بسم الله الرحمن الرحيم

Republic of the Sudan

Ministry of Higher Education and Scientific Research

University of Shendi

*Faculty of Graduate Studies and Scientific Research*

# Effect of Environmental Storage on Red Blood Cells Parameters at Elmak Nimer University Hospital Blood Bank

*A thesis submitted for partial fulfillment of the degree of M.Sc. in medical Laboratory Sciences*

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**August – 2018**

# الآيــــة

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**صدق الله العظيم**

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# Dedication

**I would like to dedicate dissertation to who gave me love, comfort throughout my life.**

**My mother**

***To who is always there for me and never let me need.***

**My father**

***To my***

**Sisters, Brother, teachers**

***Whom are always there when I need them.***

**To those help me to complete this research.**

**To all my colleagues in Shendi university and Elmak Nimer University hospital.**

# *Acknowledgment*

First, I would like to express my deepest thanks and praise to Allah for enabling me to accomplish this work.

*And pray for Prophet Mohammed peace be Upon him*

Thanks are extended to my supervisor:

**Dr. Mohammed Osman Ali Mohammed**

for helping, supporting, assistance and advice.

Also my thanks to all staff of Hematology Department.

Finally, I sincerely thank everyone who helped me to accomplish this research.

# List of abbreviations

| | |
|---|---|
| ACD | Acid Citrate Dextrose |
| AS | Additive solution |
| ATP | Adenosine triphosphate |
| BCSH | British Committee Standardization of Haematology |
| BSMS | Blood stocks management Scheme |
| COPD | Chronic Obstracttive Pulmonary Disease |
| CPDA | Citrate phosphate Dextrose Adinin |
| DNA | Deoxy Nucleic Acid |
| DPG | Diphosphoglycerat |
| FAD | Food and Drug Administration |
| Hb | Haemoglobin |
| HCT | Haematocrit |
| HIV | Human Immunodeficiency Virus |
| HSL | Hypothermic storage lesion |
| MCH | Mean cell haemoglobin |
| MCV | Mean Cell Volume |
| MCHC | Mean cell haemoglobin concentration |
| PCV | Packed Cell Volume |
| PRBCs | Packed red blood cells |
| RBCs | Red Blood Cells |
| RDWCV | Red Cell Distribution Width Coefficient varies |
| RPM | Round Per minute |
| SAGM | Saline adenine glucose mannitol |
| SNO-Hg | S-nitroslation of hemoglobin |
| TRAI | Transfusion-related acute lung injury |
| UK | United Kingdum |
| USA | United States of America |
| WBCs | White Blood Cells |

# الخــلاصـــة

أجريت هذه الدراسة ببنك دم مستشفى الملك نمر الجامعى بجامعة شندى في الفترة ما بين شهر ابريل وشهر اغسطس 2018م وهدفت لدراسة اثر بيئة التخزين على معاملات خلايا الدم الحمراء اثناء فترة التخزين خلال ثلاثة فترات مختلفة منذ قبل التخزين وحتى اليوم 35 من التخزين باستخدام محلول ( CPDA1 ) كمادة حافظة.

عندما يخزن الدم خارج الجسم قد تحدث تغيرات لمعاملات الخلايا الحمراء ينتج عنها انخفاض بقائها وهذا يمثل عائق مهم عندما تنقل للدورة الدموية للمستقبل.

تم جمع عدد50 رجاجة دم من المتبرعين فى بنك الدم فى الفترة مابين يوم16–24 من شهر أبريل للعام 2018 . بعد تحضير الزجاجة تم اخذ (6) مل منها وقسمت لثلاثة اجزاء كل جزء يحتوى على 2 مل . أحد العينات نم تحليلها مباشرة. العينتين الأخريتين تم حفظهما في ثلاحة بنك الدم بدرجة حرارة ما بين (2– 6)$^{O}$ م وثم تحايلهما لاحقا في اليوم ال17 وثم اليوم 35 من التحزين.

تم تحليل مستوى الهيموقلوبين وتعداد الكريات الحمراء والهيماتوكريت ومتوسط حجم الخلية ومتوسط هيموفلوبين الخلية ومتوسط تركيز هبموفلوبين الخلية ومتوسط المعامل التفريقى لتوريع الخلايا عن طريق جهاز تعداد الدم الكامل ووجد أن متوسط مستوي الهموقلوبين والكسر الحجمى للخلايا الحمراء ومتوسط حجم الكرية ومتوسط هيموقلوبين الخلايا يكون مختلفا وبدلالة احصائية من المتوسط قبل بداية فترة التخزين.

كذلك أظهرت النتائج عدم وجود فرق ذو دلالة احصائية فى بداية فترة التخزين ثم يصبح الفرق ذو دلالة احصائية فى نهاية التخزين وذلك فى ومتوسط تعداد كريات الدم الحمراءh,ومتوسط تركيز هيموقلوبين الخلية ووجود فرق ذو دلالة احصائية فى بداية فترة التخزين وفرق ذو دلالة غير احصائية فى نهاية فترة التخزين وذلك فى متوسط المعامل التفريقى لتوريع الخلايا.

# Abstract

This study was done in Elmak Nimer University Hospital blood bank, Shendi University during the period from April to August 2018, to determine the effect of environmental storage on red blood cells parameters in different periods of time ( at 3 storage periods from zero time up to35 days ) using CPDA-1 solution as apreservative .

When blood store outside the body, the parameters of Red Blood Cells can be affected during storage, result in a reduction of red cells survival ,which is important drawback when transfused into the circulation of arecipient.

Fifty blood donors who's attended the blood bank, during the period from 16 to 24 April, 2018 were included in the study. Six ml were taken from each blood bag and divided in to 3portions, in plain tubes 2 ml in each . Blood in one of these tubes was analyzed immediately, the other 2 tubes were stored in blood bank refrigerator at 2-6°C and analyzed later at day 17 and 35.

The blood sample were analyzed for hemoglobin, HCT, RBCs count,MCV, MCH , MCHC and RDW- CV. By Hematology analyzer. The results showed a significant variation during storage period in the Hb level, HCT , MCV and MCH ,while there was no significant variation in RBCs count and MCHC in the early period of storage, but the variation was clearly observed at the end period of storage (P.value ≤ 0.05). Also there was significant variation in RDW-CV in the early period of storage, but it tends to disappear at the end of storage and this is might be due to the lysis of old cells.

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# Chapter One

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# 1.1 Introduction

Blood transfusion is a life-saving treatment for patients with massive blood loss and chronic anemia and a supportive therapy to optimize oxygen delivery and tissue perfusion in critical illness [1,2] . The clinical benefits of blood transfusion were made possible through the development of techniques to preserve cell viability ex vivo, allowing the blood donation and transfusion to be separated in time and space [3]. In the 1960s, with the introduction of plastic blood bags [4], whole blood transfusion was replaced for specific blood component therapy red blood cells (RBCs), platelets and plasma components translating the life-saving benefits of one whole blood donation to up to four transfusion recipients [5]. Currently packed RBCs (pRBCs) the most highly used blood component, are produced by two common component manufacturing methods: the whole blood filtration method and the buffy coat method [6,7] . The general procedure is ( 400-500 ) ml of whole blood in Citrate Phosphate Dextrose Adinin ( CPDA1 ) is centrifuged , plasma and RBCs are separated , and RBCs can be resuspended in an additive solution, commonly accompanied by leukoreduction [7] .

Additive solutions, such as saline - adenine - glucose-mannitol (SAGM) and additive solution 3 (AS3), contain nutrients RBCs need to survive ex vivo and have effectively extended RBC ( 2 - 6°C) [4]. SAGM is widely used in blood banks in Europe, Australia and Canada [8].

During storage of pRBC units, the quality of stored RBCs progressively decreases during hypothermic storage. RBCs undergo a series of biochemical and biomechanical changes, collectively known as the ‘hypothermic storage lesion’(HSL) [9]. Characteristics of the HSL includes RBC membrane remodeling, decreased metabolites such as ATP and 2,3-DPG, loss of intracellular potassium, oxidative injury of protein structures and lipid peroxidation, membrane loss, vesiculation, and ultimately hemolysis (the fragility leads to the release of cell free haemoglobin and formation of

microparticles submicron haemoglobin containing vesicles and additional haemolysis [10,11,12] .

There are increasing concerns regarding the effect of the HSL on hemorheology, including RBC aggregability, deformability and membrane remodeling, effects that could potentially lead to impairment of the oxygen delivery capacity of transfused blood [13,14,15] .

## 1.2 Rationale

Red blood cells (RBCs) concentrates are the most transfused blood component worldwide.

For having a sufficient and available blood supply blood can be stored in asolution of Citrate-Phosphate-Dextrose-Adenine (CPDA1) as a combined anticoagulant and energy source for up to 35 days at( 2-6 )°C.

This study focuses on an analysis of storage related changes in RBCs parameter with duration of storage up to 35 days that may lead to impairment of the oxygen delivery capacity ( decrease active desirable substances such as hemoglobin and viable red blood cells) of transfused blood.

The importance of this study is, its result valuable for the quality control in hospital blood bank.

# 1.3 Objectives

## General objective:

To evaluate the effect of environmental storage on Red Blood Cells parameters in Elmak Nimer University Hospital Blood Bank.

## Specific objectives:

- To measure haemoglobin level during red blood cells storage.

-To determine Red Blood Cells count during red blood cells storage.

-To Calculate hematocrit (Hct) during red blood cells storage.

-To estimate mean corpuscular volume (MCV) during red blood cells storage.

-To measure mean corpuscular Hgb (MCH) during red blood cells storage.

-To estimate mean corpuscular Hgb concentration (MCHC) during red blood cells storage.

-To estimate Red blood cell distribution width-Coefficient varies (RDW -CV) during red blood cells storage.

# Chapter Two

## Literature Review

# 2 Literature Review

## 2.1 Blood transfusion:

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 [16, 17].

A 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 [18].

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 edition 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) [19,20].

Blood is collected into a plastic bag for blood collection consist of 450 ml of blood mixed with anticoagulants, these include citrate – phosphate dextrose (CPD), acid – citrate dextrose (ACD), with adenine to prolong red cell storage [18,21].

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 collected is tested for hepatitis B and C, Human Immune Virus (HIV), malaria and other infectious diseases, the only blood that tests negative for these are given to patients [17, 22 – 24].

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 [25-27],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 [17,28-30].

While the granulocytes are some times used to fight infections, although their efficacy is not well – established, they must be transfused with 24 hours of donation [31, 32] .

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 [21] .

## 2.2 Red Blood Cells:

RBCs manufactured in the bone marrow , RBCs are enucleated biconcave discs that are continuously being produced [33] .

Once circulating, theses RBCs serve a great purpose of delivering oxygen to tissues; however, overtime these RBCs brake down, lose their efficiency and ultimately are eliminated. The biconcave disc shape is crucial to the function of RBCs, presenting a maximal surface area for the capture of oxygen in the lungs and its subsequent release to the tissue beds [33] .

The cells are flexible and able to change their shape in order to traverse the tiny tubules of the capillary beds [33] .

Since the cells are enucleated and lack mitochondria, they are unable to carry out cellular repair of damage or enzyme inactivation and therefore must rely on anaerobic glycolysis for energy [33] .

Structurally, RBCs depend on an intact membrane and an internal cytoskeleton to function normally. This cytoskeleton, the structural support that maintains the RBC's biconcave shape, is made of protein, microfilament, intermediate filaments, and microtubules [33] .

Functioning RBCs have very high levels of 2,3 diphosphoglycerate ( 2.3 DPG ), in which 2,3, DPG binds the beta chain of de oxyhemoglobin in apH

dependent environment. Adequate levels of 2,3 DPG are necessary to lower the oxygen affinity for hemoglobin thereby increasing oxygen tissue delivery [33].

Therefore, RBC function centers on the ability of an RBC to bind oxygen , if the RBC has a normal biochemical environment and sound structure or morphology, it functions normally by releasing the carried oxygen to the tissues. Furthermore, as part of this process each RBC must have an energy supply to survive and maintain its integrity and function [33] .

Adenosine triphosphate (ATP) is such an energy molecule that the cell depends upon to maintain its integrity and function [33]

## 2.3 Haemoglobin:

Haemoglobin is iron-containing protein attached red blood cell that transport oxygen from the lungs to the rest of the body. Haemoglobin bonds with oxygen in the lung exchanges it for carbon dioxide at cellular level, and then transport the carbon dioxide back to the lung to be exhaled [34].

Each red blood cell contains approximately 640 million haemoglobin molecules. Each molecules of normal adult haemoglobin consist of four poly peptide chains two alpha ($a_2$) and two beta ($\beta_2$) each with it is own haem group. The molecular weight of haemoglobin 68.000 [34].

Haem synthesis occurs largely in the mitochondria by a series biochemical reactions commencing with the condensation of glycin and succinyle co-enzyme A under the action of the key rate-limiting enzyme δ-amino laevulinic acid synthase. Byridoxal phosphate (vitamin $B_6$) is a co-enzyme for this reaction which is stimulated by erythropoietin which gives δ-amino laevulinic acid inside mitochondria which gives prophobilinogen outside the mitochondria to give uroporphyrinogen which give coproporphyrinogen to protoporphyrin which combine with iron to form the heam part [35].

Gloin synthesis occurs largely in ribosome which contain from poly peptide chain [35].

Globin synthesis from amino acids such as glycin, lysine, leucin, glutamic acid, arginin, asparatic and … etc [35].

Each molecule of haem combines with globin chain made on poly ribosomes. A tetramer of four globin chains each with it is own haem group in a ' pocket ' then formed to make up haemoglobin molecule [35].

Whether haemoglobin binds with oxygen or carbon dioxide depend on the relative concentration of each around the red blood cell. When it reaches the oxygen-rich lung, it releases the less abundant carbon-dioxide to bind with oxygen, when it goes back out into the body where cells areproducing carbon dioxide, it release the oxygen and bind with carbon dioxide this is called the Boher effect [34].

When carbon monoxide is present, it competes with oxygen at the haem binding sites. And since haemoglobin is 200 times more likely bind with carbon monoxide, forming very bright red form of haemoglobin as low as 0.02% in the air can cause nausea and headache, 0.1% causes un consciousness and death (compare that with normal 20% oxygen saturation of the air) persons, who expose themselves regularly to carbon monoxide, may have as many as 20% of their hemoglobin's oxygen sites blocked by carbon monoxide [34].

Haemoglobin abnormalities result in very serious hereditary disease, such as sickle cell anemia and thalassemia [34].

Haemoglobin is made up of four subunits with a haem (iron-containing) group in each for oxygen binding. There are slightly different haemoglobin in adult when compared to children fetuses [34].

High 2–3 diphosphoglycerate levels are found in people who live in high altitudes, this chemical allows larger amount of oxygen to be delivered to the tissue, preventing altitudes sicknes [34] .

Like all proteins the ' blue print' for haemoglobin exists in DNA (the material that makes up genes). Normally, one individed has four genes that two genes code for the alpha chain. Two other genes code for the beta chain (two additional genes code for gamma chain in fetus) [36].

The alpha chain and the beta chain are made in precisely equal amount, despite the differing number of genes. The protein chain join in developing red blood cells, and remain together for the life of the red blood cell [35].

There are hundreds of haemoglobin variants that involve genes both from the alpha and beta gene clusters [35].

### 2.3.1 Types of haemoglobin:

#### 2.3.1.1Normal types:

Embryo haemoglobin which found in the first weeks of gestation

- $Gower_1$ which contain of two epsilon($\varepsilon_2$) and two zeta ($\zeta$) chain
- $Gower_2$ which contain of two epsilon ($\varepsilon_2$) and two alpha ($\alpha_2$) chain
- Portland which contain of two Zeta ($\zeta_2$) and two gamma ($\gamma_2$) chain

**Haemoglobin F (Fetal haemoglobin)**

This type is major respiratory pigment in intrauterine life which found in fetuses and new born babies, it compose from two alpha ($\alpha_2$) chain and two gamma chain ,and its replaced by haemoglobin A,shortly after burth ,only small amount of haemoglobin F are made after birth [35].

Some disease, such as sickle cell anemia, a plastic and leukemia have abnormal types of haemoglobin and higher amount of haemoglobin F [35] .

**Haemoglobin A" adult haemoglobin"**

This is the most common types of haemoglobin found normally in adult. Some disease, such as sever from thalassemia, may cause haemoglobin F level to be high [37].

**Haemoglobin A <sub>$_2$**

This is normal type of haemoglobin contain of two alpha and two Delta($\delta$ )chain [36].

Haemoglobin $A_2$ is found in small amount in adult about 2% [36] .

#### 2.3.1.2 Abnormal types of haemoglobins:

**Haemoglobin S**

Is the most common of abnormal haemoglobin and the basic of sickle cell trait and sickle cell disease, differ from normal adult haemoglobin only by single

amino acid substitution, valine replacing glutamic acid $6^{th}$ position of the beta chain globin [35].

**Haemoglobin C**

Is abnormal haemoglobin with substitution of lysine for glutamic acid at $6^{th}$ position of the beta globin [38].

**Haemoglobin E**

Is abnormal haemoglobin, It formed when glutamic acid is replaced by lysine at $26^{th}$ position of beta chain of globin [38]

**Punjab haemoglobin D**

Is abnormal haemoglobin with substitution of glutamine residue for glutamic acid at 121th position of the Beta globin chain [38]

**Haemoglobin Arab**

Is abnormal haemoglobin with substitution of lysine residue for glutamic acid at 121th position of the Beta chain [38]

**Haemoglobin H**

Is abnormal haemoglobin containing from four beta ($\beta_4$) chains and this haemoglobin is unsuitable for life [38].

**Haemoglobin Bart**

Is abnormal haemoglobin containing from four alpha chains and this haemoglobin is unsuitable for life [38]

### 2.3.2 Haemoglobin derivatives:

**Oxygenhaemoglobin**

Is a normal forms of haemoglobin that a attaches to oxygen by ferrous iron ($Fe^{+2} - o^{-2}$) [39]

**Carbo-amino haemoglobin**

Is a normal form of haemoglobin that attaches to the carbon dioxide(40).

**Carboxyhaemoglobin**

Is a normal forms of haemoglobin that a attacks to the carbon monoxide instead of oxygen or carbon dioxide . High amount of this type of abnormal haemoglobin prevent the normal movement of oxygen by blood [39] .

**Sulfohaemoglobin**

Is an abnormal form of haemoglobin that cannot carry oxygen. It may result from certain medicines such as phenaccetin or sulfonamides [39] .

**Met haemoglobin**

When the iron that is part of haemoglobin is changed to ferric state so that doesn't carry oxygen [39] .

## 2.4 : Packed cell volume:

The haematocrit or packed cell volume are on the measures of the proportion of blood volume that is occupied by red blood cells. It's normally 45±7 (38-52%) for males and 42±5 (37-47%) for females [40] .

**Elevated PCV**:

In case danger fever, where the full blood counts done, Daily, high haematocrit is danger of an increased risk of dengue shock syndrome [39]

Polycythaemia Vera is associated with elevated haematocrit [39] .

Smoking, COPD, and other pulmonary condition associated with hypoxia may elicit an increased production of red blood cells, this increase is mediated by the increased level of erythropoietin by the kidney in response to hypoxia[39].

There have been cases where the blood for testing was in advertently drawn from the same arm with the intravenous running in a transfusion of packed red cells. In this sample the haemoglobin measurement will be high because it is measuring the fluted being transfused (that is mostly red blood cells) rather than the diluted serum, in this case, the haematocrit measurement will be artificially very high [40] .

**Lowered PCV:**

Lowered haematocrit can imply significant haemorrhage. MCV, RDW can be quiet help full in evaluating lower than normal haematocrit , because can help the clinician determine whether blood loss is chronic or acute. The MCV is the size of red blood cell and RDW is relative measure of the variation in size of the red cell population. A low haematocrit with a low MCV with high RDW suggest a chronic iron deficient erythropoiesis, but normal RDW suggest blood loss that

is more acute such as haemorrhage [11]. Conversely, if blood for haematology testing a drawn from proximal to that of an intravenous infusing line fluid into patient, the blood sample will be diluted by those fluid and the haematocrit will be or artificially low [40].

**Estimation of PCV:**

The packed cell volume can be determined by centrifuging heparinized blood in a capillary tube (also known as a micro haematocrit tube). Is typically centrifuged at10.000 RPM for five minute,This Separates the blood into layers (packed cell, Buffy coat ¯$WBC_s$ + platelet, plasma). And the tube read by haematocrit reader [40].

And estimated haematocrit as a percentage may be derived by multiplying haemoglobin concentration in g/dl three times and dropping the units [39].

## 2.5 Red blood cells count:

Total red blood cells is the number of red cells is given as an absolute number per litre [38] .

Haemoglobin - The amount of hemoglobin in the blood, expressed in grams per decilitre. (Low hemoglobin is called anaemia.)

Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells [38] .

## 2.6 Red Cell Indices:

### 2.6.1 MCHC:

The MCHC gives the concentration of haemoglobinin g/l in 1 litre of packed red cells. It is calculated from the haemoglobin (Hb) and PCV.

A guideline reference range for MCHC in health is 315–360 g/l (31.5–36.0 g/dl.

* Low MCHC values are found in iron deficiency anaemia and other conditions in which the red cells are microcytic and hypochromic (MCHC may be normal in thalassaemia trait).

*An increased MCHC can occur in marked spherocytosis but this is a rare condition. Arise MCHC is more often due to a calculation error or an incorrect haemoglobin or PCV [38].

### 2.6.2 MCV:

The mean red cell volume (MCV) provides information on red cell size. It is measured in femtolitres (fl) and is determined from the PCV and electronically obtained RBC count ,A guideline reference range is 80–98 fl.[38]

- **Low MCV values**: are found in microcytic anaemias particularly iron deficiency anaemia, anaemia of chronic disease and thalassaemia. The MCV is low in infancy (about 70 fl at 1 year of age) .
- **Raised MCV values**: are found in macrocytic anaemias, marked reticulocytosis [38] .

### 2.6.3 MCH:

The MCH gives the amount of haemoglobin in picograms (pg) in an average red cell. It is calculated from the haemoglobin and electronically obtained RBC count [38] .

A guideline reference range for MCH in health is 27–32 pg [38] .

**Low MCH values:** are found in microcytic hypochromic anaemias and also when red cells are microcytic and normochromic, In thalassaemia minor the MCH is low even when anaemia is mild (MCHC is often normal) [38] .

Raised MCH values: are found in macrocytic normochromic anaemias. MCH is also raised in neoborns [38] .

## 2.7 Red blood cell distribution width (RDW):

It is an index that is calculated by the analyzers by two methods, based on the values of the MCV and the RBCs. The first is referred to as the RDW- CV, which is the ratio of the width of the RBCs distribution curve at 1 SD divided by the MCV. The normal value for adults is11-14.5% . Microcytosis tends to increase its value, while macrocytosis minimizes the changes in the RDW-CV. The second method refers to RDW- SD, that is a direct measurement of the RBCs distribution width taken at the 20% frequency level normally( RDW-SD = 42 ± 5 fL). It is more sensitive to the appearance of minor populations of macrocytes or microcytes. This index reflects a state of anisocytosis (heterogeneous population of RBC) [38] .

## 2.8 Red Blodd Cells Storage:

Peyton Rous was the first person to store red blood cells. He had learned from Roger Lee that citrate was an anticoagulant [41] .

He kept rabbit red blood cells in a mixture of citrate and glucose for 4 weeks in a refrigerator and observed that they did not haemolyse [42] . When these stored red blood cells were infused back into the donor rabbits, they raised the haematocrit and did not cause haemoglobinuria or bilirubinuria [43].

Two years later, in military hospitals adjacent to World War I battle fields, Rous's post-doctoral fellow, Oswald Robertson used this solution to store human red blood cells for up to 26 days and used this 'banked' blood to resuscitate soldiers in shock [44,45]. However, Robertson's US Army colleagues became concerned about the possibility of bacterial contamination of the stored blood, and the commission that approved stored blood transfusion for general use approved it only for storage in citrate without glucose and only for days of storage [46] . Robertson, in his private writings, noted that this restriction both limited the utility of blood banking and reduced the quality of stored blood, because some units ran out of glucose in less than 5 days [46] .

The controversy between those who seek longer red blood ran out of glucose in less than 5 days cell storage for logistical reasons and those who have concerns about the safety and efficacy of stored blood continues. Storing red blood cells for longer times does have advantages. It allows the accumulation of inventory, takes advantage of economies of scale in collection, processing and testing, and allows the development of quality controls. Longer cold storage reduces potential transmission of syphilis and reduces transfusion-associated graft-versus-host disease. However stored red blood cells lose functional capacity during storage:

they lose membrane, and they eventually become nonviable [4] .

Stored red blood cells can be frankly dangerous with high potassium concentrations, bacterial overgrowth, and the lytic elaboration of toxic lipids [4].

### 2.8.1 Standards for red blood cell storage:

#### 2.8.1.1 recovery survival and haemolysis:

The first standards for red blood cell storage were that the cells did not haemolyse in the bottle and that they appeared to circulate when reinjected into the donor or were transfused into a recipient [47]. In a sense, these remain the only standard [47].

They are now formalized in the US licensure requirements that at the end of the approved storage period, an average of at least 75% of the cells remain in the circulation 24 h after infusion and that haemolysis be less than 1% For 50 years, labelling red blood cells with chromium-51 has been the accepted way to measure their recovery and survival [47]. The recovery is the fraction of the injected cells that circulate after infusion and their survival is the length of time that either the average cell or the longest surviving cell circulates. With the recognition of the high frequency of post-transfusion hepatitis, autologous recovery and survival

measures, where a volunteer donor's own red blood cells are evaluating blood storage systems. Such studies have the added advantage that as the infused red blood cells are the donor's own, antibody-mediated clearance of the cells does not typically interfere with the observations, and documented reductions in recovery or survival can be presumed to be the result of damage to the cells inflicted by the storage system or the passage of time[48]. The standard measure is now the 24-h post-infusion in vivo recovery, with the survival measured as the half-life of the radioactive label. One laboratory has developed and used a system for measuring recovery of allogenic red blood cells by measuring the fraction carrying alloantigens by flow cytometry [49] .

The establishment of 75% as the recovery USA came out of historical experience [50]. Whole blood stored for 3 weeks in acid–citrate–dextrose solution had an approximately 75% autologous in vivo recovery. With 3-week storage of whole blood in CPD solution, this improved to 79% [51].

When adenine was added to CPD solution to make CPDA-1, the licensure study showed 81% autologous in vivo recovery after 5-week storage as whole blood, but only a 72% autologous in vivo recovery after 5-week storage as packed red blood cells [51]. The solution was licenced recovery, but because of the sense that performance had actually gotten worse, the Food and Drug Administration (FAD) raised the standard to 75% in 1985. All of the red blood cell additive solution storage systems licenced subsequently in the USA, AS-1, AS-3, and AS-5, have met this higher standard [52].

Furthermore, in a large review of licensure trials by Dumont USA, AS-1, AS-3, and AS-5, and AuBuchon, all appeared to be equivalent, with approximately 82% 24-h in vivo recovery when stored as red blood cells in additive solution for 6 weeks [4]. When the red blood cells are leucoreduced at the time of initial processing, the red blood cell recovery is about 2% higher [53]. The survival of red blood cells that circulate for 24 h has been normal with a half-life of about 60 days in all systems where it has been measured [53].

The problem with 24-h in vivo recovery as a red blood cell storage quality standard is that its measurement is time consuming, expensive to conduct, requires exposing volunteers to modest amounts of radiation, and gives quite different results from one volunteer to another [54]. Examination of the results of a large number of such studies shows that the population distribution has a large standard deviation and negative skew with a long lower tail [53]. Examinations of cross-over studies where individual volunteers are measured several times show that some volunteers' red blood cells consistently store better than others [54].

Measures of haemolysis are easier to perform, and several very large series are available from national blood service quality-assessment programmes. Typically, red blood cells in additive solution have 0·2–0·4% haemolysis after 5–6 weeks of storage, and 1–4% of such cells typically exceed standards Leucoreduction tends to reduce storage haemolysis by about 50 % [55].

### 2.8.1.2 Other changes occurring during storage:

**The red blood cell storage lesion.**

There are many other changes that occur during red blood cell storage that have not served as conditions of storage system licensure in the past [4]. These changes include shape change, slowed metabolism with decreased concentrations of adenosine 5′-triphosphate (ATP), acidosis with resulting decreased concentrations of DPG, loss of cation pumping with loss of intracellular potassium, oxidative injury with changes in band 3 structure and lipid peroxidation, and apoptotic changes with membrane phospholipid racemization and membrane loss [55].

Storing living red blood cells in a closed plastic bag means that the products of ongoing glycolytic metabolism, lactic acid and protons accumulate over time (55). Other metabolic processes, such as the breakdown of adenosine by adenosine deaminase, mean that other breakdown products accumulate as well, but the generally small amounts of ammonia and inosine formed do not seem to be clinically important for themselves. The protons, however, decrease the pH in the blood bag and alter glycolysis, first leading to a rapid drop in DPG concentrations with a concomitant burst in ATP production, followed by an increased slowing of glycolysis and falling ATP production as acid accumulates. DPG is typically gone by the 10th day of red blood cell storage, whereas ATP concentrations initially increase or are stable during the first 2 to 4 weeks of storage with generally declining concentrations thereafter. New experimental solutions may be able to extend high concentrations of ATP longer [56].

Acidification and decreasing ATP concentrations both affect red blood cell shape [55]. Acidosis causes the initial manifestations of red blood cell shape change during storage, the development of bumps that grow to become the typical surface protrusions of echinocytes. Most of the early aspects of echinocytic shape change appear to be reversible with red blood cell warming and certainly disappear when stored red blood cells are incubated in a neutral pH solution of nutrients, a process called rejuvenation ,however, as red blood cell

ATP concentrations fall, irreversible changes associated with increased red blood cell calcium concentrations develop [13,15].

These include the loss of phospholipid asymmetry, the development of negatively charged phospholipid rafts on the cell opment of negatively charged phospholipid rafts on the cell surface, and their shedding as microvesicles [13,15]. Membrane loss during red blood cell storage would appear to be permanent. As storage progresses, red blood cells become more rigid and more adherent to endothelium [13,15]. Red blood cell concentrates are not a pure product, being derived from whole blood by simple centrifugation techniques. Many red blood cell concentrates are still made this way with the white blood cells left behind as a buffycoat when the platelet-rich plasma is removed.

When these white blood cells are exposed to the acidic conditions of storage and refrigerated, they respond with activation and cytokine production before they die [57]. After they die, the white blood cells break down and release constituents including enzymes such as phospholipase-A2 [57] . Phospholipase-A2 in turn attacks and breaks down phospholipids released by red blood cells, creating lysophospholipids such as the dialkylglycerol platelet-activating factor. The longer the red blood cells are stored, the more of these biologically active lipids are produced. Leucoreduction of red blood cell concentrate shortly after collection markedly reduces the concentrations of lysophospholipids. Leucoreduction also decreases the changes that cause stored red blood cells to stick to endothelial cells in culture and probably to post-capillary venules in the circulation [58].

Oxidative damage also occurs to red blood cells during storage [59]. The haemoglobin in venous blood is partially saturated with oxygen, so the oxygen is constantly leaving one haemoglobin molecule and binding to another. This reaction is not perfectly reversible, and occasionally, the leaving oxygen takes an electron with it, forming ferric methemoglobin and superoxide. Normally, methemoglobin is reduced and superoxide is desmuted without consequences, but occasionally superoxide interacts with iron and water in the

Fenton reaction to form hydroxyl radical which can attack and damage proteins and lipids. Damage to spectrin and glycoprotein band 3 can occur, and interaction with triacylglycerols can lead to deacylation and the formation of lysophospholipids. While damage to glycoprotein band 3 appears to have consequences as a determinant in the natural 120- day lifespan of red blood cells in the circulation its much slower rate during cold storage probably reduces its importance as part of the storage lesion. On the other hand, the slow accumulation of lysophospholipids in the blood bag during storage, without an opportunity for their continuous removal and detoxification, remains as a safety concern.

**2.8.1.3 Safety of stored red blood cells:**

There are several circumstances in which transfusion or even reinfusion of stored red blood cells are associated with bad outcomes. Deaths have been associated with the overgrowth of red blood cell units by cold-growing bacteria, with the rapid central infusion of older units with high concen various causes, and from transfusion-related acute lung injury( TRALI) from oxidation-induced lysophospholipids . There may also be hypercoagulation associated with the infusion of microvesicles exposing negatively charged phospholipids. One in 2000 units of blood is contaminated from skin or blood at the time that it is drawn [60,61]. Despite leucoreduction and cold storage, about 1 in 30 000 stored red blood cell units can be demonstrated at some point to be bacterially contaminated. Infections related to bacterial contamination could be demonstrated in 1 in 5 million red blood cell units , and in a typical year, about one of the five annual deaths from bacterially contaminated blood products is reported to be associated with a unit of red blood cells [62]. Most bacterial organisms do not survive in the cold, but a few such as serratia marcesans, Yersinia enterocolitica, and Aeromonas species can grow at refrigerator temperatures [63]. They tend to grow slowly in cold blood, dividing about once a day and so to take approximately 27 days for a single organism to grow to organisms and present with an overwhelming infection or endotoxic shock.

Examination of units of red blood cells for evidence of haemolysis or a dark colour indicative of bacterial consumption of oxygen is a routine blood bank procedure.

The activity of the sodium potassium-dependent ATPase pump' on the red blood cell surface is highly temperature dependent [4] . In the cold, it does not have the activity to overcome diffusive cation loss. Red blood cells therefore leak potassium, and, in additive solutions, the extracellular potas sium concentration of stored units increases at a rate of about 60 mEq/l each day.The rate is greatest early on when the intra- to extra - cellular concentration gradient is highest, then slows as an equilibrium is reached. As the equilibrium point is about 60 mEq/l, most units never achieve this concentration in 42 days of storage, so the approximately 1 mEq/l/day rule is usefu. Deaths have been reported when such units were infused through central lines into infants or used to prime cardiopulmonary by pass or other high-flow devices [64]. As the red blood cells will reabsorb the potassium as soon as they warm and equilibrate to body pH and osmolality, the problem is not the total potassium load, but its local extracellular concentration in the older stored units and its delivery to the central circulation where it can be associated with cardiac arrhythmias [65,66].

Rules to provide young units of red blood cells to small infants and for bypass priming or to use washed red blood cells when young units are not available largely prevent these incidents when the rules are followed infusion of haemolysed red blood cell units can cause reactions that look like immune haemolytic transfusion reactions [65,66].

Typically, they are less severe, because they do not cause the complement activation associated with antibody mediated haemolysis, but they can be associated with acute renal failure or hyperkalemic sudden death. Such reactions are more frequently associated with older units, because such units have had more time for mishaps of storage to occur . As noted above, lytic and oxidative damage to red blood cell membrane phospholipids and the elaboration of lysophospholipids occurs continuously during red blood cell storage. Silliman

and his colleagues have shown that this can be a mechanism of acute lung injury, and Gajic and his colleagues have shown that concentrations of lysophospholipids in stored red blood cell units are associated with increased rates of lung injury in intensive care patients [65,66]. However, rates of TRALI are markedly reduced when plasma from women donors is removed from the blood supply, so the role of lysophospholipids in causing clinically important lung injury is not clear [67] .

Microvesicles from stored red blood cells are shed in relatively greater numbers toward the end of storage when ATP concentrations are low [68]. These vesicles expose negatively charged phospholipids on their surfaces that are potentially proinflammatory and procoagulant. Although there are suggestions that transfusion is associated with increased inflammation in studies of transfusion and multiple organ failure and with thrombosis in critically ill patients , these are deeply confounded st-tudies of very sick patients receiving many kinds of therapy [69].

**2.8.1.4 Efficacy of stored red blood cells:**

The suggestion that stored red blood cells lose efficacy is generally based on claims that they do not flow or they do not deliver oxygen [69]. Suggestions that they do not flow are based on direct observation of the microvasculature or reologic studies in various instruments and are associated with membrane stiffness, membrane loss, and the loss of secre- tion of local vasodilators such as ATP and nitric oxide. Suggestions that stored red blood cells do not deliver oxygen are usually based on their low concentrations of DPG [70] .

Red blood cell flow is reduced after prolonged storage in capillary system [70]. Their deformability is reduced in electrocytometers [71] .

The artificial capillary systems tend to be exquisitely sensitive to membrane loss and the ectocytometers to membrane rigidity. Both would be expected to reduce flow in the living capillary systems. The problem is that the same cells, stored in solutions of nutrients that maintain ATP concentrations, tend to have

normal flow despite the membrane loss, suggesting that the reduced flow is a function of the red blood cells' interaction with its environment [72].

Since rejuvenating solutions rapidly restore red blood cell ATP concentration and ATP is important for membrane fluidity, by facilitating cytos- keletal rearrangement, and for vascular flow, by the secretion of ATP in response to shear effects resulting in local vasodilation, it seems plausible that ATP is involved. Considerable work on the development of the next generation of red blood cell storage solutions is aimed at improving red blood cell ATP concentrations at the end of storage to prevent these kinds of problems. Nitric oxide bound to the sulfur of B-93 cysteine (SNO-Hb) is also rapidly lost during red blood cell storage, is not regenerated in rejuvenating solutions, and not involved in the artificial capillary systems. It takes several hours to regenerate SNO-Hb after returning cells the body, so the prompt restoration of flow associated with better-stored red blood cells suggests that it is not critical to flow regulation [73] .

2.3 Diphosphoglycerate intercalates between the β globin chains of deoxyhemoglobin, stabilizing the deoxy form and moving the base of the oxygen equilibrium curve to the right [74] . However, an attempt to reproduce this work in larger numbers of animals was not able to demonstrate any difference in critical oxygen delivery between fresh and stored red blood cells [75] .

### 2.8.2 Temperature required for storage and transport of red blood cells:

The requirement in the UK and Europe is that red cell components must be stored with their core temperature in the range 2 to 6°C, whereas AABB Standards state 1 to 6 °C [76,77,19,78]. Exceptionally, it is allowed that the core temperature may extend from 1 to 10°C, providing that this deviation has happened on one occasion only, and that the duration is no longer than five hours [77]. In addition, the UK Guidelines allow surface tempreratures up to 10 °C for up to 12 hours during transport, although currently it is not stated on howmany occasions [19].

The Council of Europe Guidelines [19] allow up to 10 °C for 24 hours dur- ing transit. The AABB Standards and AABB Technical Manual state that blood storage and transit temperature should not exceed 10 °C but no time limit is stated. None of the published guidelines on transport of red cells state on how many occasions during the shelf-life of a red cell this may occur, and are unclear about whether these recommendations relate to blood centres or hospitals or both [78].

The EU Directive and Blood and Safety Quality Regulations state that tr- ansport and distribution of blood and blood components at all stages of the transfusion chain must be under conditions that maintain the integrity of the product .These storage and transportation regulatory requirements are in place to (a) inhibit the growth of any bacteria introduced into the bag at the point of collection, processing, or storage; and( b) to preserve red cell quality [78].

**2.8.2.1 The '30 minute' rule :**

The UK Guidelines do not give any guidance on how long blood can be out of controlled temperature before transfusion is commenced, the British Committee for Standarisation in Haematology (BCSH) guidelines [76] · state that 'If red cell units are out of temperature controlled storage for more than 30 minutes they should not be put back into storage for reissue. The BCSH guidelines also recommend that transfusions are completed within 4 hours of removal from a controlled temperature, apart from neonatal transfusions which can be up to 4.5 hours to allow for 4 hours for the transfusion it self in order to allow for transfusions up to 20 mls/kg at a rate of 5 ml/kg/hr It is likely that the 30 minute rule originated as a result of the 1971 puplication of Pick and Fabijanic [79] .

who investigated the time taken for a unit of cooled blood to reach 10 °C when removed from the refrigerator, they found that, whether the unit was handled or not, the surface temperature reached 10 °C between 15 and 30 minutes after removal into ambient conditions, whereas the core temperature took 45 to 60 minutes to reach 10°C . Thirty minutes thus would appear to be a reasonable cut-off to ensure that the core temperature did not rise above 10 °C. Since this

original work, there have been a number of studies that have confirmed the rate of warming, in increasingly sophisticated ways[80-83]. It is not clear why Pick and Fabijanic chose 10 °C as the upper limit, it may have been on the basis of data published by Hughes-Jones [84] that showed reduced, but acceptable, recovery of red cells following transfusion when stored at10 °C for 34 days. In addition, 10 °C may have been chosen as a practical limit based on the wet ice type of transit containers that were available at that time. The relevance of short-term exposures to 10 °C,and thus the relevance of the30 minute rule, is therefore worthy of review.

The 30 minute rule can result in wastage of red cells in two respects:

a) If a patient is not ready to receive a planned transfusion, and red cells are out of controlled storage for more than 30 minutes they cannot be ret urned to stock for issue to that or another patient.

b) Red cells sent to a location remote from a blood refrigerator or off-site in case a transfusion is needed, cannot be returned to stock if not transfused within 30 minutes of removal from controlled storage.

There is general concern among blood services and hospitals that a considerable number of RBCs are lost unnecessarily as a result of the 30-minute rule. Data from the UK Blood Stocks Management Scheme (BSMS) repeatedly shows approximately 10, 000 RBCs are discarded every year due to out of temperature control excursions outside of the laboratory, and this represents almost one quarter of all red cell wastage [85] . In a recent survey of hospitals by the BSMS, over 96% of respondents indicated that extending the 30 minute rule to 60 minutes would enable most of their out of temperature control units to be re-issued [86].

Storage of red cells at 4 °C decreases the metabolic rate of the cell blood to be stored for longer periods[87].

## 2.9 Previous studies:

Study conducted by Dr. Sonia Chhabra, Dr. Saurav Chaudhary , Dr. P.K. Sehgal , Dr. Sunita Singh , Dr. Monika Gupta, Dr. Rajeev Sen.

This study done in India in July 2017 aims to study the efficacy of stored whole blood for a period of 28 days and to delineate the changes that occur in RBCs indices in CPDA stored whole blood. Samples were collected and tested for various hematological parameters:

(haemoglobin, RBC count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution-coefficient of variance, ) at days 1 and 28 respectively on Mindray BC 5800 (5 part analyser). Statistically significant changes(increased) were observed in mean corpuscular volume ($p < 0.05$) while statistically non significant changes were observed in other parameters ($p > 0.05$) [88] .

Study conducted by Ahmed Y. Dallal Bashi, Bashar M. Saleh.

In Dept. of Medical Biochemistry, College of Medicine, Mosul University Dept. of Clinical Biochemistry- Mosul Central Blood Bank, Nineveh Health General Office, Mosul.

This study was done to determine certain hematochemical effects on blood when stored during different periods of time (at 7 storage periods (from zero time up to 35 days) in both sexes using CPDA1 solution as preservative. Fifty blood donors (25 males and 25 females) who were attending the Central Blood Bank, Al-Zahrawi Hospital, Mosul (IRAQ) during the period from 1st October 2002 to 31st March 2003. A blood sample consisted of 50 ml was taken from each blood bag and this was divided into 7 portions, each contained about 7 ml of blood added into plain tubes. Blood in one of these tubes was analyzed immediately. The other six tubes were analyzed later on at intervals of 3 days, 1, 2, 3, 4, and 5 weeks. The blood samples were analyzed for Hb, PCV %. The results of this study showed that there was a significant decrease ($P<0.05$) in Hb, packed cell volume [89] .

Study Conducted by Behrooz Ghezelbash, Azita Azarkeivan, Ali Akbar Pourfathollah, Mohammadreza Deyhim, Esmerdis Hajati, Alireza Goodarzi in Laboratory Hematology and Blood Bank, Blood Transfusion Research Center, High Institute for Research and Education inTransfusi- on Medicine, Tehran, Iran.In 2007-2008.

This study was planned to observe the biochemical and hematological changes in pre-storage leukoreduced RBCs compared with unfiltered RBC during in vitro storage.

Ten unit RBCs were collected, processed and stored . Every unit was split into two equal parts, unfiltered RBC and filtered. Samples were collected and tested on weeks of storage. hematology analyzer was used to monitor the change of RBC indices such as (MCV), (MCH) and (MCHC). The RBC indices remained within the expected levels in both groups [90].

Study conducted by : Karama M. I. Al – Nuaimy B.D.S., M. Sc. Department of Basic Sciences / College of Dentistry University of Mosul 2008. This study was conducted to determine the effect of storage for varying periods on some haematological parameters.in this study hemoglobin and (P.C.V.) were significantly affected (decreased) by storage period of blood but (P.C.V.) is more affected than hemoglobin [91].

Study which was conducted by Akbar Hashemi Tayer PhD1, Naser Amirizadeh PhD1, Mahtab Mghsodlu MD1, Mahin Nikogoftar PhD1, Mohammad Reza Deyhim PhD2, Minoo Ahmadinejad MD1.In**:**

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran Biochemistry lab, 2.Iranian Blood Transfusion Organization, Tehran, Iran.in 2017 The aim of the study was to evaluate various storage quality measures in RBC concentrates during storage under blood bank condition. In this descriptive study, twenty leuko-depleted packed RBCs bags from healthy donors were prepared and stored at 4°C for up to 42 days. Samples were withdrawn at seven different times and evaluated for

various hematological measures. The assessment of RBCs during cold storage showed significant **:** increase in hematocrit (Hct) [92] .

Study which conducted by Teddy C Adias and his college in college of health ,health technology, Nigeria 2012. Sample were collected and tested for haematological red blood cells parameters and no significant changes were observed during the storage period [93] .

# Chapter Three

## Materials and Method

# 3. Materials and methods

## 3.1 Study design:

This is prospective analytical cross sectional study conducted in Elmak Nimer University Hospital blood bank, and aimed to study the effect of storage on haematological parameters of Red Blood Cells.

## 3.2 Study area:

Shendi is city located in northern Sudan, situated on the east bank of the Nile River 150 Km northeast of Khartoum city. Shendi is also about 45 Km southwest of the ancient city of Meroe. Located in the River Nile Wilayah, Shendi is the center of the Jaaliin tribe and an important historic trading center. Amajor traditional rout across the Bayuda Desert connects AL-Matamma to Marawi and Napata, 250 Km to the north west.

The University exist within the city from 1990 with several faculties. There are 4 hospital within the city:

1- Elmek Nimer university hospital.

2-Shendi teaching hospital.

3- Molecular medicine center.

4- The military hospital.

There are also two blood bank within the city :

1-Elmik Nimer university hospital blood bank .

2- Shendi teaching hospital blood bank.

## 3.3 Study population sampling:

450 ± 50 ml whole blood was collected into blood bags containing CPD A1 anticoagulant solution (63 ml) from healthy donors in Elmik Nimer university hospital blood bank .

Anon probability blood samples were tacken from afresh collected blood bags, which were already collected from donors for the purpose of clinical routein work after physicion approval as fit person,suitable for donation .

**3.3.1-Inclusion criteria:** Healthy donors

**3.3.2.Exclusion:** Ages out of range between 18-45 years , weight less than 45.4 Kg,the subject donate blood in at last 56 dayes and with presence of chornic disease such as hypo/ hypertension, metabolic syndorm, upper respiratory tract infection , to have Jaundice ,hyperlipidemia, anemia, vitaminB12 or vitamin D deficiency, leukocytosis, leucopenia or any hematological or serological abnormalities.

## 3.4 Data collection tools:

1.Structure Questionnaire and observation of lab experiment results.

## 3.5 Ethical consideration:

The permission for collection of sample taken from Shendi University, Faculty of Graduate Studies & scientific Research, Elmak Nimer University Hospital, Blood Bank and verbally from the donors, also identification number is used.

## 3.6 Data analysis:

SPSS statistics is a software package used for statistical analysis it is now officially named (IBM SPSS statistic).

Results of day (0) used as normal control.

The data was subjected to paired t - test for calculating degree of variation. p values were obtained and $<0.05$ were considered significant.

## 3.7 Method & procedure:

### 3. 7.1. Specimen collection :

Blood collection bag was labeled with donor identification number before withdrawal of blood of about 450 ± 50 ml, into blood bags containing CPD A1 anticoagulant solution (63 ml) from healthy donors.

Blood bags after being given aunique number, and after preparation of packed red blodd cells, samples from them sent as possible early to hematology laboratory to study haematological parameter at day 0.

Then sample of RBCs concentrates are stored in the refrigerator at 2-6 °C. 2 ml of stored sample was sent to hematology laboratory for analysis at (days

17, and 35). In 3 part analyze with Mindray BC 3000 automated haematology analyzer.

**3.7.2. Methodology :**

In this analytical study, 50 healthy donors who attended to Elmik Nimer university hospital blood bank in period from 16 to 24 April 2018.Ages: were ranged between 18-45 years , the weight: at least 45.4 Kg,the subject should not donate blood in at last 56 dayes , Blood pressure : (Systolic BP between 100-130 mm of Hg and diastolic between 60-90 mmHg,Tempreature : normal,Pulse : Pulse rate between 50-100/min and regular, and without having any known disorder.They were selected and serologically examined for syphilis , hepatitis B, C virus and HIV.

450 ± 50 ml whole blood were collected into blood bags containing CPD A1 anticoagulant solution (63 ml), which contain sodium citrate(2.63gm) ,citric acid(anhydrous 0.3gm ) ,sodium biphosphate (monohydrate 0.22gm ), dextrose (monohydrate 3.19 gm ), adenine(0.027 gm ),and water (63 ml) . The units were centrifuged at 1750 cycle per minute for 11 minutes at temperature of 25°C to extract plasma from the original blood donation bag . Ablood smple consistef of 6 ml from each blood bag was taken . Each sample was divided in to 3 portion , each portion consisted of 2 ml of blood was added into plain test tube.

In these conditions, RBC concentrates can be stored up to 35 dayes at special blood bank refrigators at 2-6°C.

At each testing point (days 0, 17 , and 35), samples were thoroughly mixed and was analyzed hematologically for: hemoglobin , HCT, RBCs count, MCV, MCH , MCHCand RDW-CV. By Hematology analyzer.

**3.7.2.1 Hematology Analyzers:**

The there main physical technologies used in hematology analyzers are: electrical impedance, flow cytometry, and fluorescent flow cytometry. These are used in combination with chemical reagents that lyse or alter blood cells to extend the measurable parameters. For example, electrical impedance can differentiate red blood cells(RBCS),WBCS, and Platelete by volume. Adding a

nucleating agent that shrinks lymphocytes more than other WBCS makes it possible to differentiate lymphocytes by volume.

### 3.7.2.2 Electrical impedance

The traditional method for counting cells is electrical impedance, also known as the coulter principle. It is used in almost every hematology analyzer.

Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume.

Impedance analysis returns CBCs and three-part WBC differentials (granulocytes, lymphocyte, and monocyte) but can not distinguish between the similarly sized granular leukocytes: eosinophils, basophils and neutrophils.

Counting rates of up to 10,000 cells per second can be achieved and a typical impedance analysis can be carried out in less than a minute [94].

# Chapter Four

## Results

# 4. Results

This descriptive prospective analytical cross sectional study which aimed to determine the effect of storage on Red Blood Cells parameters**.**

According to the table (4-1) the mean of haemoglobin level in day zero was (24.1 g/dl), while in day 17 was (22.6 g/dl ) then decreased to (23.5 g/dl) in day 35.

Also according to the table (4-2) the mean of red blood cells count in day zero was ($7.4\times10^{12}$ /L), while in day 17 was ($7.4\times10^{12}$ /L ) then increased to ($7.5\times10^{12}$ /L) in day 35.

The mean of haematocrit in day zero was (72.8%), while in day 17 was (69.4%) then decreased to (68.1 %) in day 35 as demonstrated in table (4-3).

While the mean of mean cell volume in day zero was (91.1fL), while in day 17 was (93.8 fL ) then incresed to (99.6 fL) in day 35 as noted in table (4-4).

According to the table (4-5) the mean of mean cell haemoglobin level in day zero was (32.3 pg), while in day 17 was (30.6 pg ) then decreased to (31.3 pg) in day 35.

Also according to the table (4-6) the mean of mean cell haemoglobin concentration in day zero was (32.5 g/dl), while in day 17 was (32.6 g/dl ) then incresed to (34.6 g/dl) in day 35.

The mean of of red distrbuation width in day zero was (15.5 %), while in day 17 was (14.1 % ) then decreased to (14.3%) in day 35 as referred in table (4-7).

**Table (4-1) Show the mean of Haemoglobin level according to the period of storage:**

| Day of storage | Mean of Hb level |
|---|---|
| Day zero | 24.1 g/dl |
| Day 17 | 22.6 g/dl |
| Day 35 | 23.5 g/dl |

**Table (4-2) Show the mean of RBCs count according to the period of storage:**

| Day of storage | Mean of RBC count |
|---|---|
| Day zero | $7.4 \times 10^{12}$ /L |
| Day 17 | $7.4 \times 10^{12}$ /L |
| Day 35 | $7.5 \times 10^{12}$ /L |

**Table (4-3) Show the mean of HCT according to the period of storage:**

| Day of storage | Mean of HCT level |
|---|---|
| Day zero | 72.8 % |
| Day 17 | 69.4 % |
| Day 35 | 68.1 % |

**Table (4-4) Show the mean of MCV according to the period of storage:**

| Day of storage | Mean of MCV level |
|---|---|
| Day zero | 91.1 FL |
| Day 17 | 93.8 FL |
| Day 35 | 99.6 FL |

**Table (4-5) Show the mean of MCH according to the period of storage:**

| Day of storage | Mean of MCH level |
|---|---|
| Day zero | 32.3 pg |
| Day 17 | 30.6 pg |
| Day 35 | 31.3 pg |

**Table (4-6) Show the mean of MCHC according to the period of storage:**

| Day of storage | Mean of MCHC level |
|---|---|
| Day zero | 32.5 g/dl |
| Day 17 | 32.6 g/dl |
| Day 35 | 34.6 g/dl |

**Table (4-7) Show the mean of RDW-CV according to the period of storage:**

| Day of storage | Mean of RDW-CV level |
|---|---|
| Day zero | 15.5 % |
| Day 17 | 14.1 % |
| Day 35 | 14.3 % |

# Chapter Five

## Discussion
## Conclusion
## Recommendations

# 5.1. Discussion

This descriptive prospective cross sectional analytical study was conducted in Elmak Nimer university hospital blood bank during the period of April to August 2018 and aimed to determine the effect of storage on Red Blood Cells Parameters. A total of voluntary donors satisfying the inclusion criteria were taken.

The results of this study showed that the mean of heamoglolobin level in day zero was (24.1 g/dl), while in day 17 was ( 22.6 g/dl) and (23.5 g/dl) in day 35. Statistical analysis showed that there was significant variation with P.value of (0.000 ) in day 17 and (0.000) in day 35.This result was similar to result of study done by Karama MI *et al* in Mosul 2008.,in which though significant fall in haemoglobin from 10 days onwards of storage ($p < 0.05$) . This decrease can be attributed to hemolysis which occurs during storage. Significant fall from 7th day onward was also observed by Ahmed Y *et al* in Mosul 2003, ( $p<0.05$).

The mean of RBCs count in day zero was ($7.4 \times 10^{12}$ /L), while in day 17 was ($7.4 \times 10^{12}$ /L) and ($7.5 \times 10^{12}$ /L) in day 35.Statistical analysis showed that there was no significant variation with P.value of (0.491) in day 17. This result was similar to result of study done by Sonia Chhabra in Indi 2017, ( $p >0.05$), and other study done by Adias TC *et al* ($p = 0.376$) in Nigeria 2012 . The statistically significant rise in the mean of RBCs count on day 35 with p.value of ( 0.008) and this might be due to the delaying in sample processing or improper mixing of blood.

The mean of HCT was (72.8 % )in day 0 ,while in day 17 was (69.4%) and (68.1%) in day 35. Statistical analysis showed that there was significant variation with P.value of (0.017 ) in day 17 and (0.017) in day 35.This result was similar to result of study done by Karama MI *et al* in Mosul 2008 and other study done by Ahmed Y *et al* ($p = 0.008$) in Mosul 2003 .

The mean of MCV in day zero was (91.1 fL), while in day 17 was (93.8 fL) and (99.6 fL) in day 35. Statistical analysis showed that there was significant variation with P.value of (0.002) in day 17 and (0.000) in day 35.This result was

similar to result of study done by Sonia Chhabra in Indi , 2017 (P<0.05) , However statistically non significant increased were observed by Adias TC *et al* in Nigeria 2012 (p=0.677) The rise in MCV is attributed to the swelling of RBCs during the storage period.

The mean of MCH in day zero was (32.3 pg ), while in day 17 was ( 30.6 pg) and (31.3pg) in day 35. Statistical analysis showed that there was significant variation with P.value of (0.000) in day 17 and (0.004) in day 35 , However statistically non significant changes were observed by Sonia Chhabra in Indi 2017 and by Adias TC *et al* in Nigeria 2012 **(**p =0.805) .

The mean value of MCHC was( 32.5) g/dl in day zero then ( 32.6 g/dl)in day 17 and ( 34.6) g/dl in day 35. Statistical analysis showed that there was in significant variation with P.value of (0.513) in day 17 , This result was similar to result of study done by Sonia Chhabra in Indi and by Adias TC *et al* **(**p =0.470) in Nigeria 2012. Also Statistical analysis showed that there was significant variation with P.value of (0.000) in day 35, this may be attributed to gradual fall in haematocrit during storage.

The mean of RDW-CV in day zero was (15.5 %), while in day 17 was (14.1 %) and (14.3%) in day 35. Statistical analysis showed that there was significant variation with P.value of (0.000) in day 17. The statistically in significant variation with P.value (0.234) in day 35 was similar to result were observed by Adias TC *et al* in Nigeria (2012), with p. value of (0.316) during the 28 day storage period ,and by Sonia Chhabra *et al* in India (2017) .

# 5-2.Conclusion

**By the end of this study we conclude that:**

- There was significant variation in Hb ,HCT,MCV,MCH and significant ,insignificant variation during storage in RBCs count ,MCHC and RDW-CV
- The mean of heamoglolobin level in day zero was (24.1 g/dl), while in day 17 was ( 22.6 g/dl) and (23.5 g/dl) in day 35.
- The mean of RBCs count in day zero was ($7.4 \times 10^{12}$ /L), while in day 17 was ($7.4 \times 10^{12}$ /L) and ($7.5 \times 10^{12}$ /L) in day 35.
- The mean of HCT was (72.8 % )in day 0 ,while in day 17 was (69.4% ) and ( 68.1% ) in day 35.
- The mean of MCV in day zero was (91.1 fL), while in day 17 was (93.8 fL) and (99.6 fL) in day 35.
- The mean of MCH in day zero was (32.3 pg), while in day 17 was ( 30.6 pg) and (31.3pg) in day 35.
- The mean of MCHC in day zero was( 32.5 g/dl) , while in day 17 was ( 32.6 g/dl) and ( 34.6 g/d) in day 35.
- The mean of RDW-CV in day zero was (15.5 %), while in day 17 was (14.1 %) and (14.3 %) in day 35

## 5-3.Recommendations

1. Further study about this topic should be done with increase sample size to obtain accurate result. With increased quality control in hematology lab.
2. Periodic follow up and monitoring of haematological parameters in stored red blood cells to assess the environmental storage .

# Chapter six

## References

## Appendices

# 6.1. References


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# 6.2. Appendix

## Shendi University

### Faculty of Graduate Studies & scientific Research

**Questionnaire about Effect of Environmental Storage on Red Blood Cells Parameters at Elmak Nimer University Hospital Blood Bank**

**Donor identification number:……**

**Name:** ……..

**Age:** …….

**Gender:**

a. Male ( ) b. Female ( )

**Residence:** ……..

**Blood grouping :……**

**Haemoglobin concentration :……**

**Weight:……..**

**Blood pressure:…….....**

**Do/did you suffer from Jaundice disease:**

**Do you suffer from any other disease:**

a. Yes ( ) b. No ( )

**If yes what are they:** ……

**Investigation result :**

**Day ( o ) :**

- temperature of storage refrigerator ( °C )

- Hb ( ) - PCV ( ) - RBCs ( )

- MCV ( ) - MCH ( ) - MCHC ( )

-RDW-CV ( )

**Day ( 17 ):**

- temperature of storage refrigerator ( °C )

- Hb ( ) - PCV ( ) - RBCs ( )

- MCV ( ) - MCH ( ) - MCHC ( )

-RDW-CV ( )

**Day ( 35 ):**

- temperature of storage refrigerator ( °C )

- Hb ( ) - PCV ( ) - RBCs ( )

- MCV ( ) - MCH ( ) - MCHC ( )

-RDWCV ( )

**Date:**

……