1. Introduction and literature review

1.1 Introduction

Blood plays a very important role in the body. It carries oxygen and important nutrients to organs and tissues.

A transfusion is putting blood or some part of it in a vein through an intravenous line. Transfusions of blood and blood products temporarily replace parts of the blood when a person has-been bleeding, or when their body can't make enough blood. The blood usually comes from another person, called a *donor*. People usually donate whole blood – blood taken right out of a vein through a needle. This whole blood may be called a *unit* or *pint* of blood, and equals about 450 milliliters. Blood has many parts such as red blood cells, white blood cells, platelets, plasma, clotting factors, and small proteins. Each component does a different job (*AABB, 2013*).

Preservation and long term storage of red Blood Cells (RBCs) is needed to ensure a readily available, safe blood supply for transfusion medicine (Koch et al, 2008).

The ability to store blood was first started in the 1915 with the discovery of sodium citrate as blood anticoagulant (Koch *et al*, 2008).

Since then, much progress had been made. currently, blood components can be stored for a prolonged time. The ability to store blood for a long time revolutionized blood transfusion practices and dramatically improved the practice of medicine and surgery. However, storing blood has pathological consequences that are collectively known as the ''storage lesions'' (Bonaventura , 2007).

Blood collection and storage systems licensed by the Food and Drug administration allow red cells to be stored up to 42 days, while the median duration of storage of transfused red cell units in the United State is 15 days (Koch *et al*, 2008).

Some studies have suggested that the risk of complications after transfusion increases when transfused blood has been stored for long periods (Koch *et al,* 2008)**.**

During storage, in fact, preserved blood cells undergo progressive structural and functional changes that may reduce red cell function and viability after transfusion (Koch *et al*, 2008)**.**

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1.2 Literature review

1.2.1 The Blood

The blood is the main transport system in the body. It carries raw materials and finished products from where they originate to where they are used and transports waste products to disposal sites. Some of the contents of the blood are traveling to a specific destination. For example, sugar (glucose) may be going from the liver to muscle to provide a source of energy for movement; coagulation factors may be carried from the liver to a cut blood vessel to ensure clotting. Integral parts of the blood are the red cells, different types of white cells and platelets. Red cells and platelets perform their functions and spend their mature existence entirely within the blood (Fang et *al,* 2005) (Spinelli *et al*,2007).

The blood accounts for about 7 percent of the body weight of a normal adult. This means that a 154-pound person (70-kilogram) has about 10 pints (5 liters) of blood. Smaller adults and children have proportionately smaller blood volumes. blood is composed of plasma and cells suspended in plasma (red cells, platelets and white cells [neutrophils, monocytes, eosinophils, basophiles and lymphocytes]). Plasma is largely made up of water in which many chemicals are dissolved (Fang *et al*, 2005) (Spinelli *et al*, 2007).

1.2.1.1 Blood function

Blood is main transportation vehicle of the body. it carries oxygen and nutrients to tissues and waste products of metabolism, eg.carbon dioxide and urea, to lungs and kidnys.most of the hormones are also carried from the endocrine glands to target organs.blood also have important hemoststic functions exemplified in following. Blood circulation helps to distribute heat around the body from metabolically active and warmer organs,eg liver and gut, to peripheral organs,thereby helping to maintain an even body tempreature.buffer in the blood, like heamoglbin, plasma protins,bicaronate and othrer,help to keep hydrogen concentration of extracellular fluid constant at a pH 7.4. Blood play vital protective function agenist infections by virtue of its leucocytes and antibodies (immnonoglobulins) in the plasma. Furthermore, injury to blood vessels is followed by blood clotting, which stops further loss of this vital fluid (Sukkar *et al* ,2000).

1.2.1.2 Blood composition

Blood consist of:

1-Formed elements (blood cells) of three types

a – Red cells – erythrocytes

b – White cells – leucocytes

c – Platelets – thrmbocytes

2 – Plasma (Sukkar *et al* ,2000).

1.2.2 Hemoglobin

Oxygen and some carbon dioxide transport depends almost totally on the presence of the red respiratory pigment hemoglonin in erythrocytes hemoglobin increases the ability of the blood to carry oxygen by 60-fold. plasma carries 20ml/100ml. This vital function makes hemoglobin one of the most important constituents of higher organisms.

1.2.2.1 Types of normal Hemoglobin

Adult Hemoglobin (Hb A)

Hemoglobin A2 (Hb A2)

Hemoglobin F (Hb F) this is the major respiratory pigment during intrauterine life (Sukkar *et al* ,2000).

1.2.3 Blood transfusion

A transfusion is putting blood or some part of it in a vein through an intravenous line (*AABB,* 2013).

1.2.3.1 Historical aspects of blood transfusion

In 1942, blood was taken from three young men and given to stricken pope innocent V11 in the hope of curing him; unfortunately, all four died. It is the first time a blood transfusion was recorded in history (*DeniseM, 2005*).

Clotting was the principal obstacle to overcome. Attempts to find nontoxic anticoagulant began in 1869; wen Braxton Hicks recommended sodium phosphate (*Denise, 2005*).

Edward E.linemann was the first to sccceed.he carried out vein-to vein transfusion of blood by using multiple syringes and a special cannula for puncturing the vein through the skin (*Denise, 2005*).

An unprecedented accomplishment in blood transfusion was achieved in 1914, when Hustin reported the use of sodium citrate as anticoagulant sodium for transfusions. Later, in 1915, Lewisohn determined the minimum amount of citrate needed for anticoagulation and demonstrated its nontoxicity in small amount. Transfusions became more practical and safer for the patient (*Denise, 2005*).

Rous and Turner in 1916 indicated that when Glucose added to citrate it would improve the preservation of citrate (Rous, 1916).
on that, the first blood bank was established and Opened in Chicago in 1937. Later on, Molison in 1943 improved blood Transfusion by adding acid citrate Dextrose anticoagulant (ACD) mixed with preservative solution that made the storage of whole blood possible up to 21 days (*Mollison et al*,1954).

Gibson introduced a new improved preservative solution in 1957 containing citrate, phosphate, dextrose (CPD) that increases the storage age up to 28 days (Gibson Metal,1957).This storage solution was modified in USA in 1978 by incorporating adenine to citrate phosphate-dextrose and called CPDA1,which is, regarded the latest blood Preservative. It increases blood storage Time up to 35 days and it is nowadays in every blood bank in the world (Aubuchon *et al*,1997).

1.2.3.2 Types of blood transfusion
Whole blood transfusion and blood components
Whole blood is rarely used for transfusion anymore, and only the components that are specifically required by the patient are used. The collected blood is therefore fractionated into its respective components by centrifugation to produce packed red blood cells, plasma, platelets, albumin, clotting Factor concentrates, cryoprecipitate, fibrinogen concentrate, and immunoglobulins (Kinnear, 2011).

Packed Red cells
A bag of RBCs have a hematocrit of between 60-70%, and an average shelf life of 35 days if properly stored. Their function is to act as carriers of oxygen and carbon dioxide in the blood. The main indications for transfusion are the correction of anemia or replacement in acute

hemorrhage, but there is no absolute level of Hb to trigger a transfusion. asingle unit of red blood cells will typically increase the Hb by 1g/dl (Kinnear, 2011).

White Blood cells (granulocytes)

Are collected by apheresis (filtration), and can only be stored for 24 hours at 20-24°C. They need to be cross-matched because they contain large numbers of RBCs,and also irradiated to remove the lymphocytes. they are indicated for life-threatening infections in neutropenic cancer patients who are unresponsive to antibiotics (John Kinnear, 2011).

Platelets

A unit of platelets is prepared from a single whole blood collection and contains at leastnThey are stored at 20-24°C under agitation and have a shelf life of 5 days. Each unit can raise the platelet count by 5-10 x 1 (John Kinnear, 2011).

Fresh Frozen Plasma

Is collected as the supernatant after centrifuging a donation of whole blood.It is frozen within 8 hours to maintain the activity of factors V and VII (John Kinnear, 2011) .

Cryoprecipitate

Is made by thawing frozen FFP at 1-6°C, and is indicated for von willebrand's Disease and severe hypofibrinaemia (John Kinnear, 2011).

Albumin

Is available as a 5% and 25% solution.

Immunoglobulin

Specific immunoglobulins can be given to treat specific infections, e.g. chicken pox and Hepatitis B. They are also given for certain immune-related diseases such as Guillain-Barre syndrome (John Kinnear, 2011).

Coagulation factor concentrates

Specific factors can be transfused individually to treat specific factor deficiencies. These include Antithrombin III, Factor VIII, Factor IX, Factor XIII, and Protein C. All of these are prepared from human plasma **(**John Kinnear, 2011**).**

1.2.3.3 Types of blood donations

Volunteer whole blood donation

Most blood donations come as units of whole blood from volunteers who have no connection to the person who will get the blood (*AABB, 2013*).

Autologous blood donation

Donating your own blood for later use is called *autologous* donation. autologous donation is most often done in the weeks before you have a scheduled surgery that will likely require blood transfusion. Your own blood can then be used during or after the operation to replace any blood you may have lost (*AABB, 2013*).

Directed donation

Donating blood for a family member, friend, or other specified patient is called directed donation.

This can be done at any blood donation center, but you should call ahead to check requirements and schedule the donation. The donor must meet the same requirements as for regular blood

Donation and the donor's blood must match the blood type of the recipient (*AABB, 2013*).

Paid donation

Plasma is the only component for which donors are sometimes paid, and it's taken by the apheresis method. Plasma can be treated for safety in ways that blood cells cannot. Plasma taken from paid donors is generally treated and processed by pharmaceutical companies into drugs. It cannot be transfused in the form of cryoprecipitate or fresh frozen plasma (*AABB, 2013*).

1.2.3.4 Donor testing

There are risks associated with both donating and receiving blood, but these can be minimised by good practice. Donors have their current health assessed by the Donor Health Check (DHC), which includes history and examination (temperature, pulse and blood pressure) to make sure that donating will not be a hazard to their health. Hematocrit or hemoglobin level is also assessed since risk of anemia is the most common reason for ineligibility. To ensure recipient safety the donor is also screened forealth risks that might make the donation unsafe. Donors are examined for risk and signs of Transmissible diseases such as HIV, malaria, viral hepatitis and variant Creutzfeldt-Jacob Disease (vCJD)

The blood that is collected is identified for blood type (ABO and Rh), but is also tested to minimize the risk of transmissible infections. The World health Organization recommends four core tests as a

minimum, which are

Hepatitis B Surface Antigen

Antibody to Hepatitis C

Antibody to HIV, usually subtypes 1 and 2

Serology for Syphilis

(John Kinnear, 2011).

1.2.3.5 Storage

Short-term storage of blood relies on a combination of refrigeration and the addition of preservatives.

The standard temperature used is 1°- 6°C, but the preservatives may vary. When using acid-citrate dextrose (ACD), citrate-phosphate-dextrose (CPD) or citrate-phosphate-double dextrose (CP2D), the storage is limited to 21 days, but with citrate-phosphate-dextrose-adenine (CPDA1) this can be extended to 35 days.

red blood cells can be stored for much longer periods, even up to 10 years, by freezing cryopreservation (Kinnear,2011).

1.2.3.6 Blood bag

Blood bag of 450 ml ± 10% (Agarry) which contains CPDA-1 was used. citrate phosphate dextrose adenine solution was developed in 1968 and shown to permit whole-blood storage for 5 weeks (Shields CE; 1969)

the citrate prevents coagulation by binding or chelating to calcium, phosphate acts as a buffer hence, maintains the pH of the blood. Dextrose serves as substrate for the blood cells, while adenine maintains high ATP level in the RBC. Most blood collection bags (adult) contain 63 ml CPDA anticoagulant which is sufficient to anticoagulate and ensure the viability of blood cells in 450 ml ± 10% blood for up to 28-35 days when the blood is stored at 2-8°C (Monica ,2000).

1.2.3.7 Blood groups

Blood groups are inherited and are determined by the antigens carried on the surface of red blood cells.
there are as many as 30 different blood groups, but the main ones are the ABO and rhesus groups, and (Kinnear,2011).

1.2.3.8 Lesions on stored blood

Despite refrigeration and the addition of preservatives, the quality of donated red blood cells
deteriorate over time as predictable cellular and biochemical changes occur that result in the 'storage
lesion' of banked blood(John Kinnear;2011).

Lesions on stored blood can be occur in:
1-Red blood cells

Red cells that are drawn at the beginning of donation are subjected to an acidic and hypotonic anticoagulant solution, which damages a small proportion of them irreversibly. Cells that survive the first 24 hours will remain viable for the rest of their expected life span, but during storage they depend on anaerobic glycolysis for energy production and viability. Their shelf life requires that at least 70% of them remain viable in the recipient's circulation 24 hours after transfusion (Kinnear, 2011).

Other cellular elements granulocytes become non-functional after 24 hours of storage, but still retain antigenic potential toncause febrile reactions. Some lymphocytes may remain viable for several weeks. Platelet function declines to zero after only 48 hours of storage (Kinnear,2011).

2- Oxygen affinity

Stored blood has depleted levels of 2, 3 Diphosphoglycerate (DPG), which causes the oxygen dissociation curve to shift to the left, so increasing affinity of Hb for O2 (less O2 release to tissues).

the 2, 3 DPG levels rise rapidly following transfusion and normal oxygenaffinity is usually restored Within a few hours (Kinnear,2011).

3-Coagulation

Stored blood contains an anticoagulant, commonly in the form of citrate, but this usually does not affect recipient coagulation status. the labile coagulation factors V and VIII have a 50% decline in activity within the first 72 hours of storage, but all other factors remain normal (Kinnear,2011).

4-Temperature

The optimum storage temperature of 2°- 6°C is well below normal body temperature (Kinnear, 2011).

5-Potassium

During storage there is a constant leak of potassium out of cells, and levels may sometimes

5-Exceed 30mmol/l. This is usually not a problem since the potassium rapidly re-enters red cells as they

Begin active metabolism after transfusion (Kinnear, 2011).

6-Calcium

Each unit of blood contains approximately 3g citrate, which binds ionized calcium. However, the liver is able to metabolize 3g of citrate every 5 minutes, so that only when transfusion rates are greater than one unit per five minutes or in the presence of impaired liver function is there a risk of Citrate toxicity and hypocalcaemia (Kinnear,2011).

7-Acid – Base

During storage there is a gradual accumulation of lactic acid which can result in an acid load of 30-40mmol/l and a resultant fall in pH. However, this and the citric acid load is usually metabolized rapidly. Because the

citrate is metabolized to bicarbonate there can be a paradoxical metabolic alkalosis associated with transfusion (Kinnear, 2011).

1.2.4 Automated blood count techniques

A variety of instruments for performing blood counts are in wide spread use. Automated instruments usually have a high level of precision for cell counting and cell-size techniques. If instruments are carefully calibrated and their correct operation is ensured by quality control procedures, they produce test results that are generally accurate (lewies *et al*, 2006).

1.2.4.1 Hematological parameters

Hemoglobin concentration most automated counters measure Hemoglobin by a modification of manual HiCN method with cyanide reagentor with a nonhazardous chemical such as sodium lauryl sulphate, which avoids possible environmental hazards from disposal of large volumes of cyanide-containing waste .modifications include altration in the concentration of reagents and temperature and Ph of Reaction. Anonipnic detergent is included to ensure rapid cell lysis and to reduce turbidity caused by cell membrane and plasma lipids.measurements of absorbance are made at set time interval after mixing of blood and active reagents but before the reaction is completed (lewies etal;2006).

1.2.4.2 Red blood cells count

Red cells and other blood cells can be counted in the systems based on either aperture impedance or light scattering technology .because large numbers of cells can be counted rapidly,threre is a high level of precision.consequently, elctronic counts have rendered the RBC and red cells indices derived from it (the MCV and the MCH) of much greter clinical relevance than was possible when only a slow and imprecise manual RBC was available(lewies et al,2006).

1.2.4.3 Packed cell volume and mean cell volume

Modern automated blood cell counters estimate PCV by technology that has little concentration with packing red cells by centerfugation.it is sometimes convenient to use different terms to distinguish the manual and automated tests, and for this reason the international council for

standerdization in hematology has suggested that the term Hematocrit (Hct) rather than PCV should be used for the automated measurement (lewies et al, 2006).

With automated instruments,the derivation of the RBC, PCV, MCV are closley interrelated.the passage of a cell through the aperture of an impedance counter or through the beam of light of a light-scattering instrument leads to the generation of electric pulse the height of which is proportional to cell volume. Pulse height analysis allows either the MCV or the PCV to be determined. If the average pulse height is computed, this indicative of the MCV and the PCV can be derived by multiplying the estimated MCV by the RRC. Similarly, if the pulse heights are summated, this figure is indicative of the PCV, and the MCV can, in turn, be derived by dividing the PCV by RBC (lewies et al, 2006).

1.2.4.4 Red cell indices
Red cell indices traditionally have been the derived parameters of MCV, MCH, and MCHC; more recently, red cell distribution width (RDW) has also been included and, for some instruments, hemoglobin distribution width (HDW) ((lewies et al, 2006).

1.2.4.5 Mean cell volume
In automated systems, MCV is measured directly, but in semi automated counters MCV is calculated by dividing the PCV by RBC ((lewies et al, 2006).
Mean cell Hemoglobin and mean cell Hemoglobin concentration
MCH is derived from the Hb derived by RBC ((lewies et al, 2006).
MCHC is derived n the traditional manner from the Hb and the PCV with instruments that measure the PCV and calculate the MCV, whereas when the MCV s measured directly and PCV is calculated, the MCHC is derived from the Hb,MCV, and RBC according to the following formula

$$\text{MCHC (g/l)} = \frac{Hb\ (g/l) \times 1000}{MCV\ (fl) \times RBC/L\ \times 10^{12}}$$

1.2.4.6 Total white blood cell count
The total WBC is determined in whole blood in which red cells have been lysed.the lytic agent is required to destroy the red cells and reduce

the red cells stroma to residue that causes no detectable response in the counting system without affecting leucosytes in such a manner that the ability of the system to count them is altered.various manufacturers recommend specific reagents, and for mutichannel instruments that also perform an automated differential count use of the recommended reagent is essential. (lewies et al,2006)

Relatively simple instruments are also available that determine the Hb and the WBC by consecutive measurements on the one blood sample ((lewies et al, 2006)

Fully automated multichannel instruments perform WBCs by impedance or light scattering technology or both ((lewies et al, 2006).

1.2.4.7 Platelet count

Platelets can be counted in whole blood using the same techniques of electrical or electro-optical detection as are used for counting red cells. an upper threshold is needed to separate platelets from red cells, and lower threshold is needed to separate platelets from debris and electric noise. Recirculation of red cells near the aperture should be prevented, as pulses produced may stimulate those generated by platelets. Three techniques for setting thresholds have been used: (a) platelets can be counted between two fixed thresholds; (b) pulses between fixed thresholds can be counted with subsequent fitting of a curve and extrapolation so that platelets falling outside the fixed thresholds are included in the computed count; and (c) thresholds can very automatically, depending on the characteristics of individual blood samples, to make allowance for microcytic or fragmented red cells or for giant platelets. Anew method for platelet counting by flow cytometry has been developed (lewies et al, 2006).

1.2.5 Effect of storage on blood

The goal of blood preservation is to provide viable and functional blood components for patients needing blood transfusion (Aubuchon *et al*, 1997).

Viability is a measure of in vivo red blood cells survival following blood transfusion (Aubuchon *et al*, 1997).

Decreased RBC viability is correlated with lesion of storage that is associated with various biochemical changes including decreased pH, a

buildup of lactic acid, a decrease in glucose consumption, a decrease in adenosine triphosphate levels and a loss of RBC functions (Majr E, 1999).

Rossmussen 1961 found that there is some loss of the erythrocyte during storage when stored in ACD solution. He found that the post transfusion survival virtually is 100%then this decline by slightly more than 1% per day to reach about 90% in about one week of storage and about 70% in three weeks of storage. This means that Hb concentration remains declining by increased period of storage (Rossmussen , 1997).

Approximately all of the Hb in the blood contained with the RBC and only minute amount of less than 0.025 g/L of Hb normally is released into the plasma due to destruction of erythrocyte Hb. It is believed that it may occurs as a result of transfusion reaction (Cumming *et al*, 1997) (Donna *et al,* 2003) .

The red cell volume is maintained and regulated by the bone marrow, which under steady state condition is accurately replace the red cell loss (Telen et *al,* 1996).

The hematocrit is the simplest and most widely used test by which it is possible to Estimate the size of the red cell volume (Jaffe, 1981).

Storage has a negative effect on RBC oxygen delivery (Bonaventura, 2007).

Current research indicated that the RBC hypothermic storage lesion is responsible for the association of blood transfusion with an increased length of stay in the hospital, impaired tissue oxygen use, pro-inflammatory and immunomodulatory effects, increased infections, multiple organ system failure, and ultimately increased morbidity and mortality (Bennett–Guerrero *et al*,1990).

Clinical implications, collectively known as the RBC storage medium lesion, is in part related to bioreactive substances released by leucocytes in the storage medium, such as histamine, lipids, and cytokines, which may exert direct effect on metabolic and physical changes associated with the senescence, such as membrane reticulation, decrease in cell size, increase of cell density, alteration of cytoskeleton, enzymatic desilylation, and phosphatidylserine exposure, RBCs lose potassium 2,3-diphosphoglycerate (2, 3-DPG), Adenosine Triphosphate (ATP) stores,

lipids and membrane, while becoming more rigid and demonstrating reduced oxygen off-loading (Hess, 2006).

Moreover, stored units become more acidotic and the suspending fluid has higher concentrations of free hemoglobin and biologically active lipids, and contains greater quantities of negatively charged microvesicles with proinflammatory and procoagulant activity (Hess, 2006).
Platelets circulate longer when stored at room temperature and are more activated and able to form clot more effectively when stored at 4°C (Bruce-Chwatt LJ 1972).
White cells lose their phagocytic property within 4-6 hrs of collection and become non-functional after 24 hrs of storage (Bruce-Chwatt 1972, Schubert et *al,* 2008).

The aim of this work is to study the effects of storage on blood Using CPDA1 solution as preservative by measuring several hematochemical parameters at 5 storage periods (Jaffe, 1981).

1.3 Justification

Blood transfusions are very common. Each year millions of people in Sudan need blood transfusion. Blood transfusions are given to replace blood lost during surgery or serious injury or when person's body does not produce enough blood due to certain diseases.

Elnehoud Teaching Hospital is one of large hospitals in western kodofan state and it was receive many of patients. Most of patient admitted to this hospital may require blood transfusion.

As a majority of blood banks in Sudan still practice whole blood banking; Elnehoud teaching hospital related blood bank is whole blood transfusion practice dependant.

The changes associated with the storage of blood in Elnehoud Teaching Hospital blood bank have not been reported.

I will study in this side for providing a new and local data to aid in a good patient health by good transfusion treatment.

1.4 Previous studies

Ahmed *et al,* were studied Effect of storage on certain hematological parameters at different periods of time (at 7 storage periods)with intervals of 3days,1,2,3,4,and 5 weeks was started from day zero , using CPDA1 solution as preservative. Fifty blood donors who were include. The blood samples were analyzed for hemoglobin (Hb), packed cell volume percentage (PCV %), and other parameters. The results of this study showed that there was a significant decrease (P<0.05) in Hb, packed cell volume (Ahmed *et al, 2008*).

Teddy *et al,* also were studied storage related hematological and biochemical changes of CPDA-1 whole blood in a resource limited setting and were find that there is no significant changes were observed in Hb, PCV and other hematological parameters throughout the study(Teddy *et al,* 2012).

1.5 Objectives

1.5.1 General objectives

To assess the storage duration effect on some hematological parameters of CPDA-1 whole blood in blood bank.

1.5.2 Specific objectives

1- To determine effect of storage duration on Hb, RBCs count, Hct and RBCs indices.

 2- To determine effect of storage duration on white blood cells count.

3- To determine effect of storage duration on platelets count.

2. Materials and Methods

2.1 Study design

This is a prospective cross sectional study designed to detect effect of storage duration on some hematological parameters at Elnehoud Teaching Hospital Blood Bank in Western Kordofan State.

2.2 Study population

 Volunteer donors.

2.3 Inclusion criteria

Volunteer donors satisfy the blood donation requirement were included in study.

2.4 Exclusion criteria

Volunteer donors do not satisfy the blood donation requirements were excluded from study.

2.5 Study area

Elnhoud Teaching Hospital Blood Bank in Western kordofan state

2.6 Sampling

2.6.1 Sample size: 50 volunteer donors

2.6.2 Sampling technique: simple random sample.

2.7 Data collection

- Questionnaires were used for collection of donor age and blood group

- List of variables for laboratory results

2.8 Methodology

2.8.1 Blood cell counts and indices

Blood cell counts and indices were determined by sysmex (KX-21) company-country to determine the following hematological parameters:

1 – Hb g/dl

2- HCT%

3- RBCs count $\times 10^6/\mu l$

4- WBCs count $\times 10^3 /\mu l$

5- plts count $\times 10^3/\mu l$.

6- Red cell indices:

A – Mean corpuscular volume (MCV fL)

b- Mean corpuscular Hemoglobin (MCH pg)

c- Mean corpuscular Hemoglobin concentration (MCHC g/dl)

2.8.2 Principle

There are two transducer chambers one used to count WBCs and Hb together and other used to count RBCs and platelets.

Apportion of blood is separated aspirated whole blood and mixed with diluent in pre-rest ratio, a defined amount of this dilution is sent to detection chamber and passed through a small opening known as aperture. There are also electrodes on each side of aperture – and direct current pass through these electrodes. The direct current resistances between the electrodes changes as the blood suspension pass through aperture. This resistance causes an electrical pulse change proportional to the size of blood cell. These electrical data are converted into graphical displays of volume distribution curves, or histograms.

2.8.3 Procedures

1 – The instrument was checked up for the sufficient of the solutions (all pack stromatolyser), also checked electric power supply machine has full battery and earthed connected then power key was pressed on.

2- Sample was mixed well and entered to probe then the start switch was pressed, when LCD screen was displayed analyzing the sample removed. 30sec and then the results were printed out.

2.8.4 Quality control

Quality control is of great importance for obtaining highly reliable data over a long period of time as is the consistent monitoring of instrument for preventing troubles for detection of problem. Quality Control checks are performed to monitor an instrument's performance over time.

Before sample analyzed control blood was used, control blood was analyzed twice and the mean of the two is used to evaluate analyzer performance.

EIGHTCHECK-3WP is the quality control material this product was run every 8 hours of operation and after component replacement or after a service call.

2.8.5 Material required

Blood analyzer

50 CPDA-1 blood bags

EDTA blood containers

ICT for HIV, HBsAg, HCV, and TP for donors blood screening.

Small test tubes

Syringes

2.8.6 Sample collection

Blood collection was performed as described by Monica (2000). Blood was collected from each of the donors with care and adequate safety precautions to avoid contamination and infection from blood transmissible pathogens, protective gloves were worn during collection, syringes were sterile and blood collected materials were discarded safely to avoid injury from needles. 2ml of blood was collected from each blood bag and was added to 0.5ml dipotassium EDTA anticoagulant after labeling, the sample was mixed properly and then analyzed for hematological parameters.

2.9 Data analysis

By computerized SPSS program version 11(t.test).

2.10 Ethical consideration

Ethical Approval from university Ethical committee was obtained.

Permission from Hospital targeted for study also was obtained.

Data were obtained with high confidentiality and sure that data were used for research purposes only.

3. Result

The mean age of volunteer donors (30.9 ±7.9 years) and frequency of blood groups (9, 6, 32, 3 times) for blood groups A+ve, B+ve, O+ve,-Ove respectively figure (1).

Hemoglobin levels were showed significant changes at day 7,15,21 and 28 when compared with the level on day zero(day of collection) with highest mean value 12.1g/dl(day zero),lowest mean value 10.3g/dl (at day 28) with P.value (≤ 0.05) table(1)

Highest mean value of RBCs counts $4.60 \times 10^6/\mu$ (day zero) with lowest value $3.93 \times 10^6/\mu$ (day 28). Significant decrease was showed when day 7, 15 21 and 28 were compared with day zero P.value (≤ 0.05) table (1).

Hematocrit % was showed significant decrease at day 7, 15, 21 and 28 when compared with day zero P.value ($\leq$0.05), highest mean value (39.6%) and lowest value (33.0 %) table(1).

MCV was also showed significant decrease during day 7, 15, 21, and day 28 when compared with day zero, with highest value (85.1fL) and lowest value (83.7fL), P.value ($\leq$ 0.05) table (2).

Highest mean value of MCH, 26.7pg (at day zero) and lowest value 25.6pg (at day 28).significant decrease was showed during day 7 and other durations (15,21and 28) when compared with day of collection, P.value ($\leq$0.05) table (2).

MCHC was showed significant change during first, second, third and fourth week duration when compared with zero duration with (31.5g/dL) was highest value and (30.0g/dl) was lowest value, P.value ($\leq$0.05) table(2).

White blood cells count was showed significant decrease during day 7 and other three durations (15, 21 and 28) when compared with day zero as stander, highest value of count $4.77\times 10^3/\mu l$ (at day zero), lowest value $2.87\times 10^3/\mu l$(at day 21) with P.value ($\leq$0.05) table(3).

Platelets count also was showed significant decrease during 7, 15, 21 and 28 days of duration when compared with day zero, P.value ($\leq$0.05),

highest value $(215.2 \times 10^3 / \mu l$) and lowest value $(104.8 \times 10^3 / \mu l$) table(3).
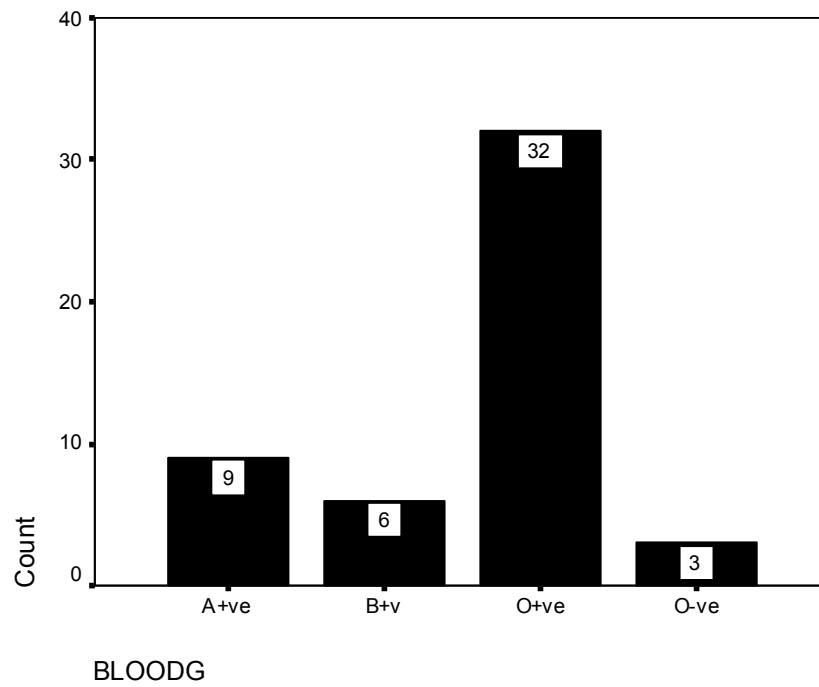
Figure (1) showing frequency of donors' blood groups

BLOODG

Table (1) showing (Hb,RBCs and HCT% at the five   durations

		Parameter mean ±SD and P.value					
Duration		Hb g/dL	P.value	RBCS count $\times$ $10^6/\mu l$	P.value	HCT%	P.value
Day1		11 2.1±2.2		4.60±.57		39.6±5.3	
Day7		11 1.2±1.8	0.01	4.27±.68	0.00	36.0±5.6	0.00
Day15		11 0.9±1.9	0.00	4.15±.65	0.00	35.1±5.7	0.00
Day21		11 0.5±2.0	0.00	3.95±.76	0.00	33.7±6.4	0.00
Day28		11 0.3±2.2	0.00	3.93±084	0.00	33.0±8.1	0.00

Significant P.value ≤ 0.05

Table (2) showing MCV, MCH and MCHC at five durations

Duration	MCV fL	P.value	MCH pg	P.value	MCHC g/dL	P.value
Day 1	85.1±3.9		26.6±2.2		31.5±2.5	
Day 7	83.6±3.6	0.00	26.0±2.2	0.01	30.8±2.5	0.00
Day 15	83.8±3.5	0.00	26.1±1.9	0.02	30.9±2.2	0.03
Day 21	83.7±4.2	0.00	26.2±2.5	0.24	30.9±2.3	0.05
Day 28	83.8±4.6	0.02	25.6±2.2	0.00	29.9±1.9	0.00

Significant P.value ≤ 0.05

Table (3) showing WBCs count and platelets count at study durations

Mean± SD and P.value				
Duration	WBCs counts × $10^3/\mu l$	P.value	Plts count× $10^3/\mu l$	P.value
Day 1	4.77±1.5		215±45	
Day 7	4.04±1.5	0.00	161±50	0.00
Day 15	3.44±1.4	0.00	136±41	0.00
Day 21	2.87±1.1	0.00	118±39	0.00
Day28	2.99±2.6	0.00	104±41	0.00

Significant P.value ≤ 0.05

4. discussion, conclusion and recommendations

4.1 Discussion

When blood was stored outside the body some hematological changes will takes place resulting in reduced red blood cells survival which is important drawback when transfused into the circulation of a Recipient (Mollison *et al*, 1954).

Hemoglobin levels showed significant changes during the 7th , 15th , 21th and 28th days of storage when compared with blood at zero time(time of collection), *this is in agree with result of Ahmed Y. Dallal Bashi, Bashar M. Salehhe were find that the Hemoglobin concentration at day 7 it started to decrease significantly (P<0.05) which can be accounted to the heamolysis that occurs during storage,(Ahmed et al,2009).The result also in agreement with the results of Donahne et al,(*Donahue *et al*, 1956*)*.

The erythrocyte hemolysis can be attributed to several causes including: old erythrocytes age hemolysis (ranged between 100-120 days) (Emmannel ,2001) (Simon *et al*, 1980), improper storage of blood (higher than 8c° in blood bank refrigerator) or blood bags not mixed periodically leading to decrease 2.3diphosphoglycerate which is very important to preserve RBC and maintains physiological functions (Wood,1973).

In addition, leucocytes break down to release their constituents such as hydrogen peroxide and proteases which damage the erythrocytes (Heaton, 1994) (Beutler *et al*, 1969) (lson, 1991). The degree of heamolysis is shown to be related to the levels of leucocytes in additive suspended units (Heaton, 1994) (lson ,1991).

The result disagree with the findings of Teddy C Adias1et al, are present No significant changes were observed in Hb and other hematological parameters throughout the study (Teddy *et al,* 2012*)*.

As RBCs count is in proportional relation to Hb concentration was show a significant decrease from 7day to 28day of storage when compared with RBCs count at first day .this may be due to Decreased RBC viability,wich is correlated with lesion of storage that is associated with various biochemical changes(Majr ,1999). A significant benefit and better blood preservation when blood components are separated at the

time of collection to liquid or freeze preserved ((Beutler *et al*, 1969) (lson E, 1991). The viability and stability of stored erythrocytes is improved (Oluyombo *et al*, 2013).

Measurement of packed cell volume was an index for real hemoglobin (Ahmed *et al,* 2009). In my study PCV was showed same changes were observed on Hemoglobin concentration at 7th, 15th, 21th, 28th when compared with fist day of collection this result is in agreement with Ahmed et al, was showed that there is a significant decrease in Hemoglobin concentration and packed cell volume and These changes continued by advancing periods of storage, (Ahmed *et al*, 2009). Teddy et al, was showed no significant changes were observed in PCV and other hematological parameters throughout the study (Teddy *et al, 2012)*.

About WBCs count, their results were showed significant decrease when compared day 7th, 15th, 21th, and 28th with first day of collection (day zero). These changes in white blood cells counts are mostly likely due to the changes in sum effects of the loss of individual cell characteristics specifically degeneration that is known to occur as the cell ages (Elemchukwu Queen *et al*,2014).
    Studies have shown that polymorphonuclear cells are reduced to 50% of their number in 48 hours and only 25% would remain after the Seventh day of storage (Rogers et *al,* 1995) (Crosby *et al*, 1940). Oluyombo *et al,* study showed 75% of granulocytes have disappeared by day 7 of storage; little benefit is therefore derived in leucopenia or agranulocytosis when blood has been stored for more than two days(Oluyombo *et al*,2013).

The study showed a significant decrease in HCT% at 7, 15, 21 and 28 days when compared with first day, this agree Oluyombo *et al*, in their study results, that are showed no significant changed in Hematocrit were noticed Until 14th day of blood storage; begin to decrease significantly after that. This could be explained partly by reduced deformability of stored Erythrocytes which are almost completely packed (Oluyombo et *al,* 2013).
Also about 1/120 of the donated erythrocytes is already at the end of its useful life because of life span of the cells (Mollison, 1961).

The study was showed a significant change in platelets count at 7, 15, 21, and 28 days when compared with platelets counts at first day this may be due to, normal life span of platelets inside the body is 7-10days (Hoffbrand *et al*, 2006). Platelets circulate longer when stored at room temperature and are more activated and able to form clot more effectively when stored at 4°C (Bruce-Chwatt, 1972).

MCV, MCH and MCHC were also shows a significant decrease at day 7 when compared with first day (P. <0.5). MCV in femtoliters = PCV divided by RBCs count per liter of blood and multiplied with 1000, MCH in pg =Hb per liter of blood divided by RBCs counts and MCHC g/dL = Hb per liter of blood divided by PCV (JOHN *et al,* 1995). Therefore all this red cell indices parameters were showed a decrease due to those calculation relations.

4.2 Conclusion

There is significant decrease in all parameters under study include:

1 - Hemoglobin concentration g/dl, Red blood cells count, White blood cells count Hematocrit, Mean cell volume, Mean cell Hemoglobin, Mean cell Hemoglobin concentration and Platelets count.

2-The possible effects of stored blood on the recipient health can be summarized by that all stored blood undergoes unavoidable hematochemical changes that lead to decrease active desirable Substances such as hemoglobin and viable red blood cells and white blood cells.

4.3 Recommendations

1- Fresh blood should be given to patient to decrease the levels of non-viable red blood cells.

2- Blood should be separated to components or leucodeplated to avoid negative effect of leukocyte enzymes on red blood cells viability.

3- Blood bags should be mixed periodically to maintain 2.3diphosphoglycerate and Adjust refrigerator at less than 8 ℃ temperature with regular check to provide good storage condition.

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Appendix

بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

College of Graduate Studies

Effect of Storage Duration on Some Hematological Parameters on CDPA-1 Whole blood at Elnehoud Teaching Hospital

Donor No……………………………

Date …………………………………………………………………..

Age…………………………………………………………………………..

Blood group……………………………………………………………………

List for laboratory results:

Day of test	Hb g/dl	RBCs count	WBCs count	HCT%	MCV fL	MCH Pg	MCHC g/dl	Plts count
Day 1								
Day 7								
Day 15								
Day21								
Day 28								