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QUALITY CONTROL MONITORING OF RED BLOOD CELL CONCENTRATES DURING STORAGE IN BLOOD BANKS

Thesis
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INTRODUCTION

Blood transfusion is an essential component of health care worldwide to save millions of lives each year. Despite encouraging results, researches are not able to introduce satisfactory artificial substitutes that can routinely replace the need for donated human blood. Therefore, blood and blood components are still required for the management of patients suffering from cancer, blood diseases, trauma and emergencies ***(Cheraghali, 2012).***

Packed red blood cells (PRBCs) are red blood cells that have been collected, processed, and stored in bags as blood product units available for blood transfusion. The collection may be from a "whole blood" (WB) donation followed by component separation, or by RBC apheresis. RBCs are mixed with an anticoagulant and storage solution which provides nutrients and aims to preserve viability and functionality of the cells , which are stored at refrigerated temperatures for up to 42 days , except for the rather unusual long-term storage in which case they can be frozen for up to 10 years ***(Ullrich et al., 2007).*** The aim of RBC transfusion is to improve the oxygen delivery to tissues when the concentration of haemoglobin (Hb) is low and/or the oxygen carrying capacity is reduced ***(Giancarlo et al., 2009).***

Red blood cells are collected ‘fresh’ from donors. The range of individual cells varies from young reticulocytes and

healthy cells in the middle of their normal lifespan to effete or damaged cells awaiting clearance ***(Hess, 2014)***. During storage, all cells get older, and some will rupture or lose the ability to circulate. Others lose functions or properties, which place a burden on the circulation when the RBCs are infused. Some of these storage-related dysfunctions are corrected with entrance of the RBCs to the circulation whereas other defects persist. Storage related changes in RBCs could have undesirable consequences on the patient safety ***(Glynn, 2014).***

Red blood cells change during storage. Visible changes include the loss of biconcave disc morphology, the formation of echinocytic spines and the blebbing of microvesicles ***(Antonelou et al., 2012)***. Chemical changes include consumption of glucose, accumulation of lactic acid, loss of intracellular potassium and gain of calcium, loss of hemoglobin-bound nitric oxide (NO) and decreases in the concentrations of adenosine triphosphate (ATP) and 2, 3 diphosphoglycerate (DPG). Enzymatic and oxidative injury to proteins, lipids and carbohydrates also occur. There are also functional changes which include decrease in the ability to deliver oxygen at conventional partial pressures and failure to survive in the circulation and remain intact ***(Doctor and Spinella, 2012).***

The method by which blood products are produced, the storage solution used, and other factors as prestorage leukoreduction have an effect on the final product and may influence quality ***(Daniel et al., 2011).*** Variation in blood

products also arises from normal biologic differences in the donor population. Product standardization is thus a challenge facing all blood biologic manufacturers ***(Jason et al., 2014).***

The quality of RBCs during storage can be evaluated by determining the recovery and the survival of radiolabelled transfused red blood cells in recipient volunteers ***(Hess, 2014).*** However, as some in vivo studies are expensive and complex to perform, several studies use in vitro tests to assess the in vivo viability, like hematocrit (Hct), hemolysis, and hemoglobin (Hb) levels ***(Acker et al., 2012)***, as well as ATP, 2, 3DPG, extracellular K and Na levels, O2 affinity, RBC indices and morphology ***(Jason et al., 2014).***

Aim of the Work

This study aims to evaluate and monitor the quality of RBC concentrates during different storage dates by determining some haematological and biochemical parameters.

Chapter 1

RED BLOOD CELL PHYSIOLOGY

Red blood cells (Erythrocytes)

Red blood cells (RBCs) are the major component of blood cells which travel in the circulation of vertebrates. They are designed for the transport of oxygen (O_2) from the lungs to the body tissues and for the removal and transport of carbon dioxide (CO_2) from the tissues to the lungs ***(Laura, 2005).***

RBCs morphology:

RBCs have a biconcave shape **(Figure 1).** This biconcavity allows them to carry oxygen and bend as they flow smoothly through the narrow blood vessels in the body ***(Khairy et al., 2008).***

RBCs lack a nucleus i.e. no DNA and no organelles & therefore cannot divide or replicate themselves like other cells. RBCs have a life span of about 120 days ***(Guyton and Hall, 2006a)***. They have an effective enzymatic system like cytoplasmic enzymes which are capable of metabolizing glucose and forming small amounts of adenosine triphosphate (ATP). These enzymes also maintain pliability of the cell membrane, maintain membrane transport of ions, it has also met-hemoglobin reductase, catalase, and superoxide dismutase, which are important in reverting hemoglobin (Hb) auto-oxidation keeping the iron of the Hb in the ferrous state rather

than in ferric form, and prevent oxidation of proteins in red cells. Oxidation leads to the formation of met- Hb, which is unable to bind O_2 ***(Ronda et al., 2008).***

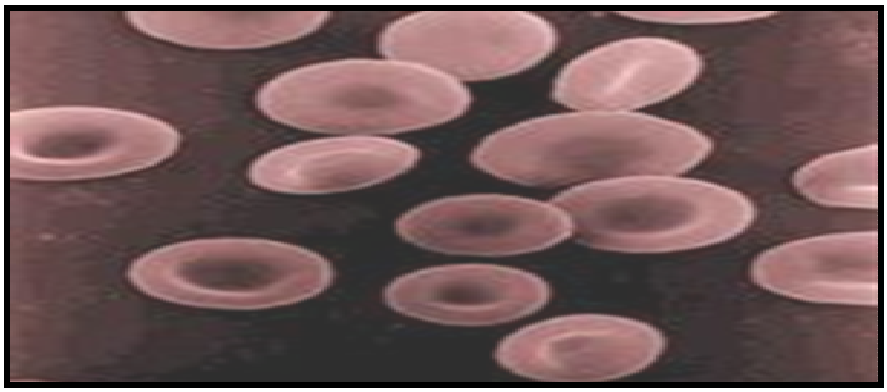

Figure (1): Human red blood cell ***(Laura, 2005).***

The diameter of a typical human erythrocyte disc is 6-8 micron, much smaller than most other human cells, with a thickness of 2.5 micrometer at the thickest point and 1 micrometer or less at the center **(Figure 2)**, and the average volume of the red blood cells is 90 to 95 cubic micrometers. A typical erythrocyte contains about 280 millions Hb molecules, with each carrying 4 heme groups ***(Brissot et al., 2004).***

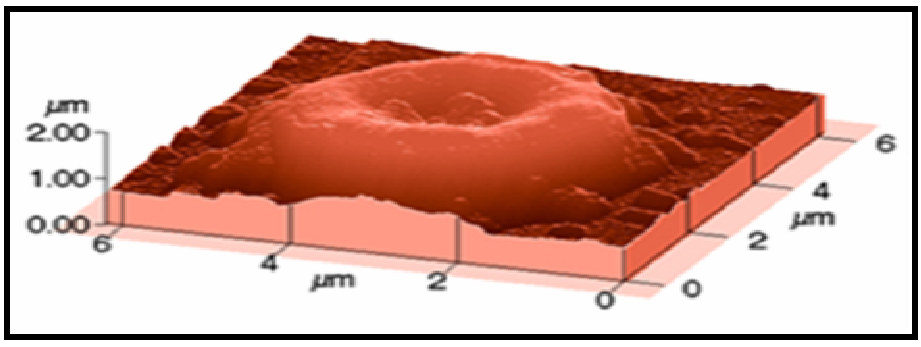

Figure (2): Red blood cell dimensions ***(Laura, 2005).***

The external membrane of the red cell contains numerous proteins that either cross the lipid bilayer one or more times or are anchored to it through a lipid tail. Many of these proteins express blood group activity; the ABO and Rhesus system (Rh). They are divided into 4 categories based on their functions; membrane transporters, adhesion molecules, receptors, enzymes, and structural proteins that link the membrane with the membrane skeleton. Some of these proteins carry out more than one of these functions ***(Daniels, 2007).*** The membrane also contains ion pumps which maintain normal homeostasis by controlling intracellular levels of sodium (Na+), potassium (K+), chloride (CL^-) and bicarbonate (HCO_3) **Figure(3)** ***(Mohandas and Patrick, 2008).***

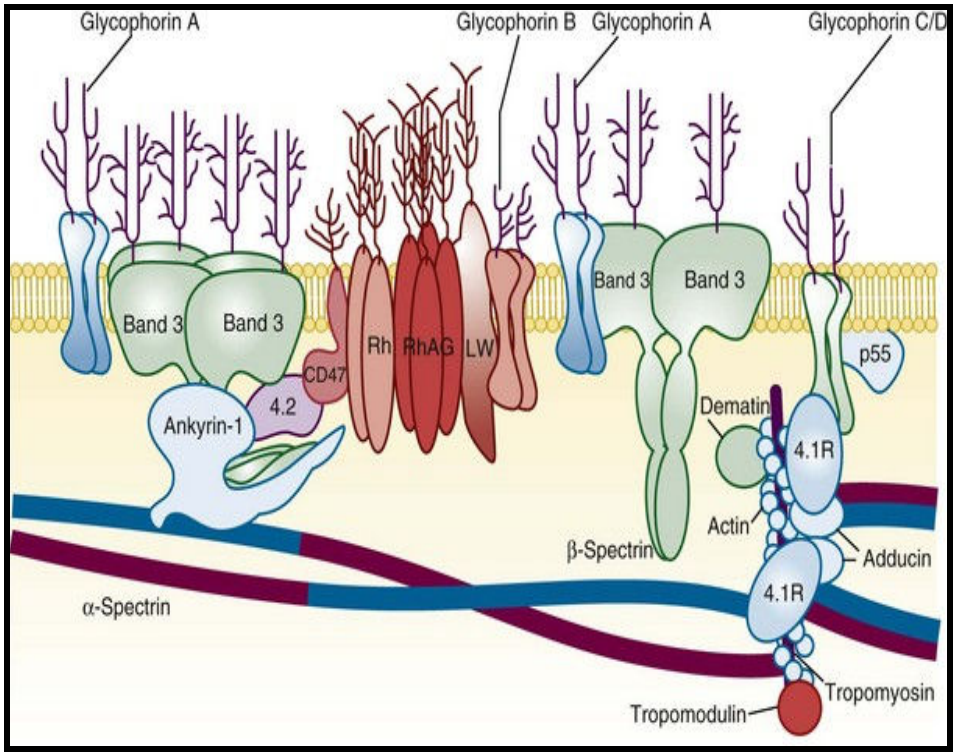

Figure (3): Red blood cell membrane structures ***(Perrotta et al., 2008).***

Erythropoiesis:

The process by which RBCs are produced is called erythropoiesis. RBCs are continuously being produced in the red bone marrow of large bones, at a rate of about 2 millions per second. In the embryo, the liver is the main site of RBC production. The production can be stimulated by the hormone erythropoietin (EPO) a glycoprotein with a molecular weight of about 34 kDa., 90% of it synthesized by the kidney in the peritubular interstitial cells in response to hypoxia and 10% in the liver and elsewhere ***(Haase, 2013).***

Fate and markers of RBCs aging:

Under normal circumstances, all human RBCs live approximately 120±4 days in blood circulation .When plasma cell membrane receptors or antigens indicating apoptosis and the metabolic system become progressively less active, the cell becomes more and more fragile then finally ruptures during passage through some tight spots of the circulation ***(Antonelou et al., 2010)***.

Many of the aging cells are self-destructed and become susceptible to recognition by phagocytes and subsequent phagocytosis in the spleen, liver, and bone marrow occurs ***(Brissot et al., 2004).***

The components of the degraded erythrocytes' Hb are further processed as follows:

- Globin, the protein portion of Hb, is broken down into amino acids which can be sent back to the bone marrow to be used in the production of new erythrocytes.

- The iron contained in the heme portion of Hb is either stored in the liver and spleen, primarily in the form of ferritin or hemosiderin, or carried through the bloodstream by transferrin to the red bone marrow for recycling into new erythrocytes.

- The non-iron portion of heme is degraded into the waste product biliverdin which is reduced into bilirubin. Bilirubin binds to albumin and travels in the blood to the liver, which uses it in the manufacture of bile. In the large intestine, bacteria breaks the bilirubin apart from the bile and converts it to urobilinogen and then into stercobilin. It is then eliminated from the body in the feces. The kidneys also remove any circulating bilirubin and other related metabolic byproducts such as urobilins and secrete them into the urine ***(Kikuchi et al., 2005)*** **Figure (4).**

Almost all erythrocytes are removed in this manner from the circulation before they are old enough to hemolyse. Hemolysed Hb is bound to a protein in plasma called haptoglobin (Hp) ***(Brissot et al., 2004).*** The cellular receptor

target of Hp is the monocyte/macrophage scavenger receptor, CD163. Following Hb-Hp binding to CD163, cellular internalization of the complex leads to globin and heme metabolism. When Hb is released from RBCs within the physiologic range of Hp, the potential deleterious effects of Hb are prevented ***(Dominik et al., 2014; Buehler and Alayash, 2008).***

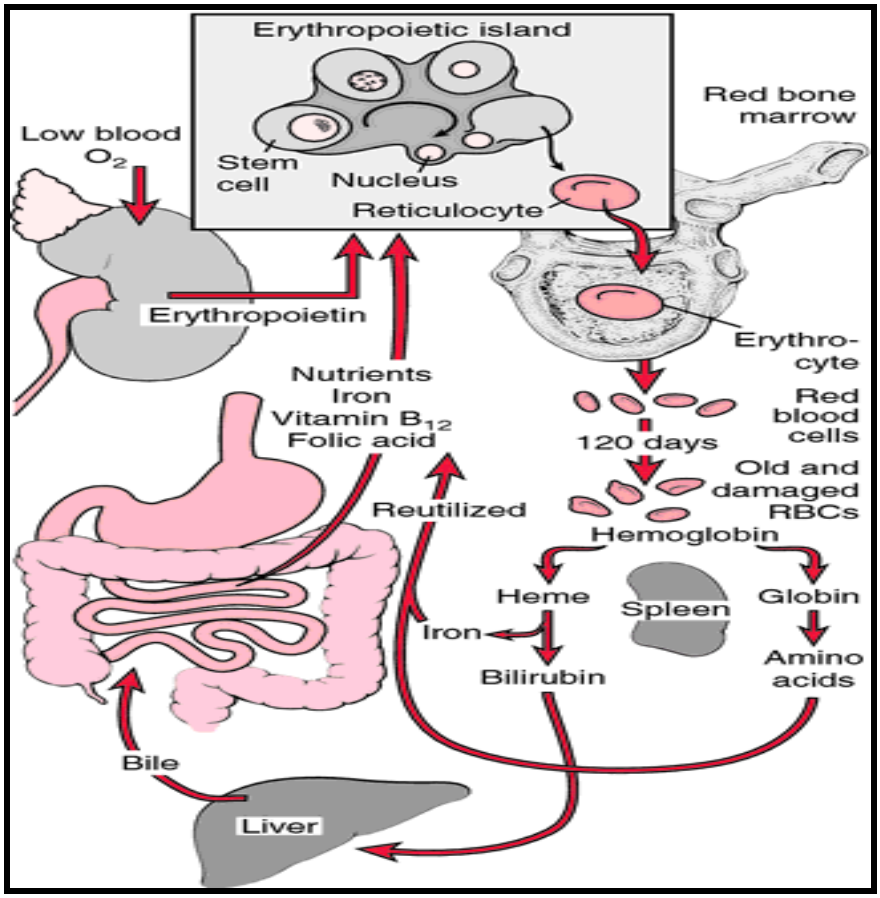

Figure (4): Life cycle of RBCs ***(Sullivan, 2008).***

RBC Metabolism:

During erythrocytes intravascular lifespan, they require energy to maintain a number of vital cell functions. These include (1) maintenance of glycolysis; (2) maintenance of the electrolyte gradient between plasma and red cell cytoplasm through the activity of ATP-driven membrane pumps; (3) synthesis of glutathione and other metabolites; (4) purine and pyrimidine metabolism; (5) maintenance of Hb's iron in its functional, reduced, ferrous state; (6) protection of metabolic enzymes, Hb, and membrane proteins from oxidative denaturation; and (7) preservation of membrane phospholipid asymmetry ***(Wijk and Solinge, 2005)***.

Because of the lack of nuclei and mitochondria, mature RBCs are incapable of generating energy via the (oxidative) Krebs cycle. Instead, erythrocytes depend on the anaerobic conversion of glucose by the Embden-Meyerhof pathway **Figure (5)** and hexose monophosphate pathway. Moreover, erythrocytes possess a unique glycolytic bypass for the production of 2,3-diphosphoglycerate (2,3-DPG), the Rapoport-Luebering shunt. This shunt bypasses the phosphoglycerate kinase (PGK) step and accounts for the synthesis and regulation of 2,3-DPG levels that decrease Hb's affinity for O_2 ***(D'Alessandro and Zolla, 2013).***

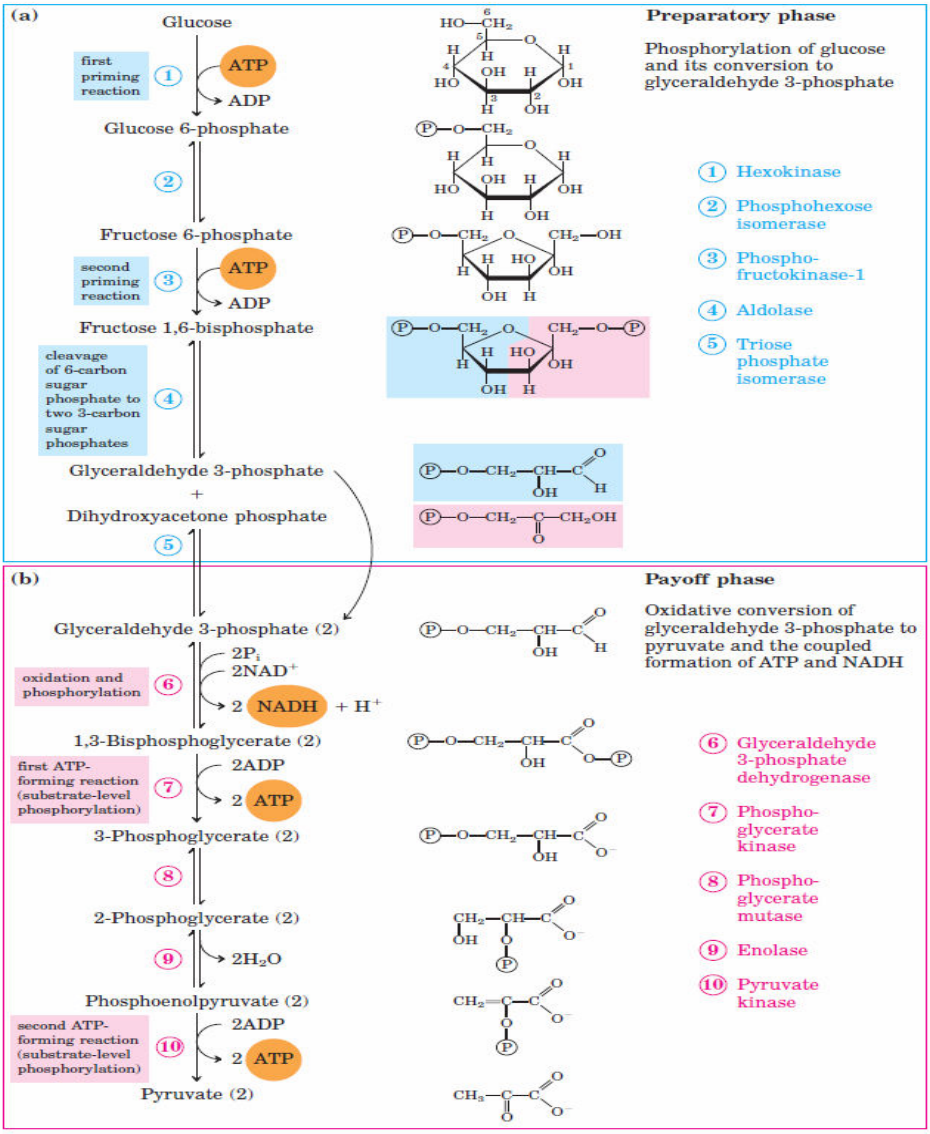

Figure (5): The Embden-Meyerhof Pathway ***(Giri, 2016)***