بسم الله الرحمن الرحيم

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Assessment the Effect of Environmental Storage on Platelet Concentrate Quality in Elmak Nimer Hospital Blood Bank

A thesis submitted for partial fulfillment of the degree of M.Sc. in Medical Laboratory Sciences (Hematology)

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الآيـــة

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قال تعالى:

﴿ وَقَضَى رَبُّكَ أَلاَّ تَعْبُدُواْ إِلاَّ إِيَّاهُ وَبِالْوَالِدَيْنِ إِحْسَاناً إِمَّا يَبْلُغَنَّ عِندَكَ الْكِبَرَ أَحَدُهُمَا أَوْ كِلاَهُمَا فَلاَ تَقُل لَّهُمَا أُفٍّ وَلاَ تَنْهَرْهُمَا وَقُل لَّهُمَا قَوْلاً كَرِيماً ﴾

صدق الله العظيم

سـورة الإسـراء – الآيـة (٢٣)

Dedication

-To my father's soul,

Who gave me love and respect,

-to my dear mother,

Who taught me the meaning of life,

-to my brothers and sisters,

Who bring happiness to my life,

-to my teachers,

Who led me to the way of success,

-to my friends and colleagues,

I dedicate this study.

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List of abbreviations

Abbreviation	Term
WB	Whole blood
PC	Platelets concentrate
CPDA-1	Citrate phosphate dextrose adenine-1
DNA	Deoxy ribonucleic acid
TPO	Thrombopoietin
GP	Glycoprotein
VWF	von Will brand factor
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
TXA2	Thromboxane A2
WBCs	White blood cells
FDA	Food and drug administration
RBCs	Red blood cells
PSL	Platelet Storage Lesion
AABB	American Association for Blood Banks
PRP	Platelets Rich Plasma
FFP	Fresh frozen plasma

ملخـــــص الدراســـــة

المقدمة: بنك الدم بمستشفى الملك نمر الجامعي هو بنك الدم المركزي بمدينة شندي وهو الوحيد المختص بمجال مشتقات الدم لاسيما الصفائح الدموية.

الأهداف: جودة الصفائح الدموية عامل مهم للتأكد من قيامها بوظائفها الحيوية بعد نقلها للمريض ,الغرض من هذه الدراسة هو دراسة معايير الجودة بالصفائح الدموية ومعرفة مدى تأثرها بالتخزين.

منهجية البحث: تم جمع ٤٠ زجاجة دم من متبرعين أصحاء وفصل الصفائح الدموية وتخزينها وقياس كل من تعداد الصفائح الدموية، معدل الحموضة,حجم الوحدة والحركة الموجية للصفائح لكل وحدة في اليوم الأول واليوم الخامس من التخزين لمعرفة مدى تأثر الجودة بعوامل التخزين.

النتيجة: متوسط تعداد الصفائح الدموية في الوحدات بلغ ٦,٤×$١٠^{١٠}$ في اللتر في اليوم الأول وتناقصت في اليوم الخامس إلى ٦,٢× $١٠^{١٠}$.

متوسط حجم الوحدات كان ٥٨ مل ولم يكن هنالك تغير خلال التخزين.

متوسط معدل الحموضة بالوحدات في اليوم الأول للتخزين كان ٧,١ وتناقص إلى ٧,٠ في اليوم الخامس.

متوسط الحركة الموجية للصفائح كان ٣ في اليوم الأول وتناقص إلى ٢ في اليوم الخامس للتخزين.

الخلاصة: كل الوحدات كانت مطابقة لمعايير الجودة العالمية.

Abstract

Introduction: platelet concentrate (PC) is a one of the blood products which obtained whole blood (WB) by several ways, it has great benefits for thrombocytopenic patients or patients with platelets function disorders.

Objective: Quality of stored platelets affect directly on viability and function of them after transfusion, so this study was done to evaluate the quality parameters of stored platelets to determine the affect of environmental storage on it.

Method: A total of 40 units of platelets concentrates were separated from whole blood donated by healthy individuals not taking antiplatelets therapy, then analyzed for volume, platelets count, swirling and pH on day 1 and day 5 of storage for the purpose of assessing the effect of storage on PCs quality.

Result: The mean platelet count of stored PC was 6.4×10^{10}/L on the 1stday of storage and decreased to 6.2×10^{10}/L by day 5th, The mean pH of stored PCs was 7.2 on day 1 and decreased to 7.0 on day 5, The mean volume of PCs was 58ml and there was no change in the volume between day1 and day5, mean swirling score was 3 on day 1 and decreased to 2 by day 5.

Conclusion: at the end of storage period all PCs units met the international quality standards.

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Chapter One

Introduction

Rationale

Objectives

1.1 Introduction

In the past whole blood (WB) was the only preparation that could be administered to replace platelets, red cells, coagulation factors, etc. in a patient. In addition to patient need, this caused unnecessary administration of unwanted cell or plasma components. Large volume of whole blood needed to achieve satisfactory replacement of a particular component also faced an important limitation. A significant advance in transfusion medicine was made when techniques became available for separation of blood components in a closed system and patient could be administered specific replacement therapy. One unit of blood can be utilized for preparation of different components and thus can benefit more than one patient. Nowadays, whole blood can be separated into several blood components and further derivatives can be obtained from plasma by fractionation. This allows administration of specific replacement therapy according to the patient's need, and avoids transfusion of unwanted blood products. One unit of blood can benefit more than one patient after its separation into red cell, plasma, and platelet components [1].

A blood donation is taken by an aseptic technique into plastic bags containing an appropriate amount of anticoagulant-usually citrate, phosphate, dextrose (CPD). Three components are made by initial centrifugation of WB: red cells, plasma and buffy coat. Platelets and plasma may also be collected by apharesis [2].

Platelet concentrates (PCs):

These are harvested by cell separators or from individual donor units of blood. They are stored at room temperature. Platelet transfusion is used in patients who are thrombocytopenic, or have disordered platelet function and

who are actively bleeding (therapeutic use) or are at serious risk of bleeding (prophylactic use)[2].

Each platelet concentrate contains a minimum of 5.5×10^{10} platelets, suspended in 50 ml of plasma. The equivalent of two or even three adult doses of platelets (minimum 2.4×10^{11} each) may be obtained from one donor, with adequate platelet counts, by an apheresis procedure lasting approximately 90 min [3].

The main function of platelets is the formation of mechanical plugs during the haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur.

There are three major platelet functions: adhesion, aggregation and release reactions and amplification. The immobilization of platelets at the sites of vascular injury requires specific platelet–vessel wall (adhesion) and platelet–platelet (aggregation) interaction [2].

1.2: Rationale

The collection process, the storage conditions, and the duration of storage of platelets concentrate lead to inducement of storage lesions. Such lesions might be mediated by alterations in the biochemical or metabolic activity of the stored platelets, leading to an ultimate compromise in vitro function. For instance, irreversible changes in the shape of platelets, elevation of cytosolic calcium, assembly of filamentous actin, loss of activity of enzymes that play a critical role in signal transduction events, loss or decrease in available receptors, or loss of adenylate energy charge are considered storage- induced lesions. These lesions singly or together will be reflected in a platelet's viability and function.Therefore, there is a great immediate need for developing an appropriate preservative to improve the function of stored platelets and a simple device that can assess the functionality of these preserved cells prior to transfusion [4].

This study concern in assessing platelets concentrates quality during storage days in Elmak Nimer hospital blood bank to determine the effect of environmental storage on PCs quality.

1.3 Objectives

1.3.1 General objective:

To assess the effect of storage on PCs quality in Almak Nimer hospital blood bank.

1.3.2 Specific objectives:

1- To measure the volume of stored PC unit.

2- To determine platelets count in the unit during storage days.

3- To assess PH of platelets concentrate unit.

4- To measure swirling of PC unit during storage days.

Chapter Two

Literature Review

2.1 Literature review

2.1.1 Blood components:

In modern medical treatments, patients may receive a pint of whole blood or just the specific components of the blood that are needed to treat their particular condition. This approach to treatment, referred to as blood component therapy, allows several patients to benefit from one pint of donated whole blood.

The transfusable components that can be derived from donated blood are red cells, platelets, plasma, cryoprecipitate AHF (cryo), and granulocytes. An additional component, white cells, is often removed from donated blood before transfusion [5].

2.1.2 Platelets:

The normal haemostatic response to vascular damage depends on a closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors.

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte – the megakaryoblast – arises by a process of differentiation from the haemopoietic stem cell. The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increases in multiples of two. Early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. At a variable stage in development the cytoplasm becomes granular. Mature megakaryocytes are extremely large, with an eccentrically placed single lobulated nucleus and a low nuclear: cytoplasmic ratio. Platelets form

by fragmentation from the tips of cytoplasmic extensions of megakaryocyte cytoplasm, each megakaryocyte giving rise approximately to 1000–5000 platelets. The platelets are released through the endothelium of the vascular niches of the marrow where megakaryocytes reside. The time interval from differentiation of the human stem cell to the production of platelets averages 10 days. Thrombopoietin (TPO) is the major regulator of platelet formation and 95% is produced by the liver. Approximaately 50% is produced constitutively, the plasma level depending on its removal from plasma by binding to c□MPL receptors on platelets and megakaryocytes . Therefore, levels are high in thrombocytopenia as a result of marrow aplasia but low in patients with raised platelet counts. The other 50% is regulated in response to platelet destruction. As platelets age they lose surface sialic acid. This exposes galactose residues that attach to the Ashwell–Morell receptor in the liver. This attachment signals for production of new TPO. TPO increases the number and rate of maturation of megakaryocytes via c□MPL receptor. Platelet levels start to rise 6 days after the start of therapy. Although TPO itself is not available for clinical use, thrombomimetic agents which bind to c□MPL are now used clinically to increase the platelet count (. The normal platelet count is approximately 250 × 109/L (range 150–400 × 109/L) and the normal platelet lifespan is 10 days. This is determined by the ratio of the apoptotic BAX and anti□apoptotic BCL□2 proteins in the cell. Up to one□third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to 90% in cases of massive splenomegaly.

2.1.2.1 Platelet structure:

Platelets are extremely small and discoid, 3.0 × 0.5 μm in diameter. The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation, which are the initial events leading to

platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein Ia (GPIa). Glycoproteins Ib (defective in Bernard–Soulier syndrome) and IIb/IIIa (also called αIIb and β3, defective in Glanzmann's throm- basthenia) are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium. The binding site for IIb/IIIa is also the receptor for fibrinogen which, like VWF, is important in platelet–platelet aggregation. The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (pre- viously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa). The platelet contains three types of storage granules: dense, α and lysosomes. The more frequent specific α granules contain clotting factors, VWF, platelet□derived growth factor (PDGF) and other proteins. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and calcium. Lysosomes contain hydrolytic enzymes. Platelets are also rich in signalling and cytoskeletal proteins, which support the rapid switch from quiescence to activation that follows vessel damage. During the release reaction described below, the contents of the granules are discharged into the open canalicular system.

2.1.2.2 Platelet function:

The main function of platelets is the formation of mechanical plugs during the haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. There are three major platelet functions: adhesion, aggregation and release reactions

and amplification. The immobilization of platelets at the sites of vascular injury requires specific platelet–vessel wall (adhesion) and platelet–platelet (aggregation) interactions, both partly mediated through VWF.

VWF is involved in shear□dependent platelet adhesion to the vessel wall and to other platelets (aggregation). It also carries factor VIII. It is a large glycoprotein, with multimers made up on average of 2–50 dimeric subunits. VWF is synthesized both in endothelial cells and megakaryocytes, and stored in Weibel–Palade bodies and platelet α granules, respectively.

Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel– Palade bodies. The stored VWF can raise the plasma levels when released under the influence of several bridges.secretagogues, such as stress, exercise, adrenaline and infusion of desmopressin (1□diamino□8□D□arginine vasopressin; DDAVP). The VWF released from Weibel–Palade bodies is in the form of large and ultra□large multimers, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to smaller multimers and monomeric VWF by the specific plasma metalloprotease, ADAMTS13.

2.1.2.3 Platelet aggregation:

This is characterized by cross□linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has GPIIb/IIIa receptors which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, enabling platelet cross linking via VWF and fibrinogen bridges.

2.1.2.4 Platelet release reaction and amplification:

Primary activation by various agonists induces intracellular signaling, leading to the release of α granule contents. These have an important role in

platelet aggregate formation and stabilization and, in addition, the ADP released from dense granules has a major positive feedback role in promoting platelet activation. Thromboxane A2 (TXA2) is important in secondary amplification of platelet activation to form a stable platelet aggregate. It is formed de novo upon activation of cytosolic phospholipase A2 (PLA2). TXA2 lowers platelet cyclic adenosine monophosphate (cAMP) levels and initiates the release reaction. TXA2 not only potentiates platelet aggregation, but also has powerful vasoconstrictive activity. The release reaction is inhibited by substances that increase the level of platelet cAMP. One such substance is prostacyclin (PGI2), which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium.

2.1.2.5 Platelet procoagulant activity:

After platelet aggregation and release, the exposed membrane phospholipid (platelet factor 3) is available for two reactions in the coagulation cascade. Both phospholipid□mediated reactions are calcium□ion dependent. The first (tenase) involves factors IXa, VIIIa and X in the formation of factor Xa. The second (prothrombinase) results in the formation of thrombin from the interaction of factors Xa, Va and prothrombin (II). The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins [2].

2.1.3 Platelets transfusion:

In the practice of transfusion medicine, it is often required and mandatory to use platelet products for the optimal care of the critically ill patients. Today with the advent of component therapy and availability cell separators, it is possible to individualize the transfusion therapy in most of the cases. Platelet preparations available include PCs and apheresis Platelets.

Platelet transfusions are available as platelet PCs or as apheresis units. The former are prepared from units of whole blood by centrifugation and the latter are collected by apheresis devices. A variety of scientific arguments has been proposed for the superiority of apheresis platelets, including reduced rates of alloimmunization and transfusion reactions. However, the only compelling reason seems to be the reduced infectious risk with apheresis platelets. Platelet concentrates are separated from whole blood by first preparing platelet-rich plasma (PRP) and then centrifuging the platelets with a second centrifugation. The contents of the platelet concentrates are highly variable depending upon technique. However, the 50 ml platelet concentrate usually has at least 5.5×10^{10} platelets. The buffy coat method of preparation is the most common method of platelet preparation. This method involves centrifugation to prepare a buffy coat, from which platelets are separated by an additional centrifugation. The white cell contamination is approximately 10^8 per bag using the PRP method of concentration, and 10^6 for platelets prepared by the buffy coat method. The relatively lower white blood cell (WBC) content in platelets prepared by the buffy coat method may be advantageous in reducing alloimmunization and febrile transfusion reactions. Pheresis platelets are collected from donors by continuous centrifugation using a large intravenous catheter which allows processing of large volume of blood and the removal of platelets using an automated system. Since a conventional transfusion dose for an adult patient is approximately 6 units of pooled platelets, collection parameters have been used to collect this number of platelets from a donor. Modern pheresis devices are equipped to predict the yield from the donor's size, platelet count and hematocrit. The leukocyte content of apheresis platelet unit depends upon the technology used, but most devices have WBC contamination of less than 106 per bag. Single donors

pose less infectious risk to the recipient than do pooled platelets simply because there are fewer donor exposures. For several years, there has been a great deal written about the benefits of pheresis platelets in reducing the risk of alloimmunization. Data from the TRAP trial have shown that leukocyte-reduced pheresis platelets provide no additional reduction in alloimmunization compared to leukocyte-reduced platelet concentrates. Platelet blood components are licensed for storage time of 5 days at room temperature. Clinical studies indicate that there is little loss of platelet function and viability with 5-day-old platelets. Maintenance of the function and viability of liquid-stored platelets is limited to a relatively short period of time because of the storage lesion that develops. There has been a great deal of interest in improving the storage condition for platelets to reduce the functional abnormalities that occur during storage. When platelets are removed from the circulation and exposed to the foreign conditions of the most carefully designed collection and storage system, a variety of changes collectively referred to as platelet activation begin. A number of variables may produce minimal changes that perpetuate the process as more platelets are recruited into the activated state. These activation changes may be reflected in platelet shape change, adhesion, aggregation, secretion of platelet granular contents, and the expression of activation antigens. The challenge of preparing platelets for transfusion has been to minimize the damaging effects of preparation and storage [4].

2.1.3.1 Indication of platelets PCs transfusion:

1. Platelet count <10,000/μl for the clinically stable patient with an intact vascular system and normal platelet function, prophylactic platelet transfusion may be indicated for platelet counts of <10,000/ μl. 2. Platelet

count <40,000/µl in patients with inborn errors of metabolism (e.g. Hurler's syndrome) for 3 months following marrow transplant.

3. Platelet count <50,000/µl

a. Severe active bleeding.

b. Major surgery (preoperative and 48 hours post- operative) or impending invasive procedure. A patient undergoing a surgical or other invasive procedure is unlikely to benefit from prophylactic platelet transfusion if the platelet count is 50,000/µl or more and thrombocytopenia is the sole abnormality.

c. Coagulopathy, including DIC.

d. Newborn <1 month of age, or preterm infant.

e. Patients with severe infections.

f. Patients with sickle cell anemia while hospitalized following marrow transplant.

4. Platelet count <100,000/µl.

a. Following cardiopulmonary bypass or use of intra-aortic balloon pump or ventricular assist devices.

b. Patients receiving extracorporeal membrane oxygenation (ECMO).

c. Neurologic or ophthalmologic surgery.

5. Excessive bleeding regardless of platelet count in patients with cardiopulmonary bypass, ECMO, neurologic or ophthalmologic surgery. 6. Acute blood loss requiring more than one blood volume replacement (adult) or 50 percent blood volume replacement (children) within 24 hours, with platelet count <100,000 µl.

7. Documented or anticipated platelet dysfunction, regardless of the platelet count, if major surgery is anticipated, or if clinically significant bleeding occurs [4].

Unlike red cells, platelets must be stored at 20– 22° C, since storage at 4 ° C results in poor survival of platelets after transfusion. Platelets have a shelf - life of 5 days, and are stored in permeable bags that allow the diffusion of oxygen into the pack, which, with constant gentle agitation, maintains aerobic metabolism and reduces the rate of fall of pH. Platelet pools provide an adequate adult dose of platelets and contain, on average, 3×10^{11} platelets, usually from four donors suspended in the plasma from one donor, preferably from a male donor, in order to avoid the risk of transfusion of donor white cell antibodies that might cause transfusion- related acute lung injury (TRALI)[3].

These cells, stored as concentrates, lose functional capability during their storage period. Past research has focused on improving the procurement of blood, formulating the ideal preservation solution, improving the processing of concentrates, designing the ideal type of storage bag, and improving the environment in which platelets are stored [4].

2.1.3.2 Platelet Dosage:

The dose of platelets should be individualized. A number of simple guidelines can be used to calculate the appropriate dose.

- A dose of 1 random donor platelet concentrate per 10 kg body weight can be expected to increase the platelet count by 5000/uL in a non-refractory patient.
- One random donor PC is expected to increase the platelet count by 5000 to 10, 000/uL in a 70 kg patient who is not refractory.
- Generally, a pool of 6 to 8 platelet concentrates or a single apheresis unit is sufficient to correct or prevent bleeding in a normal sized adult weighing up to 90 kg.

- One apheresis product is equivalent to 6 to 8 random donor PCs and therefore should increase the platelet count by 30,000/uL to 40,000/uL in a 70 kg patient.
- For pediatric patients, 5 mL/kg body weight of a random donor PC should increase the platelet count by 5000/uL. A single PC contains about 45 to 50 mL and should supply the needs of patients up to 8 kg. If the entire platelet concentrate is not used for a given patient, it is not practical to salvage the remainder of the unit.
- For children >8 kg, a standard dose of 1 unit/10 kg should be used.
- In the absence of increased platelet destruction, platelet transfusion will usually need to be repeated every 3-5 days.
- If increased platelet destruction or consumption is present, daily administration may be required [7].

2.1.3.3 Platelet concentrates Storage:

PCs prepared from whole blood and apheresis components are routinely stored at 20 C to 24 C, with continuous agitation for up to 5 days on being prepared using a system classified as being closed, bags, and/or cell separator with apheresis collections. Food and drug administration (FDA) standards define the expiration time as midnight of day 5. Although the principles for platelet preservation/storage have been developed primarily using whole blood–derived platelets, they also apply to apheresis platelets. Primarily flatbed and circular agitators are in use. There are a number of containers in use for 5-day storage of whole blood–derived and apheresis platelets [8].

Initially, platelets were stored in the cold at 1 to 6 C, based on the successful storage of RBCs, as whole blood or separated RBC components at this temperature range. A key study report in 1969 by Murphy and Gardner

showed that cold storage at 1 to 6 C resulted in a marked reduction in platelet in-vivo viability, manifested as a reduction in in-vivo life span, after only 18 hours of storage. This study also identified for the first time that 20 to 24 C (room temperature) should be the preferred range, based on viability results. The reduction in viability at 1 to 6 C was associated with conversion of the normal discoid shape to a form that is irreversibly spherical. This structural change is considered to be the factor responsible for the deleterious effects of cold storage. When stored even for several hours at 4 C, platelets do not return to their disc shape upon rewarming. This loss of shape is probably a result of micro- tubule disassembly. Based on many follow-up studies, platelets are still stored at room temperature. These studies provided an understanding of the factors that influenced the retention of platelet viability and the parameters that needed to be considered to optimize storage conditions. One factor identified as necessary was the need to agitate platelet components during storage, although initially the rationale for agitation was not understood, agitation has been shown to facilitate oxygen transfer into the platelet bag and oxygen consumption by the platelets. The positive role for oxygen has been associated with the maintenance of platelet component pH. Maintaining pH was determined to be a key parameter for retaining platelet viability in vivo when platelets were stored at 20 to 24 C. Although storage itself was associated with a small reduction in post infusion platelet viability, an enhanced loss was observed when the pH was reduced from initial levels of near 7.0 to 6.5–6.8 with a marked loss when the pH was reduced to levels below 6.0. A pH of 6.0 was initially the standard for maintaining satisfactory viability. The standard was subsequently changed to 6.2 with the availability of additional data. As pH is reduced from 6.8 to 6.2/6.0, the platelets

progressively changed shape from discs to spheres. Much of this change was irreversible. When whole blood–derived platelets were intially stored in the 1970s as concentrates, a major problem was a marked reduction in pH in many concentrates. This limited the storage period to 3 days. The reduction in pH, in the presence of agitation, was shown to be due to a decrease in plasma oxygen levels that was associated with the channeling of platelet metabolism from the aerobic respiratory pathway to the anaerobic glycolytic pathway. With glycolysis, glucose is converted to lactic acid, which depletes the plasma bicarbonate and hence the plasma constituent that allows for the maintenance of pH. The reduction in pH was associated with platelet concentration/content. With total platelet contentofapproximately5–8×10^{10}, pH levels were maintained at satisfactory levels during 3 days of storage. With content above approximately 8×10^{10}, pH levels were in most cases between 5.7–6.2 by 3 days of storage. The containers being used for storage were identified as being responsible for the fall in pH because of their limiting gas transfer properties for oxygen and also for carbon dioxide. Carbon dioxide buildup from aerobic respiration and as the end product of plasma bicarbonate depletion also influenced the fall in pH. The gas transport properties of a container is known to reflect the container material, the gas permeability of the wall of the plastic container, the surface area of the container available for gas exchange, and the thickness of the container[8].

Continuous agitation is recommended to avoid spontaneous aggregation of platelets during storage. The mode of agitation used should be vigorous to prevent aggregation but gentle enough to prevent mechanical damage and release of metabolic substances from platelets such as LDH [9] which indicate platelet fragmentation. Gentle horizontal platform agitation is recommended as the optimal method of agitation of stored platelets which avoids

mechanical damage to platelets. Though circular agitation is more effective in preventing formation of aggregates it is associated with more mechanical damage and loss of viability in platelets than horizontal agitation [10].

2.1.3.4 Quality parameter of platelets concentrates:

The recommended shelf life of PCs is 5 days stored at 22 ± 24°C with continuous agitation. It is known that platelets undergo various changes immediately after they are collected, during processing, storage and even when being administered to the patient. This may affect the effective dosage that a patient receives [11]. There is also loss of function as high 30% that is attributed to the Platelet Storage Lesion (PSL) [12]. For example, the Buffy coat method produces platelets with significantly less in vitro activation and which have a lesser residual white cell count [13]. In the laboratory the platelet quality can be assessed by using several parameters (swirling, volume, platelet count and WBC count per bag and pH changes) [14]. Most of these parameters asses in-vitro platelet function. It's difficult to determine which in vitro platelet functions are essential for in-vivo hemostasis after transfusion [10].

In this study we assess platelets concentrate quality in Almak Nimer hospital blood bank by analyzing the following parameters on day 1 and 5 of storage:

1- The volume of platelets concentrate unit.
2- pH of platelets concentrate.
3- Platelets count in the unit.
4- Platelets swirling.

2.1.3.4.1 Volume of platelet concentrates units:

The platelets must be suspended in adequate plasma volume for their metabolism and to prevent platelet clumping [15].

Sufficient plasma volume in a platelet unit is required to maintain platelet concentrate pH.

lnsufficient plasma volume results in lower pH at the end of allowable storage period. The typical final volume is 40-60ml per concentrate [10].

2.1.3.4.2 PH of platelets concentrate:

Platelets carry active mitochondria which generate 85% of the adenosine triphosphate supply through oxidative phosphorylation [16]. The remaining energy demand is largely fulfilled by anaerobic respiration leading to an accumulation of lactic acid in suspensions like concentrates for transfusion[17]

pH is the simplest parameter indicator of the platelet storage lesion and probably the most important quality parameter that gives an indication of viability and potential recovery of PCs at the end of the storage period. pH has been identified as the parameter having the highest correlation with recovery and survival platelets[18]. In absence of oxygen stored platelets revert to glycolytic metabolism with increased generation of lactic acid and consequent fall in pH within 3 days of preparation.

Platelet viability is markedly affected by pH. The final pH of platelet concentrate and hence in-vivo recovery and survival will depend on the type of storage container, storage conditions [10] and the volume of residual plasma. Therefore platelet storage bags must allow for free gaseous exchange. Platelets must be stored in sufficient plasma, whose bicarbonate content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high lactic acid level, typically at 20-25 mmol/l. lowers pH and results in loss of platelet viability.

Several methods of determining pH are in use. and most employ pH meter. The methods recommended by American Association for blood banks (AABB) use a pH meter. However use of blood gas analyzer is equally

reproducible. The major limitation in these methods is that they require obtaining the sample from the bag, which may result in contamination.

A novel method that measures pH products without sampling has been developed. This is a new pH detector system that measures pH in platelet concentrates (PCs) via a special port containing optical sensing technology. The pH can be determined in a sterile way at any moment without sampling of the PC. This method, which requires a special container showed good correlation of pH between this method and blood gas analyzer method [19].

2.1.3.4.3 Platelet count in PC unit:

According to AABB recommendations, the platelet count in a unit should be at least $5.5\times I0^{10}$ at the end of the maximal storage period or at issue [10].

2.1.3.4.4 Swirling of platelets:

Swirling is an attempt to assess donated platelets for the presence of a moving, varied color appearance when backlit. The principle behind swirling is that viable platelets in an "inactivated" state have a discoid appearance, and that shape causes light to be scattered in multiple different directions. Platelets that are activated or are in a low pH environment, on the other hand, lose their discoid shape, and lose their light-scattering abilities. Swirling was initially touted as a simple, non-invasive method for assessing platelet viability due to its correlation with pH value [20].

2.1.3.5 Standards for platelet concentrate as required by the American Association of Blood Banks (AABB):

The AABB was established in 1947. It is an international association of blood banks that includes hospital and community blood centers, transfusion and transplantation centers, and individuals involved in transfusion medicine. Its members consist of medical laboratory technicians, medical technologists, registered nurses, laboratory managers, physicians, transfusion medicine

fellows, and researchers involved in transfusion medicine. The mission of the AABB is to establish and provide the highest standard of care for patients and donors in all aspects of transfusion medicine. The AABB has published books on transfusion medicine throughout its existence; two resources that are vital to donor screening procedures are AABB Standards and AABB Technical Manual [8].

AABB standards for PC quality:

Whole blood derived PCs:

- Volume: Not available
- Platelet count (90% units): $\geq 5.5\times10^{10}$/L platelets/Single unit equivalent
-pH (90% units): ≥6.2 at the end of allowable storage [15].

2.2 Previous studies:

-A study done by Mathai J and others in Division of Blood Transfusion Services, Sree Chitra Tirunal Institute for Medical Sciences and Technology, India published in 2006 declared the suitability of swirling measurement as quality indicator of stored platelets [20].

- Other study done by. Njoroge N Richard and others in Garissa District Hospital, Ministry of Health Hematology and Blood Transfusion Unit, Department of Pathology, University of Nairobi in 2010 used the parameters: swirling, volume of the platelet concentrate, platelet count, WBC count and pH of stored platelets as quality indicator of stored platelets[10].

-A study done by Marpaung, Elida and others in Regional Blood Transfusion Unit (UTTD) Indonesia Red Cross Jakarta and Clinical Pathology Department, Medical Faculty/Cipto Mangunkusumo General Hospital Jakarta came up with the result that the three parameters: pH, platelet counts, and aggregation functions decreased on the fifth day of storage[21].

-A study done by Nahreen Tynngård at Department of Clinical Immunology and Transfusion Medicine, Linköping University Hospital, 581 85 Linköping, Sweden published in 2009 showed that The quality of platelet concentrates (PCs) is affected by the preparation method and the storage conditions including duration of storage[22].

-Other study done by Manish Raturi, Shamee Shastry, Pruthvi Raj at Department of Immunohematology and Blood Transfusion, Kasturba Medical College, Manipal University, Manipal, Karnataka, India in (2009-2013) compared the quality of in house prepared platelets concentrates with the published quality standards by analyzing the volume, pH ,platelets count, residual leukocyte count ,erythrocyte contamination and swirling [15].

- A study done by Onchaga Margare K and others at Nairobi Regional Blood Transfusion Centre in Kenya in 2017 evaluated the parameters platelet volume, count, residual WBC and Hemoglobin for assessing PC quality[23].

Chapter Three

Material and Methods

3. Materials and methods

3.1 Study design:

This is a cross sectional study carried out in Shendi Town, at Almek Nimer university hospital blood bank to assess quality of platelets concentrate during storage days during the period of May – July 2018.

3.2 Study area:

The study was conducted in Shendi town in northern Sudan ,situated on the east bank of the Nile River 150km northeast of Khartoum ,Shendi is also about 45km southwest the ancient city of Meroe. Located in the River Nile wilayah, Shendi is the center of the Ja'aliian tribe and an important historic trading center.

3.3 Study population:

A total of (40) blood bags were collected from healthy normal individuals, not having any medical condition or taking any antiplatelet therapy like aspirin.

3-4 Materials:

1-Blood bag:

Blood from a donor is collected in a sterile, disposable plastic bag (triple bag system) with capacity to hold 350 ml of blood.

2- Anticoagulant preservative solution:

The solution in the blood bag usually contains citrate phosphate dextrose adenine (CPDA)-1 (49 ml for 350 ml of blood). This solution prevents clotting of blood And also provides nutrients to maintain metabolism and viability of red cells. In CPDA-1, blood can be kept stored at 2-6° C for maximum of 35 days. Function of each component of this solution is as follows:

• Citrate: Anticoagulation by binding of calcium in plasma.

• Phosphate: Acts as a buffer to minimize the effects of decreasing pH in blood.

• Dextrose: Maintenance of red cell membrane and metabolism.

• Adenine: Generation of ATP (energy source).

3. Sphygmomanometer, weighing balance, sealing clips or sealer, artery forceps.

4. 70% ethanol, sterile cotton gauze, adhesive tape

5. Emergency drugs and equipment.

6. Blood tubes for collection of blood for testing (grouping, cross matching, screening for infectious diseases) [1].

7. Centrifuge.(Hettich-zentrifugen-Rotana- 460R)

8. Blood bag pressing device.

9. Shaker (Helmer)

10. Sensitive balance (Electronic kitchen scale-QE-400)

11. PH meter (Adwa)

12. Hematology analyzer (mindary™-BC-3000Plus)

3.5 Methods:

3.5.1 Blood collection:

For the preparation of normal, resting platelets, the blood donor must be healthy, with no signs of infection or inflammation, and must not have taken medication that have anti- thrombotic effects, such as aspirin.

Blood collection must only be performed by personnel trained in phlebotomy/venipuncture. Safety precautions for the collection and handling of blood must be employed at all time. Particular care must be taken with insertion of the needle into the vein to limit the possibility of activation of the homeostasis/coagulation system, which could compromise the quality of the blood sample. For routine venipuncture procedures, a 21-gauge needle is

recommended to minimize hemolysis[26]. Blood bag should be labeled with the identification number of the donor before withdrawal of blood. Blood is collected from a vein in the antecubital fossa. To make the veins prominent and palpable, a sphygmomanometer cuff is applied to the arm and inflated to 60 to 80 mm of Hg. The area selected for venepuncture is thoroughly cleaned with 70% ethanol and allowed to dry.

The blood collection bag is placed on a weighing balance that has been kept about 30 cm below the level of the arm. A loose knot is tied in the tubing near the venepuncture needle. Venepuncture is performed, and the needle is secured in place with an adhesive tape after ensuring free flow of blood. The pressure is reduced to 40 to 60 mm of Hg. The donor is asked to squeeze a rubber ball or a similar object slowly for the duration of donation. The blood and the anticoagulant are mixed at short intervals in the blood bag. The amount of blood collected should be monitored on the weighing balance. When the blood bag weighs 400 to 450 gm, the required amount of blood has been collected. The pressure cuff is completely deflated and the tubing is clamped with forceps about 10 cm away from the needle. The knot made earlier (close to the needle) is tightened or a sealing clip is applied. The tubing is cut between the clamp and the knot/sealing clip. The clamp is removed from the tubing and blood samples (for grouping, cross-matching, infectious disease screening) are collected in appropriate tubes. The tubing is then reclamped. Needle is removed from the vein and pressure is applied over the puncture site with sterile cotton gauze. The needle is disposed off in a special “sharps” container. Blood remaining in the tubing is non-anticoagulated and is forced back (‘stripped’) into the blood bag. Bag is inverted gently several times to mix the blood and the anticoagulant. Anticoagulated blood is then allowed to run back into the tubing. Time

required for blood collection should be between 7 to 10 minutes. Blood sample tubes should be labeled with the donor identification number. After cessation of bleeding, the venepuncture site is covered with sterile gauze and an adhesive tape. After a few minutes, the donor is allowed to sit up and taken to the refreshment area, where liquids are given. The donor is thanked for donation and is issued a donation card. Donor is given information about need to drink fluids, activities permissible, and care of venepuncture site [1].

3.5.2 Platelets preparation:

Platelets are extremely labile and are very easily activated during sample preparation. It is important to limit the extent of manipulation of the blood sample to avoid unintentional activation of the platelets. All procedures must be performed at 20–24°C to maintain platelet quality and viability. Platelets undergo cold-storage-induced activation if subjected to temperatures below 20°C. The temperature of all equipment (e.g., centrifuge) and wash buffers (if used) must be between 20 and 24°C prior to use [24].

Whole blood used for the preparation of platelet concentrates must be drawn by a single no traumatic venipuncture, and the concentrate must be prepared within 6 hours of collection.

The following is a general procedure for preparing random-donor platelets:

1. Maintain the whole blood at 20 to 24C before and during platelet preparation.

2. Set the centrifuge temperature at 22 C. The round per minute (rpm) and time must be specifically calculated for each centrifuge. It will generally be a short (2 to 3 minute), light (3200 rpm) spin. This spin should separate most of the RBCs but leave most of the platelets suspended in the plasma.

3. Platelet preparation should be done in a closed, multibag system.

4. Express off the platelet-rich plasma (PRP) into one of the satellite bags. Enough plasma must remain on the RBCs to maintain a 70 to 80 percent hematocrit level.

5. Seal the tubing between the RBC and the plasma. Disconnect the RBCs and store it at 4 C.

6. Recentrifuge the PRP at 22 C using a heavy spin (approximately 3600 rpm for 5 minutes). This will separate the platelets from the plasma.

7. Express the majority of the plasma into the second satellite bag, leaving approximately 50 to 70 mL on the platelets. The volume is important to maintain the pH above 6.2 during storage.

8. Seal the tubing between the bags and separate. Make segments for both the platelets and the plasma for testing purposes.

9. The plasma can be stored as FFP, single-donor plasma frozen within 24 hours (PF24), or liquid recovered plasma. Be sure to record the plasma volume on the bag.

10. Allow the platelet concentrate to lie undisturbed for 1 to 2 hours at 20 to 24 C. Be sure the platelet button is covered with the plasma. Platelets should be resuspended. Gentle manipulation can be used if needed.

11. Shelf-life is 5 days from the date of collection. If the system is opened, transfusion must occur within 6 hours. The volume, expiration date, and time (if indicated) must be on the label. The quality control procedures must include a platelet count (5×10^{10}/l for random donor, 3.0×10^{11}/l for single donor), pH (6.2 or greater), and volume (must be sufficient to maintain an acceptable pH until the end of the dating period) [8].

3.5.3 Quality Assessment of PCs:

Platelets concentrate quality was assessed by analyzing the volume, platelets count, swirling and pH change for each unit on day 1 and day 5 of storage.

3.5.3.1 Platelets count:

Platelets count was determined by Mindary hematology analyzer (BC3000-Plus).

The following formula was used to obtain the total Platelets count for each concentrate.

Cell count / unit= sample cell count/l × Volume (ml) ÷1000 [10]

3.5.3.2 PH measurement:

PH was determined by pH meter (Adwa-AD8000) which measures pH based on respective electrodes.

3.5.3.3 Volume:

Volume was determined using the following formula:

Volume = weight of concentrate (g) – weight of empty bag (g) ÷ 1.03

(Where 1. 03 = specific gravity of platelets rich plasma)

Volume of the concentrate= (Weight of the full bag-Weight. of empty bag)/ Specific Gravity of PRP.

Where Specific Gravity of PRP = 1.03[25].

3.5.3.4 Swirling:

Discoid platelets exposed to a light source reflect light and thus produces the "swirling" phenomenon. Swirling is routinely used to evaluate the quality of platelet concentrates (PC). Swirling determinations are performed by examining a PC against a light source while gently rotating the container or gently squeezing the PC. The presence of swirling indicates a pH value within the adequate range [26].

The swirling was evaluated by examining the units against light and scored as:

i. Score 0: Homogen turbid and is not changed with pressure.

ii. Score 1: Homogen swirling only in some part of the bag and is not clear.

iii. Score 2: Clear homogenic swirling in all part of the bag.

iv. Score 3: Very clear homogen swirling in all part of the bag [25].

3.6. Ethical consideration:

The informed consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and it is health emphasis in the future. As well as the approval of ethical committee medical laboratory sciences Shendi University.

3.7. Data analysis:

The collected data code in master sheet and proceed for analysis using (SPSS version 22) program. (Mean, standard deviation, P.value).

3.8. Data presentation:

The results of this research shown in tables.

Chapter four

Results

4. Results

The results showed that:

-The mean platelet count of stored PCs was 6.4×10^{10}/L on the 1st day of storage in shown in table (4-1) and decreased to 6.2×10^{10}/L by day 5th as demonstrated in table (4-2).

Maximum platelets count was on day 1was 10.1×10^{10}/L, minimum platelets count was on day 1was 3.4×10^{10}/L. On day 5 the maximum count was 9.9×10^{10}/L and the minimum was 3×10^{10}/L as demonstrated in table (4-1) and (4-2) respectively.

-The mean pH of stored PCs was 7.2 on day 1 and decreased to 7.0 on day 5 as showed on tables (4-3) and(4-4).

Maximum pH on day1was 7.4 and the minimum was 6.7, on day 5 maximum PH was 7.4 and the minimum was 6.2 also noted in tables(4-3) and (4-4).

- The mean volume of PCs was 58ml and there was no change in the volume between day1 and day5 as demonstrated in table (4-5).

75% of PCs volume ranged between (40-60ml).

Maximum volume of units was 92ml and the minimum volume was 47ml.

-Mean swirling score was 3 on day 1 and decreased to 2 by day 5

Table (4-1): Mean of platelets count of PCs on day 1 of storage

	Count day1(10^{10} /l)
Mean	6.463
Std. Deviation	1.227

Table (4-2): Mean of platelets count of PCs on day 5 of storage

	Count day 5(10^{10}/L)
Mean	6.262
Std.Deviation	1.234

Table (4-3): Mean of pH of PCs at day1 of storage

	pH day1
Mean	7.233
Std. deviation	.2105

Table (4-4): Mean of pH of PCs at day5 of storage

	pH day5
Mean	7.035
Std. deviation	.2851

Table (4-5): Mean volume of PCs:

	Volume (ml)
Mean	65.88
Std. Deviation	11.071

Table (4-6): Comparison of quality parameters of PCs with the quality standards of AABB.

Parameter	Mean	Median	Quality standard by AABB	PCs meeting quality criteria%
Platelets count (10^{10}/L)	6.237	6.200	>5.5	100%
Volume	65.88	64.50	Maintain pH to>6.2	100%
pH	7.035	7.150	>6.2	100%
Swirling	-	-	Present	100%

Chapter five

Discussion

Conclusion

Recommendations

5.1. Discussion

Almak Nimer Hospital blood bank is the central blood bank in Shendi town. And it is only one specialized in blood component production including PCs. There is increasing demand to blood component especially for oncology patients, so it is important to assess the quality of these products in order to achieve their desired function after transfusion.

In this study we assessed PCs quality by analyzing the parameters: volume, platelets count, and swirling and pH of PC unit during storage days and comparing them with the standards of AABB.

40 units of whole blood were collected from healthy normal donors.

WB centrifuged on light and heavy spin to separate platelets. All the procedure of platelets concentrate production was performed at room temperature,

All instruments are calibrated and temperature adjusted between 20-22 C.

Then PCs stored at 22-24 c with continuous agitation for 5 days, Ambient temperature was measured 2 times on the day.

Quality parameters of stored PCs were measured on day 1 and day 5 of storage.

90percent of concentrates showed platelets count >5.5× 10^{10}/L which meets the quality criteria of AABB of PCs. Mean count was 6.46±1.227× 10^{10}/L on day 1 and decreased to 6.26±1.234× 10^{10}/L on day 5. Statistical analysis showed that there was significant variation between counts with (P.value of 0.002). these results disagreed with the results of a study done by Njoroge N Richard and others in Nairobi in 2010 in which the mean and standard deviation for platelet count was 6.63±4.73× I0^{10} /L which was well above the minimum threshold. The large SD shows that a significant number of units

have extremely low platelet count, since only 51% meet the criteria for minimum platelet count [10]

pH values ranges between 6.2-7.4, mean pH was 7.233 on day 1 and decreased to 7.035 on day 5,100% of PCs have pH≥6.2 which meets the quality criteria.

Statistical analysis showed that there was significant variation between pH values with (P.value of 0.000). This result agreed with the result of study done by Manish Raturi and others in India at 2009-2010 showed the significant variation between pH values of PCs ($P < 0.0001$)[15].

Volume of PCS units ranges between 92-47ml, 75% of the units volume ranges between 40-60 ml, 90% of units volume >50 ml which disagreed with result done by Njoroge N Richard and others in Nairobi in 2010 in which (91%) of the concentrates were more than 60mls[10]

There was no change in volume during storage days. This agreed with the result of Njoroge N Richard and others study in Nairobi in 2010 [10].

Observation of swirling showed that all units meet the quality criteria for swirling, Swirling score in all units was 3 and decreased to score 2 on day 5.this result agreed with the result of a study done by Manish Raturi and others in India during 2009-2010 [15].

5.2. Conclusion

1. The processes of blood collection, centrifugation, separation, storage and agitation were performed according to recommended standard operating procedures.

2. 100 percent of concentrates fulfilled the minimum requirement for platelet count.

3. All the concentrates met the criteria for volume, swirling and pH.

5.3. Recommendation

1. There is need to improve standard operating procedures to achieve the maximum standards for quality especially in platelets count part.

2. Estimating platelets before donation participates in insuring higher counts in the PC unit.

3. Donor selection management is important to obtain final PCs with high quality this done by selecting donors with good health who aren't taking any antiplatlet drug.

4-Further studies need to assess the function of stored platelets.

Chapter six

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Appendix

6.1. References

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6.2: Appendix

إقـــرار بالموافقة

الاسم :----------

العمر :----------

العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة مدى تأثير التخزين على جـــودة وحدات الصفائح الدموية ببنك الدم بمستشفى المك نمر الجامعي.

ملاك السر إبراهيم عبد الله

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال على حالتي الصحية كما أنه يحق لي بدون إبداء أسباب الانسحاب مـــن هذا البحث في أي مرحلة من مراحله.

البحث بإشراف :

د. أم كلثوم عثمان حمد.