additive solution: After 'washing' to remove most of the plasma the platelets are resuspended in 200 mL of platelet additive solution (PAS). This component is indicated for patients with recurrent severe allergic or febrile reactions to standard platelet transfusions. The shelf life is reduced to 24 hours after preparation and they must be ordered specially from the Blood Service. Some platelets are lost in the washing process and the component still contains around 10 mL residual plasma.

Human leucocyte antigen (HLA)-selected platelets: Indicated for patients refractory to random platelet components because of the development of HLA antibodies after previous transfusions. The Blood Services maintain a panel of HLA-typed platelet donors who donate by apheresis. The platelets are irradiated before issue to prevent TA-GvHD.

Human platelet antigen (HPA)-selected platelets: HPA-1a/5b negative platelets are kept in limited numbers at strategically placed stock-holding units in the UK and are used for babies with neonatal alloimmune thrombocytopenia (NAIT). (Kawthalkar SM. etal, (2013))

2.2.1.3 Plasma

Plasma is obtained from whole blood donations or component donation by apheresis. Only male donors are used to reduce the risk of TRALI. The UK Departments of Health recommend that patients born on or after 1 January 1996 should only receive plasma sourced from countries with a low risk of vCJD. Imported plasma is treated with a pathogen reduction process, such as methylene blue or solvent detergent treatment, to reduce the risk of viral transmission.

Plasma components of the same ABO group should be transfused to patients wherever

possible. Plasma components do not need to be matched for RhD group as they contain no red cells or red cell stroma. They do not cause TA-GvHD and irradiation is not required.

Fresh frozen plasma (FFP): Plasma is frozen soon after collection to maintain the activity of blood-clotting factors. It can be stored for up to 36 months at –25°C or below. Standard UK FFP is issued as single-donor packs which must be thawed before use, usually in a purpose-designed water bath. Thawed units of FFP can be stored for up to 24 hours at 4°C before transfusion. Clotting factor levels vary widely between normal healthy donors and this variability is reflected in the concentrations found in individual packs of FFP.

FFP (see Table 2.4) is indicated for the treatment of patients with bleeding due to multiple clotting factor deficiencies such as disseminated intravascular coagulation (DIC). It may also be used in patients with inherited clotting factor deficiencies (e.g. Factor V deficiency) where a clotting factor concentrate is not yet available. The recommended dose is 12–15 mL/kg (minimum of four units in a 70 kg adult). However, much larger doses may be needed to produce 'therapeutic' levels of coagulation factors and volume overload is a significant clinical problem. FFP is no longer indicated for the reversal of warfarin, as a specific and effective antidote is available (prothrombin complex). FFP carries a significant risk of severe allergic reactions and should not be used as a plasma volume expander.

Table 2.4 Fresh frozen plasma

Number of donor exposures per pack	1
Mean volume (mL)	274
Mean Factor VIIIc (IU/mL)	0.83 (specification > 0.7)
Anticoagulant	CPD
Storage	< -25°C
Shelf life	36months (24 hours at 4°C after thawing)

Pathogen-inactivated fresh frozen plasma: Solvent detergent treated FFP (SD-FFP) is available as a licensed medicinal product (Octaplas®, Table 2.5). It is prepared from pools with a maximum of 1520 donations and the SD process inactivates bacteria and most encapsulated viruses, including hepatitis B and C and HIV. Donations are sourced from countries with a low risk of vCJD and a prion-reduced version, Octaplas

LG®, is now licensed in the UK. The pooling process leads to more standardised concentrations of clotting factors in each pack and probably explains the significantly reduced incidence of severe allergic and TRALI in haemovigilance reports. SD treatment reduces the concentration of fibrinogen and Factor VIIIc by 15–20%, but levels remain within the defined specification. Levels of Protein S, an anticoagulant factor, are around 30% lower and this may be important in patients with an increased risk of thromboembolism. UK guidelines recommend imported SD-FFP for plasma exchange in patients with thrombotic thrombocytopenic purpura.

Table 2.5 Solvent detergent plasma (Octaplas®)

Number of donor exposures per pack	Maximum 1520 donors per batch
Volume (mL)	200 (standardized)
Mean Factor VIIIc (IU/mL)	0.8 (specification > 0.5)
Mean fibrinogen (mg/mL)	2.6 (range 1.5 – 4.0)
Anticoagulant	Sodium citrate
Storage	< -18°C
Shelf life	4 years (transfuse immediately after thawing)
Based on data from Octapharma AG (http://www.octapharma.co.uk)	

Methylene blue treated FFP (MB-FFP): is a single-donor pathogen-reduced component available through the UK Blood Services. The process inactivates encapsulated viruses and bacteria. In the UK, the methylene blue process is used to treat packs of FFP imported from low vCJD risk countries, providing a single-donor component that is preferred by some neonatologists and paediatricians. MB-FFP has a reduced activity of fibrinogen and Factor VIII. The clinical significance of this is uncertain, although some studies in cardiac surgery have suggested the need for bigger transfusions to achieve the same therapeutic effect. Like all single-donor FFP components, the content of clotting factors varies between individual packs. (Kawthalkar SM. etal, (2013))

2.2.1.4 Cryoprecipitate

Cryoprecipitate (Table 2.6) is made by thawing UK donor FFP at 4°C, producing a cryoglobulin rich in fibrinogen, Factor VIII and von Willebrand factor. It was developed as a treatment for haemophilia but this use has now been replaced by

Factor VIII concentrate. Cryoprecipitate is mainly used as a more concentrated, hence lower volume for infusion, source of fibrinogen than FFP. It is available from the Blood Services as single-donor packs or as pools of five donations. The recommended adult therapeutic dose is two pools of five units (or one unit per 5–10 kg body weight), which will typically raise the plasma fibrinogen by about 1 g/L. Cryoprecipitate produced from imported MB-FFP is now available. Because of a lower concentration of fibrinogen, pools of six donations are issued. (Kawthalkar SM. etal, (2013))

Table 2.6 Cryoprecipitate

	Cryoprecipitate packs	Cryoprecipitate pools
0Number of donors	1	5
Mean volume (mL)	43	189
Fibrinogen (mg/pack)	396 (specification >140)	1552 (specification > 700)
Factor VIIIc (IU/pack)	105 (specification > 70)	454 (specification > 350)
Storage	< -25°C	< -25°C
Shelf life	36 months (use within 4 hours of thawing, do not refrigerate)	36 months (use within 4 hours of thawing, do not refrigerate)

2.2.1.5 Granulocytes

Although their clinical effectiveness is controversial, transfusion of granulocytes (neutrophils – phagocytic white blood cells) may be indicated in patients with life-threatening soft tissue or organ infection with bacteria or fungi and low neutrophil counts, usually in the setting of severe, prolonged neutropenia after cytotoxic chemotherapy.

There are two main granulocyte-rich components available: buffy coats derived from whole blood donations and granulocytes collected by apheresis from individual donors. Because of contaminating red cells, granulocyte components must be ABO and RhD compatible and crossmatched with the recipient. They are irradiated before issue to prevent TA-GvHD. Daily transfusions are given, with monitoring of

response, until recovery of bone marrow function. (Kawthalkar SM. etal, (2013))

2.2.1.6 Individual buffy coats

These buffy coats (Table 2.7) contain large numbers of red cells and the Hb/haematocrit of the recipient must be monitored. Usefully, the high platelet content may reduce the need for platelet transfusions.

The recommended dose is ten buffy coats daily for adults (10–20 mL/kg for smaller children and infants). (Kawthalkar SM. etal, (2013))

Table 2.7 Buffy coat (granulocytes)

Mean volume per pack (mL)	60 (10 packs = 600 mL)
Mean granulocytes ($\times 10^9$/pack)	1.0 (10 packs = 1×10^{10})
Haematocrit (L/L)	0.45
Platelets ($\times 10^9$/pack)	70
Storage	20–24°C
Shelf life	To midnight on day of collection

Pooled buffy coats (granulocytes pooled buffy coat derived in additive solution and plasma).

This component (see Table 2.8) was introduced in the UK in 2012. Although the manufacturing process is more complicated, it has the advantages of lower volume, less red cell and plasma contamination and resuspension in male donor plasma and additive solution to reduce the risk of TRALI. The dose is two packs (20 donations) for an adult and 10–20 mL/kg for children.

Table 2.8 Granulocytes pooled buffy coat derived in additive solution and plasma

Mean volume per pack (mL)	207 (175–250) mL
Mean granulocytes ($\times 10^{10}$/pack)	1.0 (1×10^{10})
Haematocrit (L/L)	0.15
Platelets ($\times 10^9$/pack)	499 (equivalent to 2.5 adult transfusion doses)
Storage	20–24°C (not agitated)
Shelf life	To midnight on day following collection

2.2.1.7 Apheresis granulocytes

The collection of a therapeutic dose of apheresis granulocytes (Table 2.9) requires the

donor to be pre-treated with steroids and/or injections of granulocyte colony stimulating factor (G-CSF). Hence, their collection is restricted to directed donors (usually a relative) for an individual patient, rather than UK Blood Service volunteer donors, and the component is only available in certain clinical centres.

Table 2.9 Apheresis granulocytes

Mean volume per unit (mL)	312
Granulocytes per unit	$>1\times10^{10}$
Haematocrit (L/L)	0.23
Platelets ($\times10^{9}$ per unit)	111
Storage	20–24°C
Shelf life	24 hours from collection

2.2.1.8 Plasma derivatives

These are licensed medicinal products manufactured from human plasma donations. Some of the main products used in hospital practice are listed below but the reader is referred to the British National Formulary (BNF – http://bnf.org/bnf) and the individual Summary of Product Characteristics for more detailed information about formulation and clinical indications. Although these products are manufactured from large donor pools, sometimes thousands of donations, all now undergo multiple pathogen inactivation steps to eradicate transfusion-transmitted viruses. Since 1999, all plasma derivatives used in the UK are derived from imported plasma (a vCJD risk-reduction measure).

Human albumin solution: Human albumin solution (HAS) contains no clotting factors or blood group antibodies and crossmatching is not required. The clinical indications for HAS are controversial. Crystalloid solutions or synthetic colloidal plasma substitutes are alternatives for use as plasma expanders in acute blood or plasma loss. HAS should not be used to 'correct' the low serum albumin level often associated with acute or chronic illness. Side effects include occasional severe hypersensitivity reactions. HAS is available in two forms:

Isotonic solutions (4.5 and 5.0% in volumes of 50 to 500 mL): Often used to replace subacute plasma volume loss caused by burns, pancreatitis or trauma, and as a replacement fluid in plasma exchange. Concentrated solutions (20% in volumes of 50 and 100 mL): Indications may include initiating diuresis in hypoalbuminaemic

patients with liver cirrhosis or nephrotic syndrome, removal of large volumes of ascites in patients with portal hypertension and to assist the reduction of high bilirubin levels by exchange transfusion in the newborn (unconjugated bilirubin binds to albumin).

Clotting factor concentrates: Single-factor concentrates are available for the treatment of most inherited coagulation deficiencies except Factor V and Factor II (prothrombin). Most patients in the UK with severe haemophilia A are now treated with recombinant Factor VIIIc, which carries no risk of viral or prion transmission.

Fibrinogen concentrate (Factor I) is, at present, only licensed in the UK for the treatment of congenital hypofibrinogenaemia but there is encouraging international experience of its effectiveness in the much more common setting of acquired hypofibrinogenaemia (e.g. DIC, traumatic haemorrhage, massive transfusion). Many coagulation experts believe that it will replace the use of cryoprecipitate for this purpose in view of its ease of administration, convenience of storage and standardised fibrinogen content.

Prothrombin complex concentrate (PCC) contains Factors II, VII, IX and X. It has replaced FFP as the recommended treatment for rapid reversal of warfarin overdose, with elevated international normalised ratio (INR) and severe bleeding, in view of its superior efficacy, ease of administration and lower risk of severe allergic reactions or fluid overload. Modern formulations of PCC do not contain activated clotting factors and have a low risk of causing thrombotic complications. PCC may also be used to treat bleeding due to the coagulopathy associated with liver disease. The dose for reversal of warfarin is 25–50 IU/kg.

Immunoglobulin solutions: These are manufactured from large pools of donor plasma: Normal immunoglobulin: contains antibodies to viruses that are common in the population. Intramuscular normal immunoglobulin may be used to protect susceptible contacts against hepatitis A, measles or rubella. High-dose intravenous immunoglobulin is used as replacement therapy in patients with severe immunoglobulin deficiency and in the treatment of autoimmune diseases such as idiopathic thrombocytopenic purpura (ITP). Specific immunoglobulins: made from selected donors with high antibody levels to the target of treatment. Examples include tetanus, hepatitis B and rabies immunoglobulins as well as anti-D immunoglobulin for the prevention of maternal sensitisation to RhD in pregnancy. (Kawthalkar SM. etal, (2013))

2.2.2 Labelling of blood components

The content of blood pack labels attached at the transfusion centre is prescribed by the Blood Safety and Quality Regulations 2005 (BSQR). Key information is present in both eye-readable and barcoded form and allows the donor origin (via a unique donation number) and processing steps of the product to be traced as well as indicating the blood group, any special requirements (such as CMV negative or irradiated), expiry date and storage conditions. Work is in progress to review the content of blood component labels and improve their clarity. Up-to-date information is available in the Guidelines for the Blood Transfusion Services in the UK (http://www.transfusionguidelines.org.uk)**.**

2.2.2.1 Blood compatibility labels

These are attached to the pack in the hospital transfusion laboratory and uniquely identify the patient for whom the component has been selected. At the final bedside check, the donation number and other details on the compatibility label must match those on the blood pack label and the patient details must exactly match those on the recipient's ID. (Kawthalkar SM. etal, (2013))

2.2.2.2 Specifications of blood components

Whole blood donations of 405–495 mL (mean 470 mL) are collected into 63 mL of citrate phosphate dextrose (CPD) anticoagulant**.**

All blood donations are filtered to remove white blood cells (pre-storage leucodepletion) to leave <1×106 leucocytes in the pack. This was introduced in 1998 as a vCJD risk-reduction measure but also reduces the incidence of febrile transfusion reactions and alloimmunisation to white cell (including HLA) antigens**.**

Indicative contents of commonly available components are noted below, based on quality assurance data from NHS Blood and Transplant.

(Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee, 2013).

2.2.3 Blood component storage:

temperature range: Blood components should be stored at temperatures in accordance with the requirements listed in the table below.

Table 2.10: storage temperature range

Blood Components	Storage temperature	Shelf life	Comments
Red Cell	2-6 ºC	Red cells: 42 days Paediatric red cells: 35 days Washed red cells: 28 days	All blood refrigerators, including theatre and other holding refrigerators, must comply with AS 3864.1, AS 3864.2 (1, 2).
Platelets	20–24 ºC	5 days	Platelets components must be agitated gently and continuously in a single layer on a .platelet agitator
Fresh frozen plasma, cryodepleted plasma, cryoprecipitate	At or below –25 ºC	12 months	Freezers must comply with AS 3864.1 & 3864.2 .((1,2
Fractionated plasma components	As per product insert	As per expiry date on product	All blood refrigerators, including theatre and other holding refrigerators, must comply with AS 3864.1, AS 3864.2 ((1, 2

(Standards Australia on behalf of Committee HE-020 etal, 2012)

2.2.3.1Blood bags

Blood bags are designed for the collection, processing and storage of whole blood and blood components. They help in providing aseptic conditions for the separation of blood components. It acts as a closed system reducing the chances of contamination. Blood bags are made with high molecular weight PVC to ensure better tensile strength and weld strength. Validated sterilization process is used. The process is monitored automatically with a data logger which confirms the product sterility. Triple filtration of anticoagulant is done and is filled in the bags automatically to ensure accuracy. Advanced and standardized coiling method is used to prevent kinks, which ensures a free flow during collection and separation. See figure **2**.2 (Kawthalkar SM. etal, (2013))

2.2.3.1.1 Components of bags

Tubing: it ensures a better yield of components without damage during blood collection. Very clear printing over the tubes ensures easy identification of samples. Stable wall thickness and inner diameter ensures the smooth flow and collection of blood. See figure 2.3

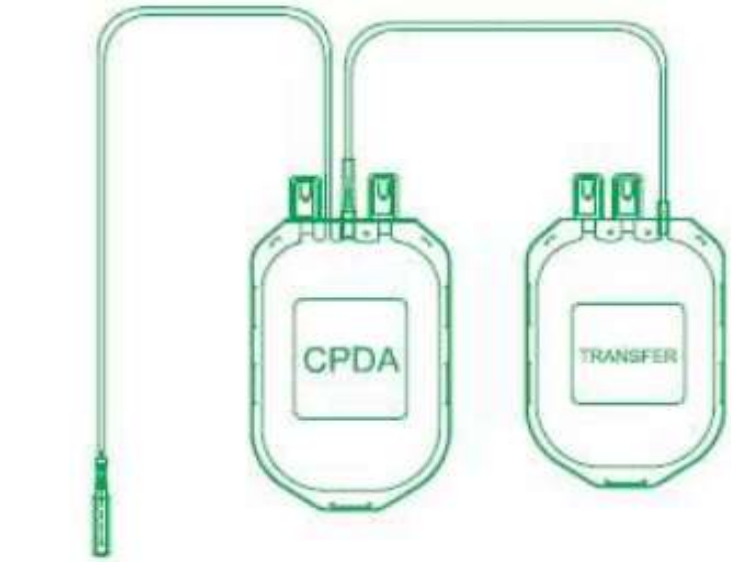
Label: Labels are clear and easy to understand. Resistant to tear, water and centrifugation force. See figure 2.4

Outlet Port Pouch: Each outlet port is fitted with a hermetically sealed protector to maintain sterility of the internal surface. Can be opened with single – handed operation. See figure 2.5

Needle: Ultra-thin walled silicone coated needle. High quality needle for smooth phlebotomy. Minimal stress to the donor. Needle Protector. Composed of two parts. The outer layer is made of hard polypropylene to ensure. Rigidity of cap. The inner layer is made of PVC to ensure hermetic closure with the hub. See figure 2.6

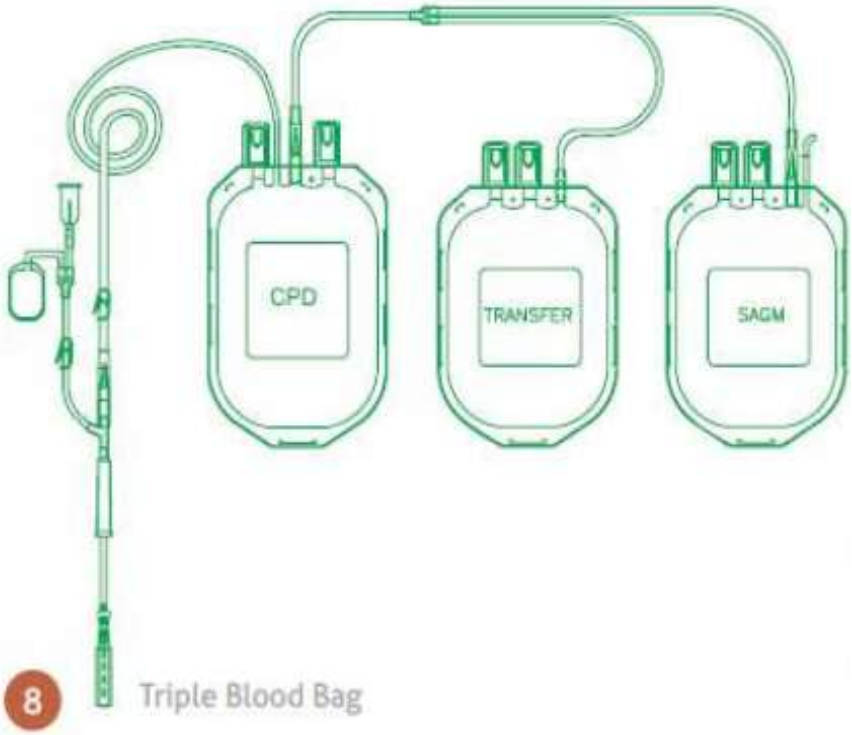
Needle injury protector: Provides immediate shielding of needle on withdrawal from vein. Reduce the risk of 7needle stick injury from both phlebotomy and sampling

needles. Improves safety in blood banks. See figure 2.7



5 Double Blood Bag

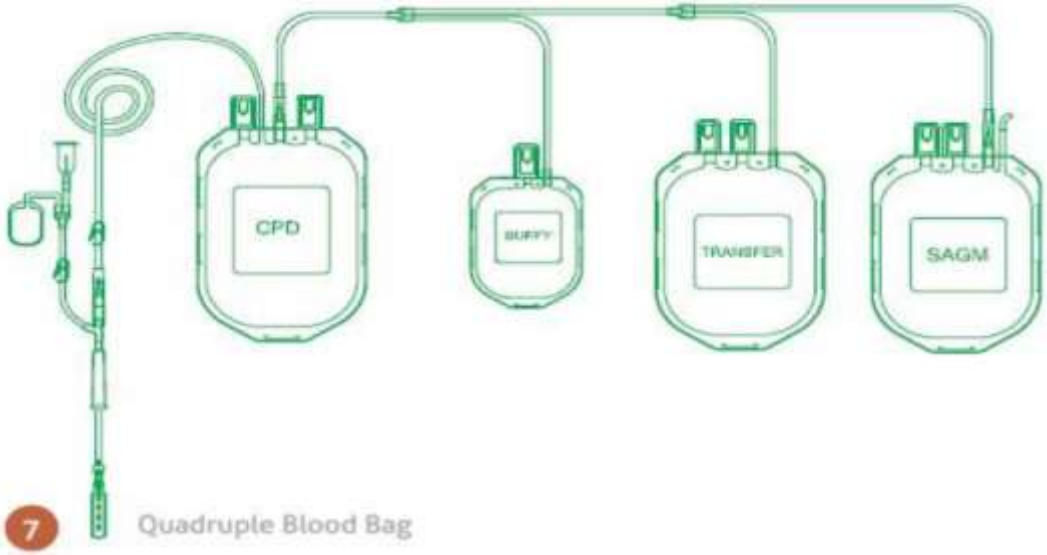
Pre-donation bag (PDB): Diverts 10-30 ml of initial blood. Enables diversion and collection of the first amount of blood which usually contains skin particles and bacteria. Risk of bacterial sepsis is minimized. See figure 2.8



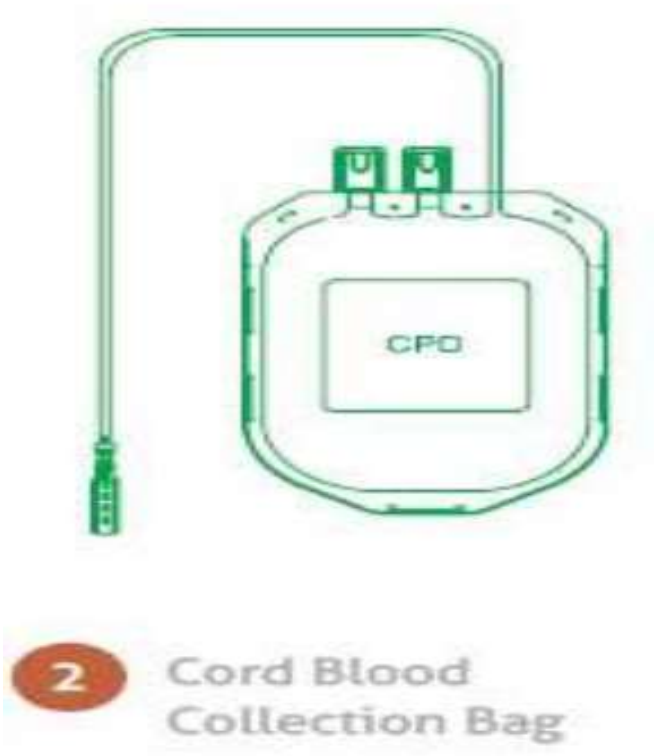
8 Triple Blood Bag

2.2.3.2.2 Types of blood bags

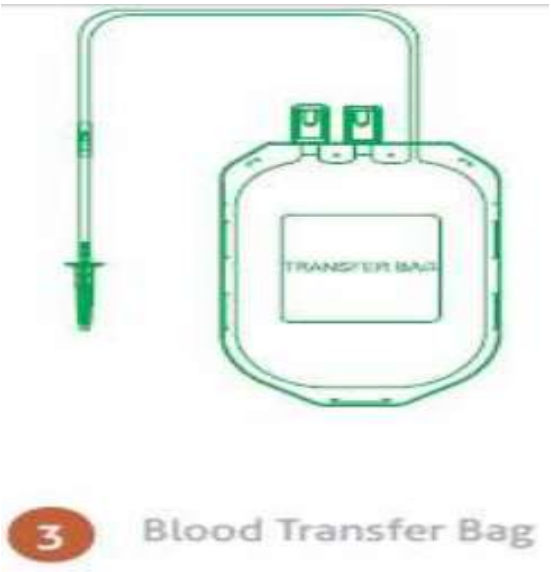
Single Blood Bag: For whole blood collection. The bag contains CPDA solution. Available in capacity of 350ml and 450ml. See figure 2.9



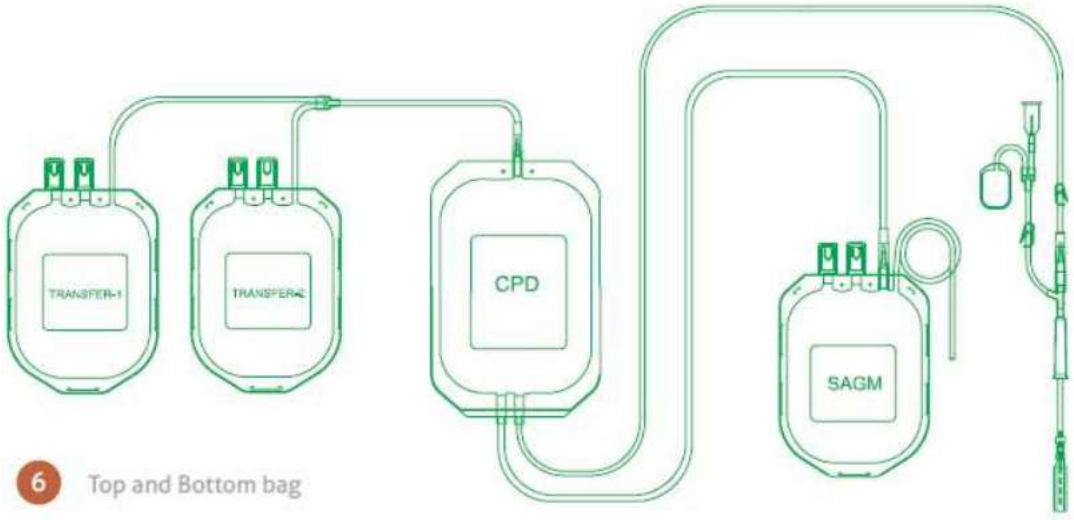
Double Blood Bag: For whole blood collection. Separation of 2 different blood components (red blood cells and plasma) obtained through the process of centrifugation and extraction. See figure 2.10



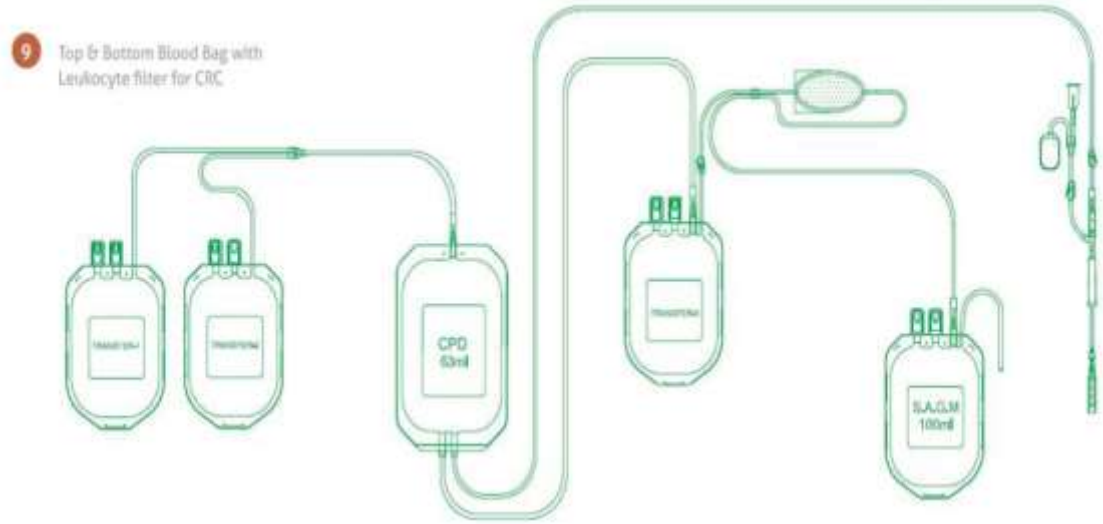
Triple Blood Bag: Triple Blood Bag with SAGM, for whole blood collection. Separation of 3 different blood components (red blood cells, plasma and platelets). The primary bag contains CPD and one satellite bag contains SAGM. See figure 2.11



Quadruple Blood bag: It comes with SAGM for whole blood collection. Separation for 3 different blood components (red blood cells, plasma and platelets) through the buffy coat method. The primary bag - CPD solution and has 3 satellite bags. One satellite bag - 100 ml capacity to prepare platelets through the buffy coat method. Valid for 5 days of platelet storage. See figure 2.12



Cord blood collection bag: Umbilical cord blood collection bag of 150 ml capacity with 22ml of CPD solution. Each bag comes with a second layer of packing of luminous foil for the convenience of cord blood collection center. See figure 2.13



Blood transfer bag: For use with blood bag for transfer or pooling of blood and blood components.

Top and Bottom Bag: Top and Bottom Quadruple blood bags are for whole blood collection and separation of three different blood components (red blood cells, plasma and platelets).The primary bag contains CPD solution and one satellite bag comes with SAGM solution for red cell preservation. Platelets are prepared from the buffy coat method. One transfer bag is valid for 5 days of platelet storage.

Top and Bottom Blood Bag with Leukocyte filter: Top and Bottom pentad blood bag with a leucocyte filter for whole blood collection and separation of 3 different blood components (leucodepleted red blood cells, plasma and platelets).The primary bag contains CPD solution and one satellite bag is attached to a leucocyte filter which comes with SAGM solution for red cell preservation. Platelets are prepared from the buffy coat method. One transfer bag is valid for 5 days of platelet storage. (Kawthalkar SM. etal, (2013))

2.2.3.3 Anticoagulant preservative solutions

Acid Citrate Dextrose (ACD), Citrate Phosphate Dextrose (CPD) and Citrate Phosphate Dextrose Adenine (CPDA-1) 15ml of ACD, 14ml of CPD or CPDA is used for preserving 100ml of blood.

Purpose: To prevent coagulation and preserve the life and survival of RBCs so as to have the maximum post transfusion survival.

Citrate: Acts by chelating Calcium, dextrose: Necessary for the metabolism of stored

RBCs.It passes from plasma into the red cells and is utilized for energy production. The principal pathway being anaerobic glycolysis, citric acid: prevents caramelization of glucose in citrate dextrose solution during autoclaving, and adenine: improves the viability of red cells. CPDA – 2 Here the amount of Adenine is increased to 0.55g and that of dextrose to 44.6g. This is a better anticoagulant preservative solution than CPDA–1. (Kawthalkar SM. etal, (2013))

2.2.3.4 Additive solutions:

Additive solutions are preserving solutions that are added to the RBCsafter removal of the plasma with/without platelets.

Reason for their development - removal of the plasma component during the preparation of RBC concentrates removed much of the nutrients needed to maintain RBCs during storage. Also overcome the problem of high viscosity of RBC concentrates. With CPD anticoagulant in the primary bag, the additive solution usedis SAGM (saline, adenine, glucose, and mannitol)

Advantages are Extends the storage of RBCs, Lowers the viscosity of packed red cells for ease of transfusion and Maximum amount of fresh plasma is harvested – platelets and cryoprecipitate. (Kawthalkar SM. etal, (2013))

Table 2.11: Composition of the primary anticoagulants used in collection of blood for transfusion

Constituent (g/L)	CPD (citrate-phosphate-dextrose)	CPDA1 (citrate-phosphate-dextrose-adenine-1)	CP2D (citrate-phosphate double dextrose)	ACD-A (acid citrate-dextrose formula ‘A’)
Trisodium citrate	26.3	26.3	26.3	22
Citric acid	3.27	3.27	3.27	8
Dextrose	25.5	31.90	51.5	24.5
Monobasic sodium phosphate	2.22	2.22	22.22	N/A
Adenine	N/A	0.28	N/A	N/A

2.3 Previous Studies

A study conducted in L.N. Medical College and J.K. Hospital, Bhopal, in collaboration with blood bank department of our institute. 450 mL of blood was drawn from 30 healthy volunteer donors into citrate phosphate dextrose adenine (CPDA-1) anticoagulant (63 mL). The blood was kept for 28 days and samples were evaluated on days 1, 7, 14, 21 and 28. The study showed a constant decline in WBC and platelet counts from day 0 to 28. RBC count, Hb, MCV, HCT showed increasing values. MCH was almost constant, while MCHC decreased. PDW increased while PCT increased till 4^{th} day and then decreased. Neutrophils, eosinophils, monocytes decreased, basophils remained constant while lymphocytes increased. (ADR Journaletc., 2016) Hematological changes in stored blood Karama M.T. AI-Nuaimy, India.

Results: Hemoglobin and (PCV) were significantly affected by storage period of blood but (PCV) is more affected than hemoglobin.

This could be due to increased (MCV) or real effect of age of blood, mean new blood is better than old blood in raising (PCV).

the many studies showed that during storage the total leukocyte count decreases, they attribute this decrease to degeneration of the granulocytes. While pletlete lose viability rapidly on storage in refrigerators, so platelets transfusion to be physiologically effective must be completed within 4 – 6 hours after blood is withdraw. The platelet viability and functions are affected by white blood cells present in stored platelet concentrate. White blood cells have adverse effect on platelets function and post transfusion recovery (21, 27). In fresh whole blood platelets are about 60 % effective at 24 hours and almost completely ineffective after 48 hours (27 – 31). This rapid disintegration of platelets during storage may be due to a rapid disappearance of the white blood cells; their hydrolytic enzyme the affect the platelet membrane may cause to platelet degeneration (27) (J.Edu Journal etc., 2008)

Rationale

During storage of blood several hematological and biochemical changes take place that may affect viability of blood cells and other components. In Sudan (to our knowledge) there is no enough information about these changes and there are some gaps in the studies that have been conducted, it did not include all hematological parameters, thus the aim of the present study is to study the various hematological changes occurring in stored whole blood.

Objectives

General objective:

To study the various hematological changes occurring in stored whole blood.

Specific objective:

To estimate of Hb, RBCs, HCT, MCV, MCH, MCHC, RDW, WBCs, Differential leukocyte count by using Sysmex KX-21.

To determine the stability of hematological parameters like RBC count, WBC count, differential count, platelet count, MCV, MCH and MCHC during extended 28 days.

To compare changes of various hematological properties in stored blood during different intervals (day7, day14, day21 and day28) with control (day0) at 4℃.

CHAPTER III

Materials and methods

4.1 Study design

This is cross-sectional study conducted at Central Blood Bank , Khartoum state. The study conducted in the period from October 2019 to February 2020, included 100 Sudanese adults donors at defined age group (18-48), excluded individuals who are not met the criteria for blood donation.

4.2 Data collection and Sampling technique

The data was collected using laboratory investigation to obtain complete blood count also a questionnaire was used. (See appendix A) by Non Probability Voluntary sampling technique.

4.3 Specimen collection

Three milliliters venous blood sample was collected by disposable plastic syringe from fresh blood at the time of collection as control (day 0). Then same amount of blood was taken from stored blood (350 mL blood + 49 mL CPDA-1), at different intervals (day7, day14, day21and day28). The blood was slowly poured into 2 tetra acetic acid (EDTA) anticoagulant blood containers. -gentle and adequate mixing of sample was applied by mixture to avoid hemolysis, clot or platelet aggregation.

4.4 Ethical consideration

It was considered that all information obtained from participants was kept as highly confidential data and specimen's results were not permitted.

The participants were provided with information about the study and any risk which may be raised especially when the collection technique was applied.

4.*5*. Material and Method:

Measure of CBC by automated cell count (Sysmex device) appendix B table (4.1):

EDTA blood samples were analyzed for CBC by Sysmex automated hematological analyzer.

4.6 Automated technique (principle and method):

A blood cell counters Sysmex KX-21 was used. The whole blood mode sample without pre-dilution. The sample number was entered before each sample.

A well-mixed ant coagulated sample was set to the sample probe, and the start switch

was pressed till the aspirating process was finished. (Volume aspirated approx. 50 µl).The sample was removed straight down and the sample probe was automatically cleaned. The aspirated sample was then automatically suspended into the different detector blocks and different parameter was measured. The results of parameters were then viewed on the screen and subsequently printed out.

4.6.1Method for HB, WBCs, and platelet counts:

Two type of blood are use Whole Blood Mode: 50 µL or Pre-diluted Mode: 1:26 Diluted Sample 200 µIn WBC and HGB analysis, the volume of WBC and hemoglobin in the blood are measured. The flow of WBC/HGB analysis is described below:

Blood is aspirated from the sample probe into the sample rotor valve.

2.6 µL of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/HGB lyse is added to prepare 1:500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is hemolysis and platelets shrink. At the same time, hemoglobin is converted into red colored met hemoglobin. Of the diluted/hemolysis sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.

500 µL of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).In RBC/PLT analysis, RBC and platelet count in the blood are measured.

The flow of RBC/PLT analysis is described below:

Blood is aspirated from the sample probe into the sample rotor valve.4.0 µL of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 mL of diluent and brought to the mixing chamber as diluted sample. (1st step dilution)Out of the 1:500 dilution sample, 40 µL is measured by the sample rotor valve, diluted into 1:25000 with 1.960 mL of diluent, and then transferred to the RBC/PLT transducer chamber. (2nd step dilution)250 µL of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT are counted by the DC

detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

4.7 Data analysis

Statistical Package of Social Sciences (SPSS) software program was used for Statistical analysis one way ANOVA and Independent T-test were used to Obtain P-value significant level was set at ≤ 0.05.

CHAPTER IV

Results

4.1 Demographic Data:

This study was conducted at the central blood bank in the state of Khartoum on one hundred adult donors at defined age group (18-48). all of them are males, beginning in August and ending in February, to study hematological changes that occur during storage of whole blood (450ml from blood+49ml from CPDA-1)

All samples collected from donors who met the specifications required to donate blood. The CBC was performed on it immediately after first collection, and this was considered as the control, then CBC performed at 1,2,3,4 week. The results of each week were compared with the control. All conditions related to blood storage are kept in all stages of the examination.

Our study shows different results at different stages.

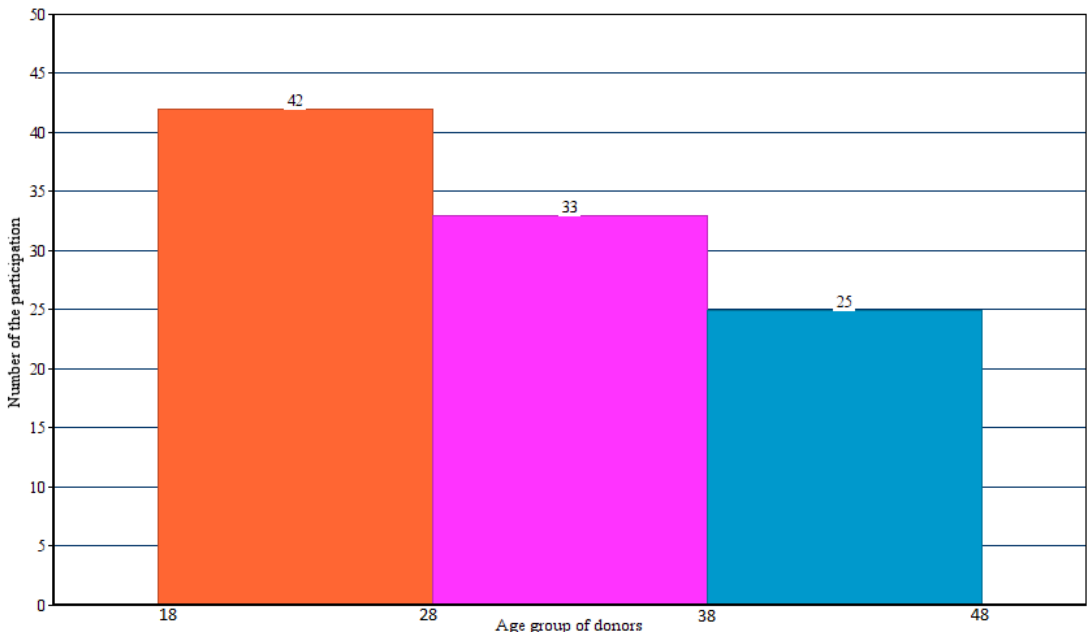


Figure 4.1: Show distribution age of participants in the study

The mean value(X`) +/_Standard Error Mean (SEM) are performed

In the first week there was insignificant changes in all hematological parameters except for platelets, there was a decrease significantly (mean of plt in first week =2.086 and p value =.005) compared to the control (mean of plt in control = 3.286) showed in tables 4.1.

Table: 4.1 show statistical results of CBC and their P-values in first week

	Control	First week	p-value1
RBCs $x10^{6\mu l}$	5.1121	5.0963	0.872
Hb g/dl	15.1777	14.539	0.115
PCV%	45.856	44.137	0.794
MCV/ fl	87.846	86.359	0.138
MCH P.g	30.4091	29.130	0.281
MCHC g/dl	32.428	31.513	0.183
PLT $x10^{3\mu l}$	3.2863	2.0867	0.005
TWBCS $x10^{3\mu l}$	6.3804	5.9330	0.114
Neutrophil $x10^{3\mu l}$	2.7432	2. 538	0.108
Lymphocyte $x10^{3\mu l}$	2.9929	2.2753	0.162
Monocyte $x10^{3\mu l}$	.3519	.3339	0.987
Basophil $x10^{3\mu l}$	0.2935	.2575	0.158
Eosinophil $x10^{3\mu l}$	0.2875	.1319	0.077

In the second week there was a insignificant changes in all hematological parameters except for platelets, there was a decrease significantly (mean of plt in second week =1.049 and p value = .001) compared to the control (mean of plt in control = 3.286) showed in tables 5.2.

Table: 4.2 show statistical results of CBC and their P-values in Second week

	Control	Second week	p-value2
RBCs $x10^{6\mu l}$	5.1121	4.9601	0.071
Hb g/dl	15.1770	14.196	0.096
PCV%	44.8561	43.721	0.089
MCV/ fl	87.8460	87.947	0.074
MCH P.g	28.4091	28.837	0.091
MCHC g/dl	32.4284	31.085	0.092
PLT $x10^{3\mu l}$	3.2863	1.049	0.001
TWBCS $x10^{3\mu l}$	6.3804	5.0185	0.051
Neutrophil $x10^{3\mu l}$	2.7432	2.103	0.095
Lymphocyte $x10^{3\mu l}$	2.0729	2.007	0.104
Monocyte $x10^{3\mu l}$	.3519	0.2954	0.516
Basophil $x10^{3\mu l}$	.2333	0.2108	0.089
Eosinophil $x10^{3\mu l}$	.1875	0.2009	0.063

In the third significant changes occurred in all hematological parameters showed in tables 4.3.

All changes that occurred with a significant decrease, except for the MCV, in which the change occurred with a significant increase (mean in third week =89.946, p value=0.026 and mean of control 87.846)

Table: 4.3 show statistical results of CBC and their P-values in Third week

	Control	Third week	p-value3
RBCs $x10^{6\mu l}$	5.1121	3.752	.044
Hb g/dl	15.1777	13.263	0.042
PCV%	45.856	42.520	0.038
MCV/ fl	87.846	89.964	0.026
MCH P.g	30.4091	27.270	0.045
MCHC g/dl	32.428	29.172	0.033
PLT $x10^{3\mu l}$	3.2863	0.902	0.001
TWBCS $x10^{3\mu l}$	6.3804	4.856	0.049
Neutrophil $x10^{3\mu l}$	2.7432	1.054	0.037
Lymphocyte $x10^{3\mu l}$	2.9929	1.543	0.025
Monocyte $x10^{3\mu l}$	.3519	0.1722	0.034
Basophil $x10^{3\mu l}$	0.2935	0.1843	0.039
Eosinophil $x10^{3\mu l}$	0.2875	0.1996	0.028

In the fourth significant changes occurred in all hematological parameters showed in tables 4.4. a significant

All changes that occurred with a significant decrease, except for the MCV, in which the change occurred with increase (mean in fourth week =90.725, p value =.001 and mean of control 87.846)

Table: 4.4 show statistical results of CBC and their P-values in Fourth week

	Control	Fourth week	p-value4
RBCs $x10^{6\mu l}$	5.1121	3.012	0.003
Hb g/dl	15.1777	12.572	0.001
PCV%	45.856	41.610	0.015
MCV/ fl	87.846	90.725	0.001
MCH P.g	30.4091	26.941	0.006
MCHC g/dl	32.428	28.100	0.003
PLT $x10^{3\mu l}$	3.2863	0.054	0.00
TWBCS $x10^{3\mu l}$	6.3804	3.296	0.009
Neutrophil $x10^{3\mu l}$	2.7432	1.001	0.001
Lymphocyte $x10^{3\mu l}$	2.9929	1.020	0.000
Monocyte $x10^{3\mu l}$	.3519	0.1095	0.00
Basophil $x10^{3\mu l}$	0.2935	0.1415	0.000
Eosinophil $x10^{3\mu l}$	0.2875	0.1675	0.001

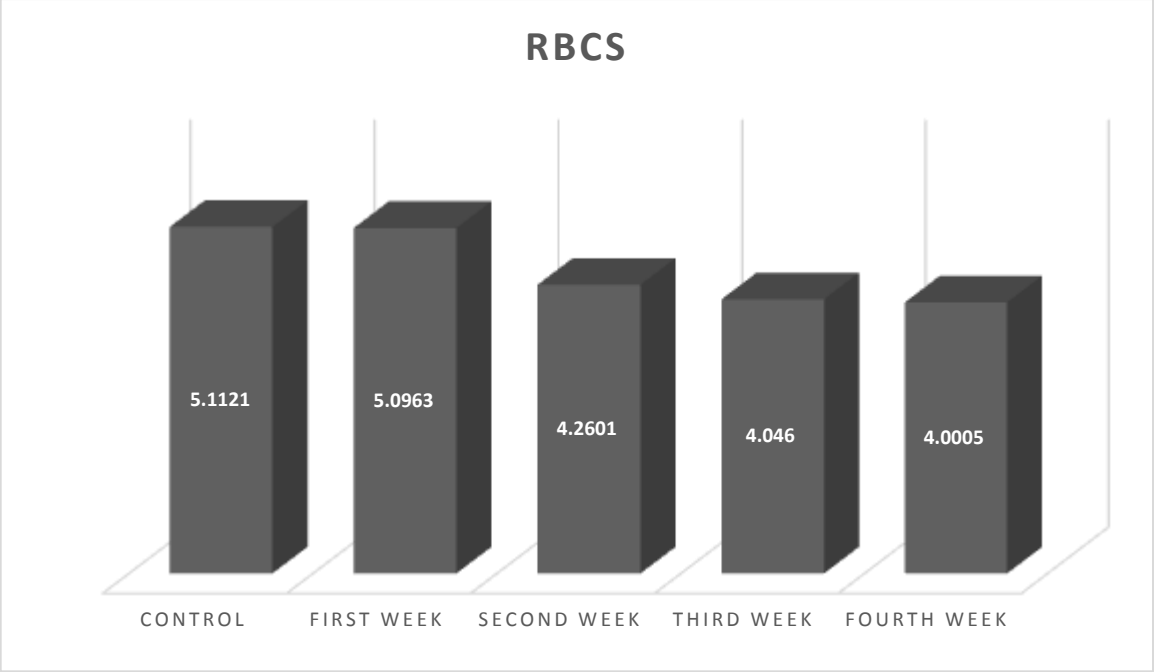


Figure 4.2 Show RBCS during four weeks

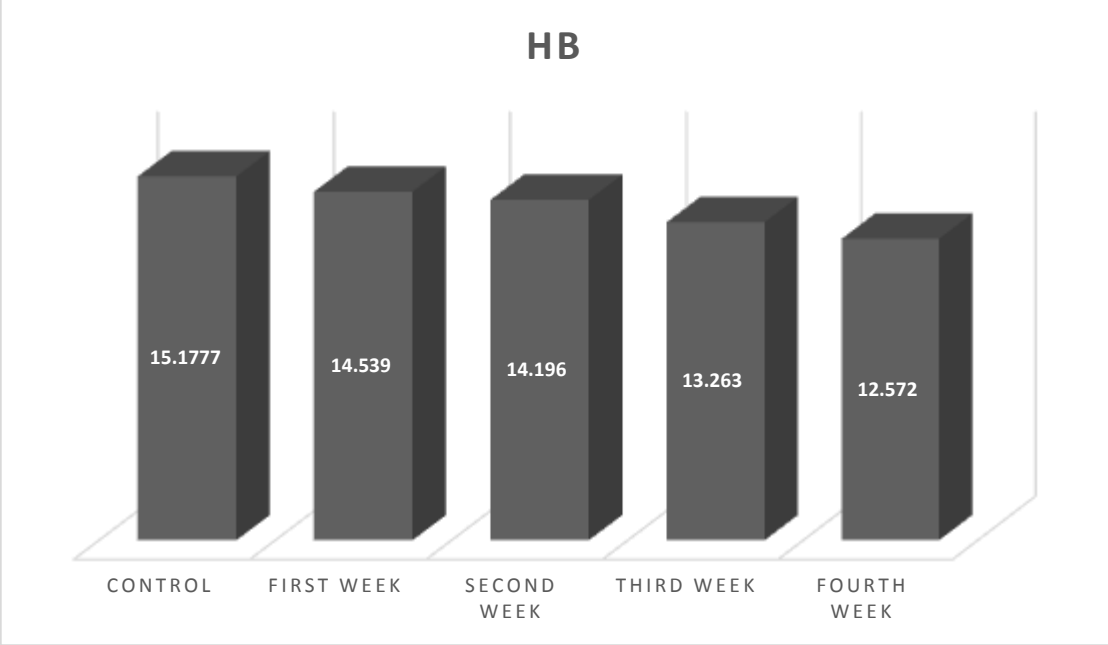


Figure 5.3 Show HB during four weeks

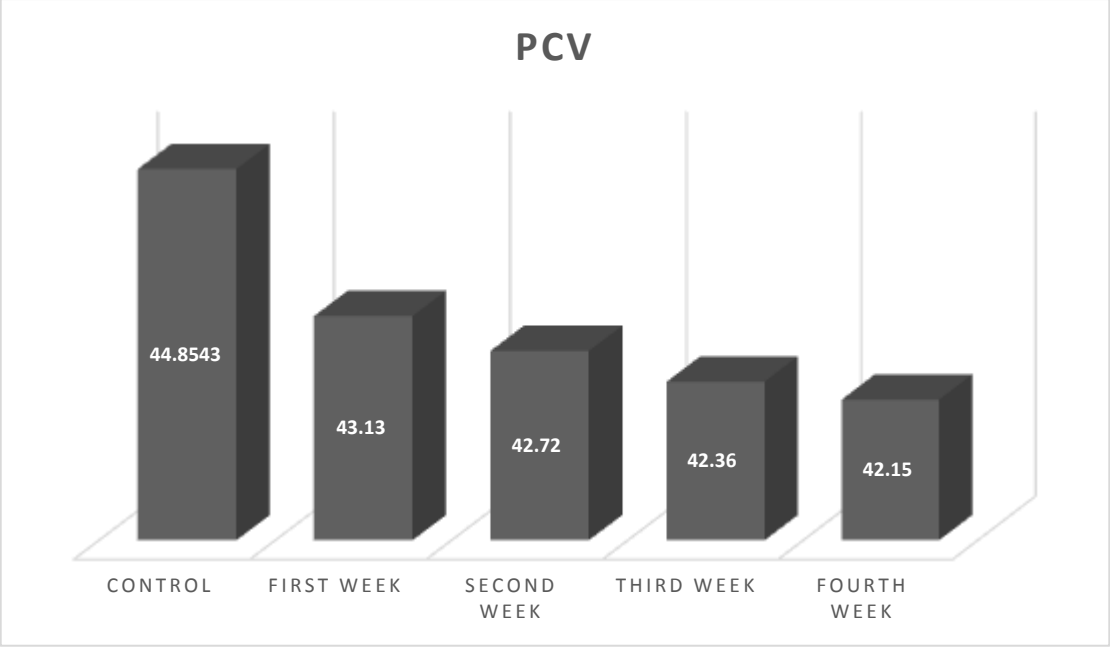


Figure 4.4 Shows PCV during four weeks

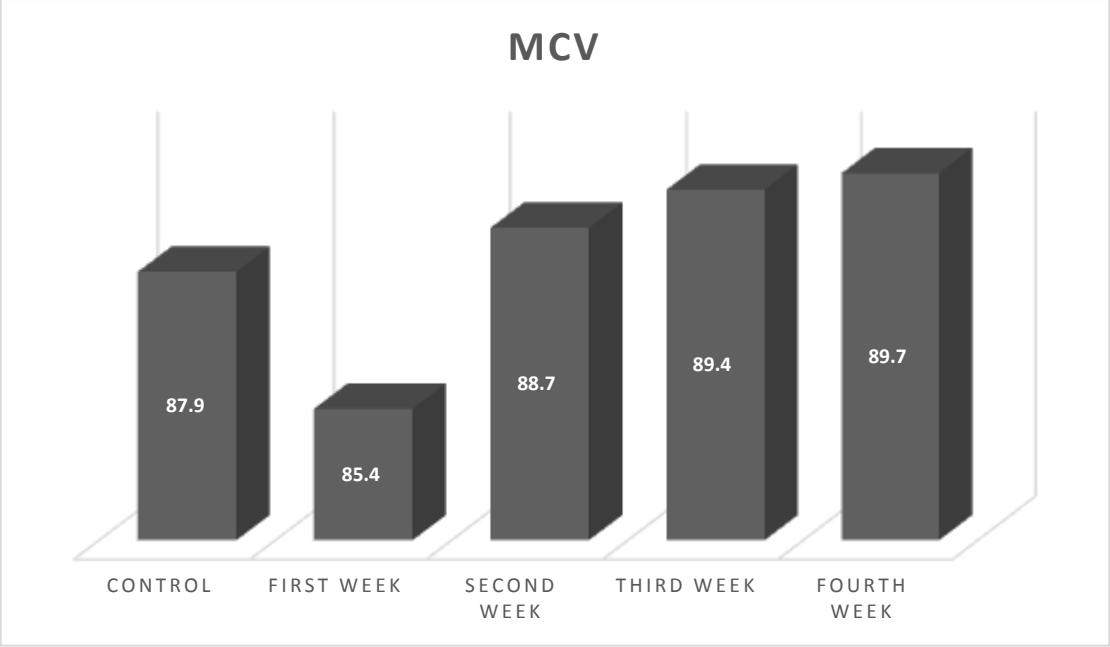


Figure 4. 5 shows MCV during four weeks

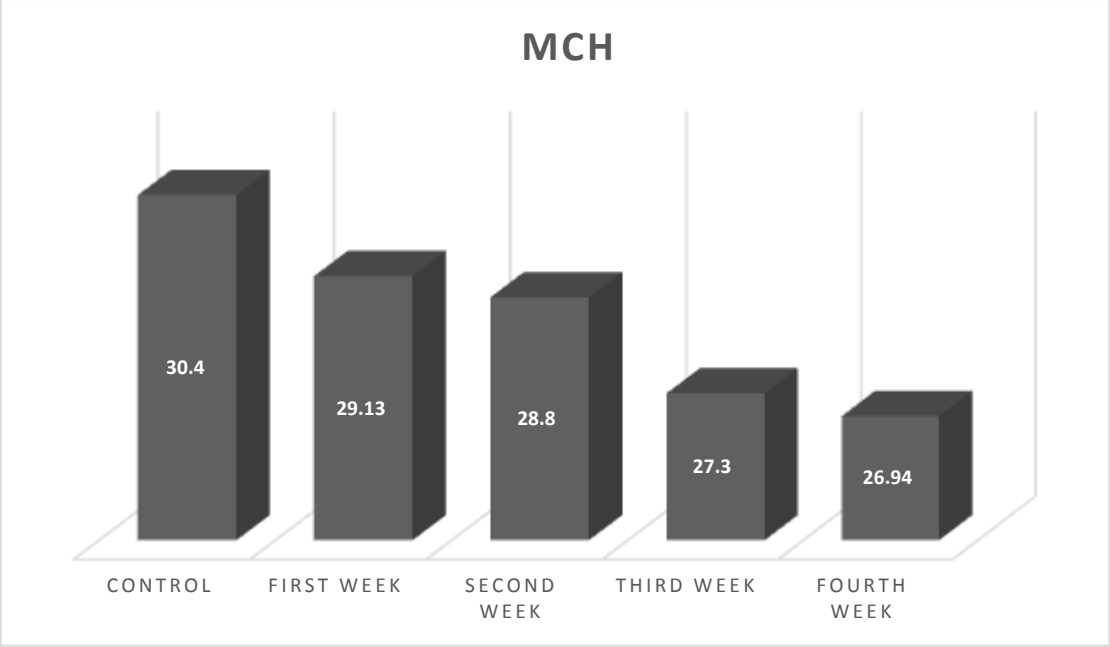


Figure 4.6 Shows MCH during four weeks

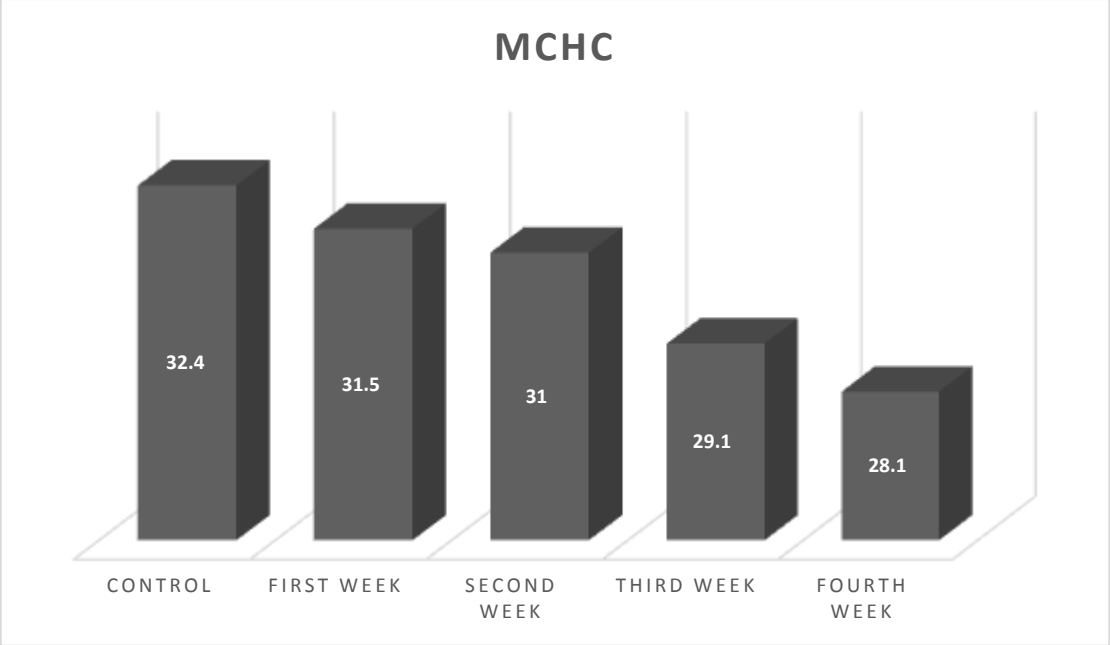


Figure 4.7 shows MCHC during four weeks

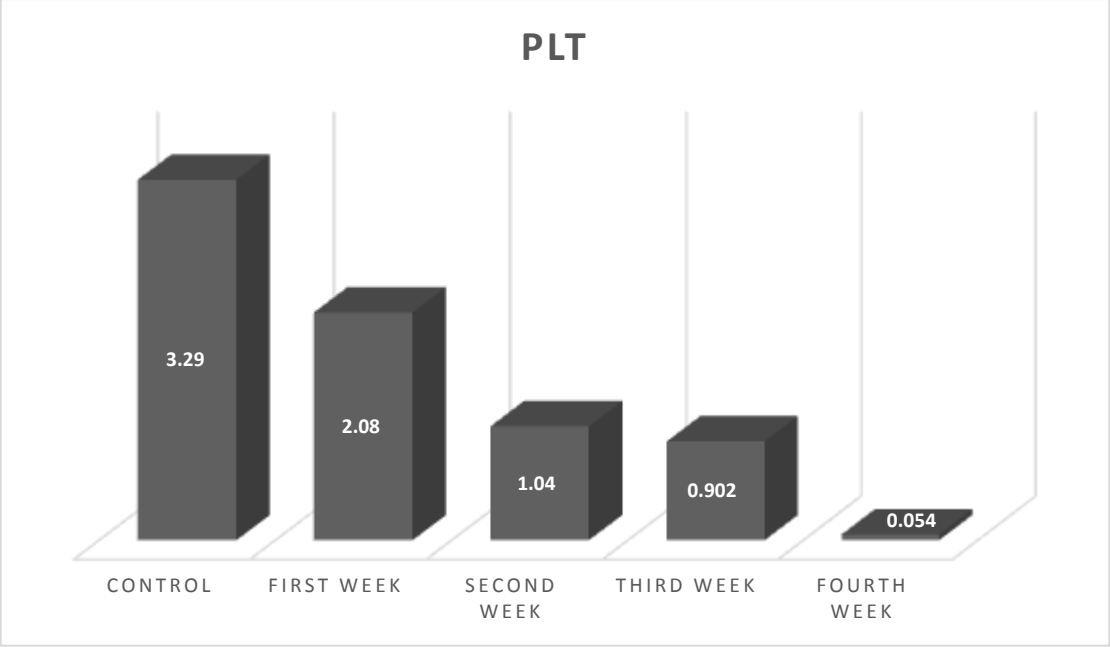


Figure 4.8 shows PLT during four weeks

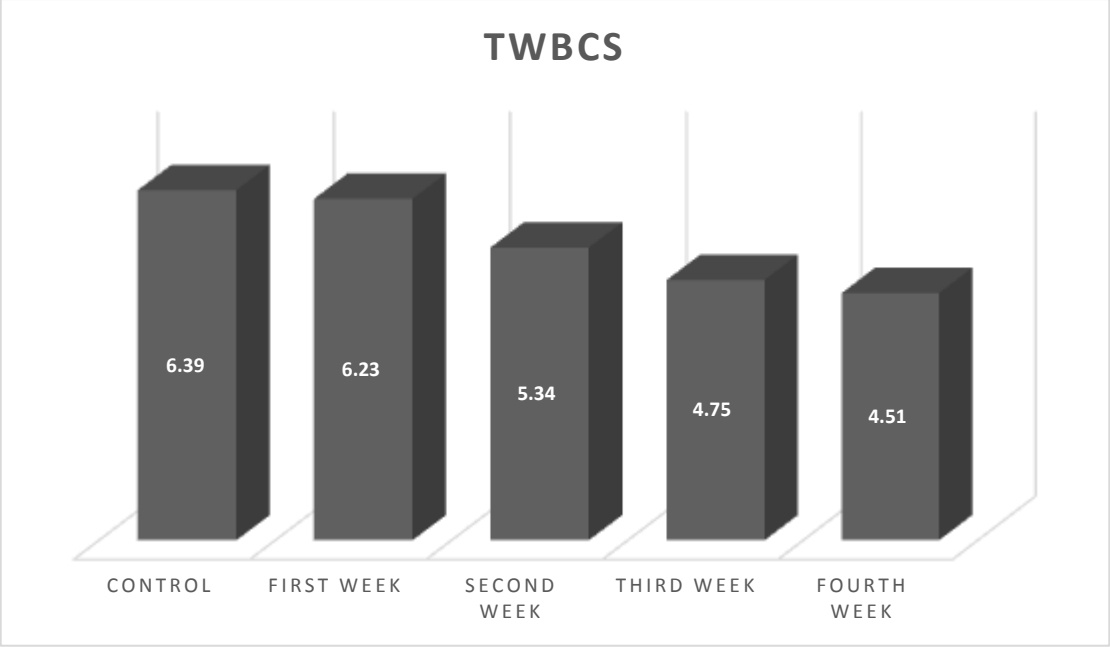


Figure 4.9 shows TWBCS during four weeks

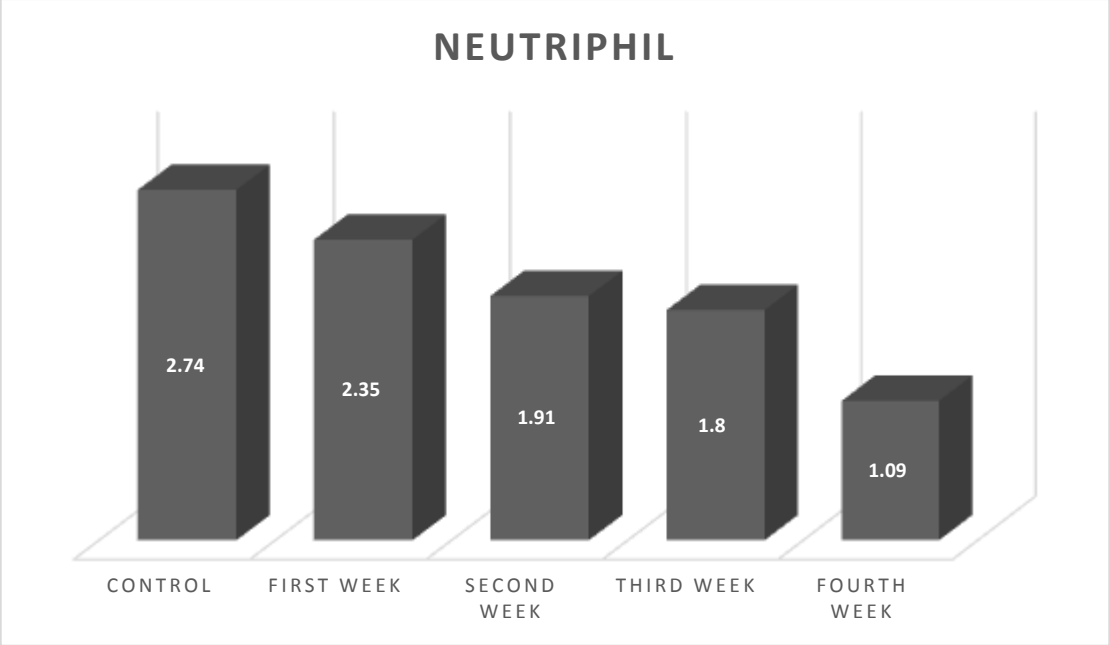


Figure 4.10 shows NEUTRIPHIL during four weeks

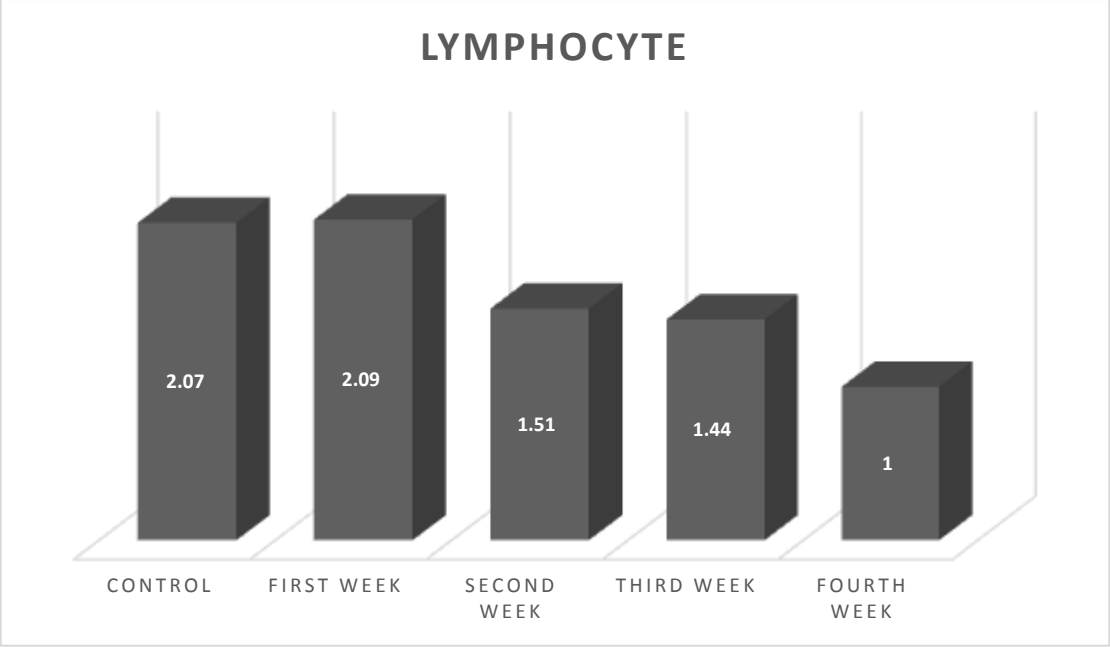


Figure 4.11 shows LYMPHOCYTE during four weeks

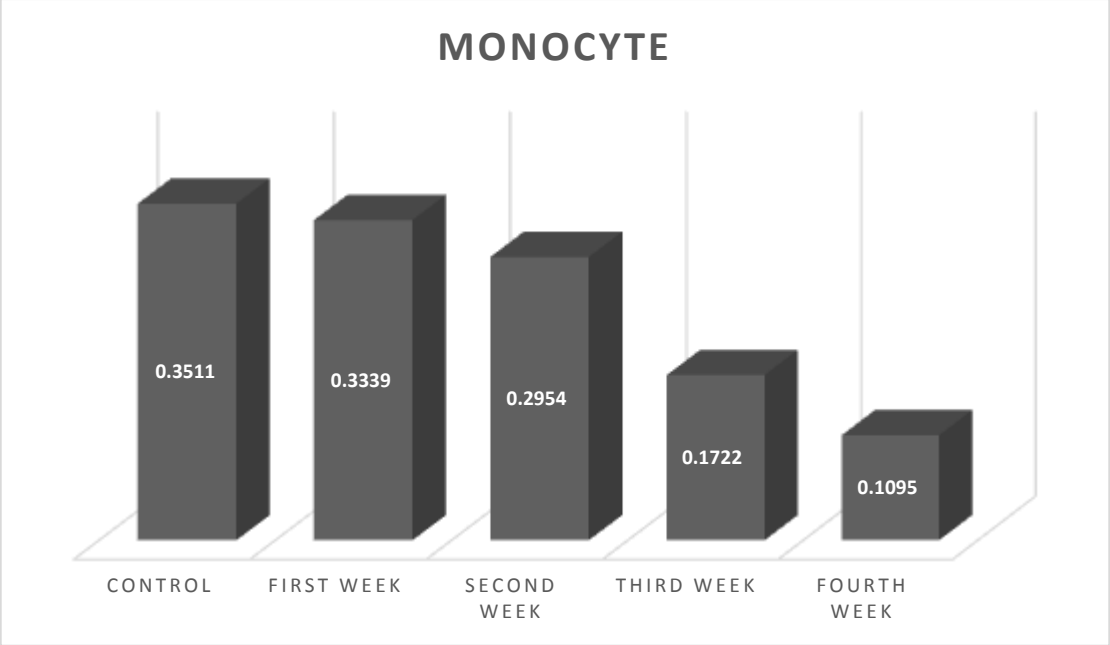


Figure 4.12 shows MONOCYTE during four weeks

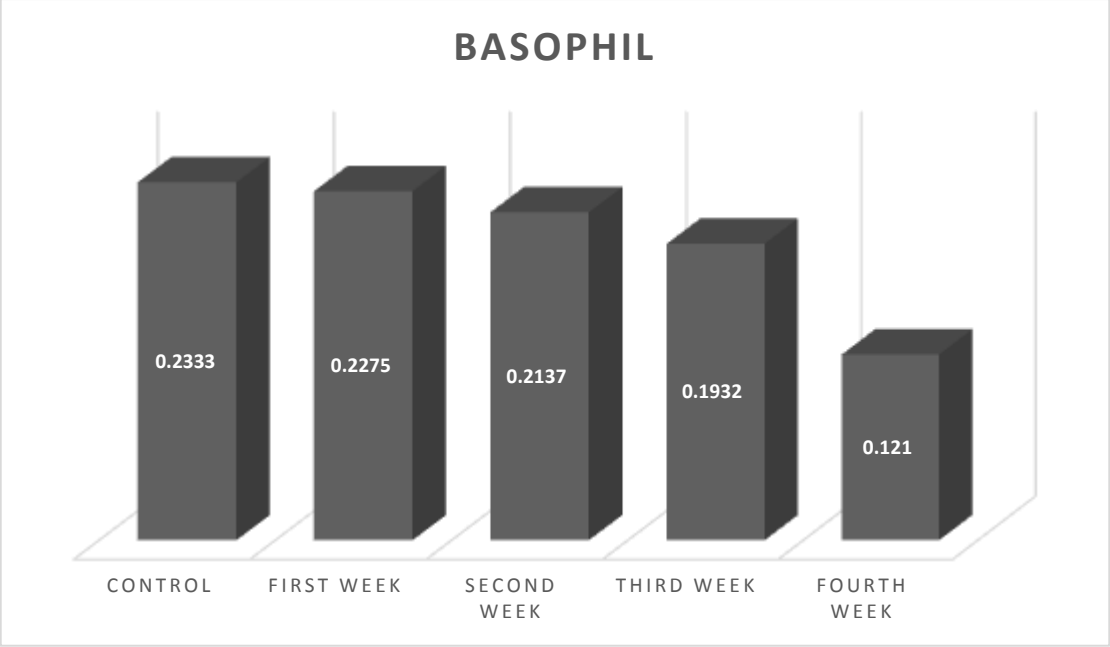


Figure 4.13 shows BASOPHIL during four weeks

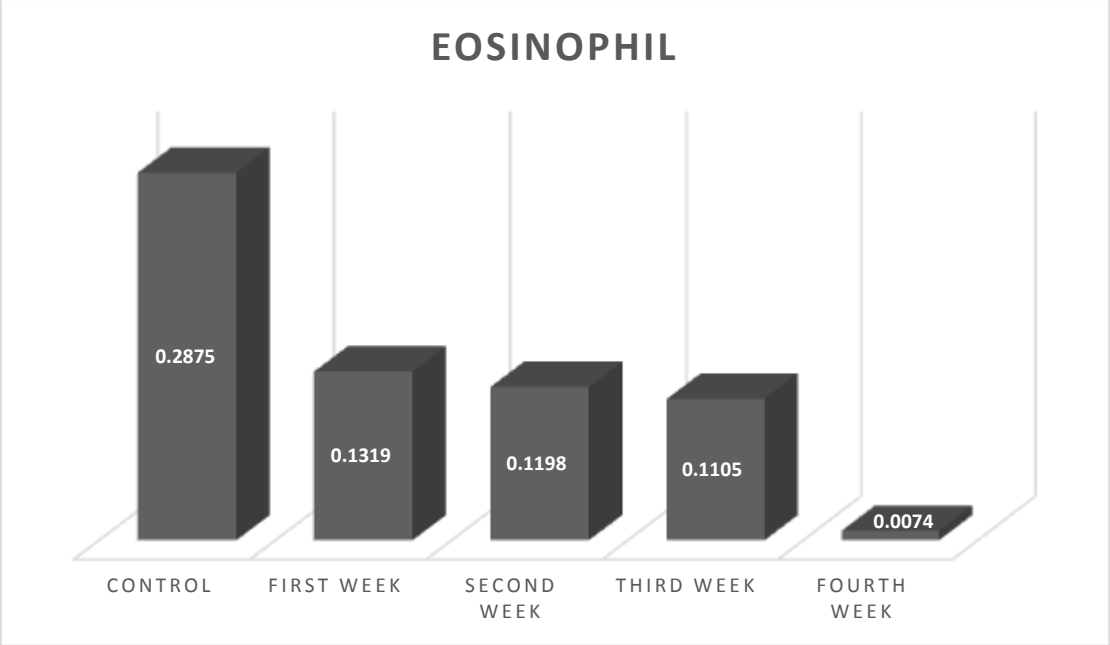


Figure 4.14 shows ESINOPHIL during four weeks

CHAPTER V

Discussion, conclusion and Recommendations

5.1. Discussion

This study was conducted to determine the effect of storage varying periods on some hematological parameters.

The study includes 100 blood samples admitted to central blood bank in Khartoum State. All samples collected from donors who met the specifications required to donate blood. The CBC was performed on it immediately after first collection (day zero). Day zero was considered as the control, and then CBC performed at 1,2,3,4 weeks. The results of each week were compared with the control.

In the first week and the second week, there was insignificant changes in all hematological parameters except for platelets, there was a decrease significantly (mean of plt in first week =2.086 and p value =.005, while mean in second week =1.049 and p value = .001) compared to the control (mean of plt in control = 3.286) showed in tables 5.1 and 5.2,

A study done on the platelets in the central blood bank in Iraq Mosul, and it found:

In fresh whole blood platelets are about 60 % effective at 24 hours and almost completely ineffective after 48 hours (mean of plt in first day =261.3±13.45 and p value = NS, while mean in second day =202.4±9.12 and p value = .01) compared to the control (mean of plt in control = 267.2±13.82)

This rapid disintegration of platelets during storage may be due to a rapid disappearance of the white blood cells; their hydrolytic enzyme the affect the platelet membrane may cause to platelet degeneration.

This is study agree with my results.

It is believed Platelets affected by the hydrolytic enzymes released by the white blood cells which distributes platelets membrane may couse platelets degeneration. Platelets lose viability rapidly on storage in refrigerators, the platelet viability and functions are affected by white blood cells present in stored platelet concentrate. White blood cells have adverse effect on platelets function and post transfusion recovery.

In the third and fourth week significant changes occurred in all hematological parameters showed in tables 5.3 and5.4.

All changes that occurred with a significant decrease, except for the MCV, in which the change occurred with a significant increase (mean in third week =89.946, p value=0.026 while mean in fourth week =90.725, p value =.001 and mean of control 87.846).

Another study was conducted in hematological changes in stored blood in India, showed: Hemoglobin was significantly affected by storage period of blood but PCV. Is more affected than hemoglobin. This could be due to increased MCV (mean of HB in second day =16.50±0.35 and p value = NS, while mean in tenth day =14.72±0.25 and p value = 0.05) compared to the control (mean of HB in control = 16.50 ±0.35), also showed: PCV was significantly affected by storage period of blood but PCV. Is more affected than hemoglobin. This could be due to increased MCV (mean of PCV in second day =44.1±0.79 and p value = NS, while mean in tenth day =42.7±0.86 and p value = 0.01) compared to the control (mean of PCV in control = 44.3 ±0.72)

It is believed due to the swelling of red blood cells resulting from the imbalance of electrolytes.

This is study agree with my results in PCV and Hemoglobin but against in Total white blood cells (mean of WBCs in first day =5.1±0.25 and p value = NS, while mean in second day =4.2 ±0.21 and p value = 0.01) compared to the control (mean of WBCs in control = 5.2 ±0.28)

The significant decrease in hemoglobin concentration and total red blood cells can be attributed to the hemolysis that occurs during storage.

While white blood cells have an abbreviated life span in stored blood and transfusion proved ineffective in elevating the leukocytes count, the many studies showed that during storage the total leukocyte count decreases, they attribute this decrease to degeneration of the granulocytes.

Another study was showed:

In Nigeria, another study was conducted, Blood bags were screened Comparison of day 1 versus day 7 revealed that the granulocytes were drastically reduced, there was significant changes in WBC, differential and absolute leucocytes,

No significant changes were observed in Hb, PCV and other hematological parameters throughout the study.

This is study agree with my results in PCV , Hemoglobin and other hematological parameters but against in Total white blood cells.

5.2. Conclusion

All hematological parameters that evaluated above affected by storage, but platelets are more affected which have significant decrease in Day 7.

5.3. Recommendations

- Due to the presence of significant changes in stored blood that may adversely affect recipients, it is recommended not to store blood for more than 7 days if the PLT is the target.
- Another Anticoagulant also can be used to preserve a blood for long time. Such as additive solutions.
- Blood components (PLT and FFP) are preferable than whole blood.

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Appendix A

رقم ( )

التاريخ...../...../..... الرقم التسلسلي:.....

بيانات شخصية: -

الاسم:...../...../...../.....

النوع: ذكر ☐ انثى ☐

العمر :.....

العنوان:..... رقم الهاتف:.....

هل تعاني من احد الامراض التاليه: -

ارتفاع ضغط الدم : نعم ☐ لا ☐

السكري: نعم ☐ لا ☐

امراض القلب: نعم ☐ لا ☐

زيادة تركيز نسبه الدم: نعم ☐ لا ☐

البرقان نعم ☐ لا ☐ كم كان عمرك /.....

هل قمت بعملية جراحية خلال سته اشهر الماضية : نعم ☐ لا ☐

هل تناولت الاسبرين خلال 72 ساعه نعم ☐ لا ☐

هل تتعاطى اي نوع من الدواء حاليا نعم ☐ لا ☐

هل قمت بالتدخين خلال الساعه الماضية نعم ☐ لا ☐

هل سبق ان نقل لك دم او احد مشتقاته نعم ☐ لا ☐

الفترة الزمنية/.....

هل تعاني من اي مشاكل صحيه اخري نعم ☐ لا ☐

Appendix B

Table (4.1) shows materials used in the study:

Automated Hematological analyzer Sysmex.	EDTA containers (vacotainer)
Plastic pipette	Lishman's stains
Slides	Light microscope