"ASSESSMENT OF STORAGE RELATED HEMATOLOGICAL AND BIOCHEMICAL CHANGES OF CITRATE PHOSPHATE DEXTROSE ADENINE-1 WHOLE BLOOD"

By

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Dissertation submitted to the

BLDE University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

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Date: **Dr. Bhaswanth.P**

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LIST OF ABBREVATIONS USED

CPDA-1	Citrate Phosphate Dextrose Adenine-1
WBCs	White Blood Cells
RBCs	Red Blood Cells
HGB	Hemoglobin
WHO	World Health Organisation
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
ACD	Acid Citrate Dextrose
ATP	Adenosine Triphosphate
2,3 – DPG	2,3 – diphosphoglycerate
NACO	National Aids Control Organisation
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug administration
SAGM	Saline Adenine Glucose Mannitol
TRALI	Transfusion related acute lung injury
Fig	Figure

ABSTRACT

INTRODUCTION

Blood transfusion is life saving, being administered to approximately 5 million patients per year in India. Blood can be stored under refrigeration in preservative solutions such as citrate phosphate dextrose adenine (CPDA- 1) solution for up to 35 days. These storage solutions have met the requirements mandating that red blood cells (RBCs) transfused at the end of the approved storage period should have at least 75% of the cells remaining in the circulation 24 hours after infusion, and that the hemolysis in the stored bag be <1%. Upon storage, RBCs undergo numerous biochemical and physiological changes with the concomitant release of potentially hazardous bioactive products referred to as RBC storage lesions.

OBJECTIVE

A cross-sectional study was carried out on 50 blood donors who donated blood voluntarily in the blood bank of Shri B.M.Patil Medical College, Hospital and Research Centre from December 2014 to June 2016.This study was done to determine certain hematological & biochemical changes in blood when stored during different periods of time (at 5 storage periods from zero to 28 days) using CPDA-1solution as preservative.

MATERIALS & METHODS:

Prospective blood donors who fulfilled standard blood donor selection were included in the study. The study was carried out on donors who come to donate blood voluntarily in our blood bank. After blood donation of 350/450 ml of whole blood, a blood sample consisted of 15 ml from each blood bag was taken. Each sample was divided into 5 portions, each portion consists of 3 ml of blood was added into plain test tube. One of these tubes was analyzed on the day of collection, which is regarded

as control. The other tubes are kept in blood bank refrigerator at 4-6^0 C to be analyzed on day 7, day 14, day 21, day 28.

Each sample was analyzed using the 5 part differentiated automated Hematoanalyzer for hematological parameters like RBC count, WBC count, Platelet count, Hemoglobin, Hematocrit (PCV), Mean cellular hemoglobin (MCH) and Cobas C 311 biochemical analyzer to measure Plasma Potassium.

RESULT

Analysis of variance (ANOVA) showed that at the end of 28 days, there was significant decrease in WBC count ($P<0.01$), Platelet count ($P<0.01$) among hematological parameters and plasma potassium levels ($P<0.01$) was significantly increased compared on day 0 to day 28. No significant changes were observed in other parameters throughout the study.

CONCLUSION

The present study indicates that rapid degeneration of WBCs could lead to immunomodulation. Therefore, Whole blood or RBC concentrates should be leukodepleted before storage. Blood banks should also check for baseline K level on the day of collection and blood bags should be selected for transfusion by harmonising low K with the first in first output (FIFO) system. Potassium absorption filters should be used during transfusion to prevent transfusion related hyperkalemia.

KEY WORDS: Potassium, white blood cells, Red blood cell storage lesion

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INTRODUCTION

Blood is considered as the living force of our body, it is an essential element of human life and there are no substitutes for it.[1-2] Blood is made up of plasma, platelets, white blood cells (WBCs) and red blood cells (RBCs) which are life saving components.[3] Blood transfusion is required for trauma cases, major surgeries and patients in need of long term therapies.[1] Every year with an increase in population, life-expectancy, urbanization and increased demand for blood products, the burden of requirement of safe blood is increasing.[4]

Blood transfusion is the process of transferring blood or blood based products from one person into the circulatory system of another to treat certain medical conditions.[5] Many trials were done until the year 1914, at which began the modern era of blood transfusion.[6] The first anticoagulant preservative (citrate-glucose solution) was introduced by Rous and Turner in 1916.[7] In 1918, Rous Turner's solution used for storage of human blood during the First World War.[7] The next important development occurred in 1943 when acid citrate dextrose (ACD) solution was introduced for clinical use by Mollison.[8] In 1957 Gibson developed an improved preservative of citrate-phosphate-dextrose (CPD), that increases the storage time upto 28 days.[9] In 1978 Citrate-phosphate-dextrose-Adenine (CPDA-1) was developed which is regarded as one of the best preservative that increases the blood storage time upto 35 days and it is now a days used in every blood bank in the world.[6]

The goal of blood preservation is to provide viable and functional blood components for patients who need a blood transfusion.[10] Viability is a measure of in vivo red blood cells survival following blood transfusion.[10] During storage, preserved blood cells undergo progressive structural and functional changes like decreased pH, an increase of lactic acid, decrease in adenosine triphosphate (ATP) levels and

decrease glucose consumption, that may reduce red cell function and viability after transfusion.[6] These changes are collectively known as RBC storage medium lesion, which is related to bioreactive substances released by leukocytes in the storage medium such as histamine, lipids and cytokines. These may exert direct effect on metabolic and physical changes associated with senescence, such as membrane regulation, decrease in cell size, increase in cell density, alteration of cytoskeleton and phosphatidylserine exposure, RBCs lose 2,3 – diphosphoglycerate (2,3 – DPG), and few components of the cell membrane, while becoming more rigid and demonstrating reduced oxygen off-loading.[6]

In 1962, Rossmusen found that there is some loss of erythrocyte during storage. He found that the post transfusion survival viability is 100%, later this declined by about 90% in about one week of storage and about 70% in three weeks of storage.[10] This means that hemoglobin concentration continues to decline with increased period of storage.[10] Platelets are viable longer when stored at room temperature, more activated and able to form clot more effectively when stored at 4^0C. WBCs also lose their phagocytic property within 4-6 hrs of collection and become non-functional after 24 hrs of storage. It is important to remember that they do not lose their antigenic property and are capable of sensitizing the recipient to produce non-hemolytic febrile transfusion reactions and few lymphocytes may remain viable even after 3 weeks of storage.[6]

Despite Indian population of more than one billion, National AIDS Control Organisation (NACO) statistics show that the annual rate of blood donation in India is about 9.3 million units, against the requirement of 10 million units. This shows the vast gap between demand and supply of safe blood and blood products in India.[11,12]

Considering above discussed facts, in the present study, we studied and analyzed the hematological and biochemical changes of stored blood in BLDEU Shri B.M.Patil Medical College, Hospital and Research Centre blood bank to make blood transfusions safe.

AIMS AND OBJECTIVES

To assess the hematological and biochemical changes in stored CITRATE PHOSPHATE DEXTROSE ADENINE – 1 whole blood.

REVIEW OF LITERATURE

Introduction

The past two decades have witnessed increased scrutiny regarding efficacy and risk of the once unquestioned therapy of RBC transfusion. Simultaneously, a variety of changes has been identified within the RBC and storage media during RBC preservation that are correlated with reduced tissue oxygenation and transfusion associated adverse effects.[13]

These alterations are collectively termed the storage lesion and include extensive hematological, biochemical, biomechanical, and immunologic changes involving cells of diverse origin.[13]

History of blood donation:

Since as early as 2,500 B.C. humans know about the blood. At that time Egyptians used to draw blood out of the body in an attempt to cleanse the body of diseases. In 500 B.C. Greeks used to perform human dissections in attempts to better understand how the blood flows through the body.[3]

In 1628, British physician William Harvey discovered how blood circulates throughout the body, while in 1665, the first recorded successful blood transfusion was done in England by Physician Richard Lower who kept a dog alive by transfusing blood from another dog.[3,14]

In 1818, British obstetrician James Blundell performed the first successful human blood transfusion for treatment of a postpartum haemorrhage.[3,14]

In 1901, Karl Landsteiner, discovered the first three human blood groups.[14]

In 1939-1940, Landsteiner along with Alexander Weiner, Philip Levine and R.E. Stetson discovered the Rh blood group system.[14]

In 1907. Reuben Ottenberg performs the first blood transfusion using blood typing and cross matching.[14]

In 1939-1940, The Rh blood group system was discovered by Karl Landsteiner, Alexander Wiener, Philip Levine and R.E. Stetson.[14]

In 1947, ABO blood-typing and syphilis testing were performed on each unit of blood. [14]

In 1957, The American association of blood banks formed its committee on inspection and accreditation to monitor the implementation of standards for blood banking. [14]

In 1969, S.Murphy and F.Gardner demonstrate the feasibility of storing platelets at room temperature, revolutionizing platelet transfusion therapy. [14]

In 1992, testing of donor blood for HIV-1 and HIV-2 antibodies is implemented. [14]

In 2002, Nuclei acid amplification test (NAT) for HIV and Hepatitis C virus (HCV) licensed by FDA. [14]

Blood donation process:

The blood donation process mainly involves four basic stages:

1. Donor Registration
2. Medical history questionnaire and mini-physical examination
3. Collection of blood
4. Recovery stage

Donor registration

During this stage blood bank staff signs in the donor with basic information like name, age, sex, address, and contact number. Educational material is given to the donor in the language which he/she understands and a general idea about the whole

process of blood donation is given to the donor. The donor is also asked to show a valid identification document to confirm his/her identity. [3]

Questionnaire and physical examination

The health history questionnaire helps to determine donor eligibility and requires the donor to reveal private health information as well as places they have traveled. The questionnaire also helps to determine the level of knowledge and attitude of donor towards blood donation process. This is also done to ensure the integrity and safety of the blood being donated. [3]

Physical examination includes; measurement of temperature, pulse and blood pressure. A blood sample is drawn to determine the blood hemoglobin levels. These measures are taken for the safety of donors.[3]

Collection of blood

Before the blood is collected from the donor, he/she should meet the blood donation criteria which are established for the protection of the blood donor and the transfusion recipients.

Criteria for blood donation (As per FDA guidelines) followed at our blood bank

- Age : 18 to 60 years
- Weight: above 50 kilogram
- Normal vital signs (Pulse rate, blood pressure and respiratory rate)
- Minimum hemoglobin concentration of 12.5 g/dl
- Minimum of 3 months interval between two consecutive blood donations
- No evidence or history of infections like hepatitis, malaria, HIV/AIDS, tuberculosis, typhoid and sexually transmitted diseases

- No evidence or history of any heart disease, lung disease, kidney disease, diabetes, jaundice, cancer/malignancy, epilepsy and abnormal bleeding tendency
- No history of medication within past 72 hour like antibiotics, aspirin, alcohol, steroids and vaccination.
- No history of a dog bite or rabies vaccine within the past 1 year.
- No history of any surgery/ blood transfusion in past 6 months.
- For women donors: No evidence of pregnancy, abortion in the past 3 months, having a child less than 1 year old, breast feeding and a minimum 3 days gap after menstruation.

Blood is collected in such a manner that the risk of bacterial contamination is minimized. The skin at the site of venepuncture is prepared with an antibacterial scrub. Whole blood is collected into sterilized bag sets containing anticoagulant and attached satellite bags to facilitate component separation in a closed system. Phlebotomy is done and the rate of blood flow should be sufficient to prevent clot formation within the tubing. The volume of blood withdrawn should be less than 10% of the donor's expected blood volume. So, typically 350ml is withdrawn for whole blood collection and 450 ml for component preparation.[3,15]

Blood components can also be collected by apheresis which has the advantage of greater volume of the desired components being obtained from a single donor. The most common use of the apheresis donation is the collection of platelets, commonly called as 'single donor platelets'. Plasma can also be collected concurrently with platelets. Apheresis donation allows for the collection of two units of red blood cells from suitable donors. Leukocytes can also be collected by apheresis. This is most

commonly done for the collection of hematopoietic progenitor cells for either autologous or allogeneic transplantation.[15,16]

Autologous donation is the collection of blood from a patient in advance of scheduled surgery for transfusion during or after the procedure to compensate for expected blood loss. Autologous transfusion prevents the transmission of blood-borne pathogens from allogeneic donors. Candidates for autologous transfusion must not be anaemic. Candidate must not have heart disease. Typically, two or three units can be collected several weeks before surgery. Optimally, there should be sufficient time from donation to surgery to allow for recovery of a substantial portion of the collected red cell mass. [16]

Some patients prefer to select their own donors, i.e. they bring their own donors (directed or designated donation) rather than receiving blood from the community blood supply. This provides patients perception of greater safety, although there remains a risk of transfusion transmitted diseases. These donors must meet all the criteria for allogeneic blood donation and must be compatible with the intended recipient. Directed donation is desired in cases like rare blood group and limitation of donor exposures for patients with long-term expected transfusion requirements such as aplastic anemia.[16]

Recovery stage

This stage involves a recovery period of about 10-15 minutes. This time allows blood bank staff to observe the donor for any physical reaction or complication as a result of the donation process. This recovery stage also allows the donors to receive refreshments in order to rehydrate their body due to fluid loss during donation. This time also allows blood bank staff to interview donor on one on one basis to determine the level of knowledge and attitude about blood donation. Blood bank staff

can clear donors' misconceptions and doubts about the process of blood donation and can also get to know about the experience of the blood donation during this time. [3]

Hemoglobin levels in donors

Blood donors are required to have hemoglobin level of at least 12.5 g/dl or hematocrit (PCV) of 38% in order to donate blood. This is to ensure that donors have an adequate number of red blood cells (RBCs) for donation as well as adequate iron stores for erythropoiesis following donation.[16]

Being deferred from donation due to a low hematocrit during screening does not always mean the patient is anaemic or has a medical problem. For example, male donors with a hematocrit below the acceptable level of 38% are considered anaemic, but non-anaemic women within the normal hematocrit range of 36-37% are not able to donate blood.[16]

Although this practice turns away non-anaemic women from donating blood, it reduces the chance of depleting their iron stores and potentially causing anaemia following donation. Men are allowed to donate when slightly anaemic because it is much easier for them to replace the iron lost during donation.[16]

Hemoglobin screening methods

There is no consensus among blood banks on the best method for blood donor anaemia screening. In hospitals and laboratories, the gold standard for hemoglobin estimation is the hemoglobincyanide method provided by automatic haematology analyzers.[17]

Screening tests for potential blood donors however require quicker, easier, and more cost-effective testing methods that do not require a venepuncture.

The tests which are commonly used for primary screening are as follows-[17]

1. Copper sulfate method
2. Microhematocrit method
3. Hemacue method
4. Automated hematology analyzer

Though the first three tests used for haemoglobin estimation are quick, easy and relatively inexpensive; their sensitivity, specificity, and accuracy are lower than that of automatic haematology analyzers.[17]

Separation of components

Whole blood is separated into components to improve their utility and storage. For this, 450ml of blood is collected in a triple bag. This facilitates the treatment of different patients with requirements for RBCs, plasma proteins or platelets. The aim of components separation is to maintain viability and to prevent detrimental changes or contamination of the desired constituents. One unit of whole blood (450ml) gives one unit each of Packed Red Blood Cell (PRBC) (200ml), fresh frozen plasma (200ml) and platelets (50ml).[17]

Procedure

The process starts with the collection of 450 ml of blood under all aseptic precautions in a triple bag. It is then kept for one hour at room temperature for settlement of RBCs which then undergoes the process of light spin on blood bag centrifuge. Prior to this, the centrifuge is balanced with two blood bags kept in opposite compartments. Then the blood bags are centrifuged at 1800 rpm for 20 minutes (light spin) at room temperature. This separates RBCs from platelet rich plasma. RBCs settle down and then platelet rich plasma is expressed out into the first empty bag with laminar flow method using plasma expressor instrument. Then the

RBCs remaining in the main bag are hot sealed and separated from other two bags.[18,19]

The bag containing platelet rich plasma along with the second empty bag then undergoes the process of heavy spin on blood bag centrifuge after the centrifuge is balanced. The bags are centrifuged at 2600 rpm for 20 minutes (heavy spin). This separates platelets from plasma. The plasma is expressed out into the second empty bag with laminar flow method using plasma expressor instrument and hot sealed.[18,19]

This process separates 200ml of packed red blood cells, 200 ml of plasma and 50 ml of platelet concentrate from 450 ml of whole blood.[18]

Packed red blood cells (PRBC)

RBCs are prepared from whole blood by centrifugation and separation of plasma. Anti-coagulant commonly used is CPDA-1. Dextrose and Adenine are added to it to preserve red cell ATP levels. RBC in CPDA-1 can be stored for up to 35 days at 1-6˚C. This period can be extended up to 42 days with use of SAGM as anti-coagulant. Red cells undergo senescence changes during storage similar to aging in vivo, so that post-transfusion a portion of transfused red cells are rapidly cleared by spleen. The maximum allowable storage time for RBC is calculated as the time required for recovery of 70% of transfused cells, 24 hours after transfusion. Changes occurring during storage are leakage of intracellular potassium along with the decrease in 2, 3-DPG levels.[20]

Fresh frozen plasma

Plasma can be stored in a frozen state for 1 year at -60˚C. Fresh frozen plasma (FFP) is separated from red blood cells and should be placed at -18˚C or lower within 8 hours of collection. In the liquid state loss of labile clotting factors, particularly factor VIII and factor V occurs. Before transfusion, FFP is thawed at 37˚C in a water

bath and then it must be transfused within 24 hours of thawing. Thawed plasma can be stored at refrigerator temperatures for up to 5 days and still maintain adequate levels of factor V and VIII.[18,21]

Platelet concentrate

Platelet concentrates are prepared from whole blood by heavy spin after separation of RBCs and platelet poor plasma is expressed out in other satellite bag. Platelet concentrates must contain at least $5.5x10^{10}$ platelets per unit to be effective. They are stored at room temperature (20-24˚C) on platelet agitator because platelets have greatly diminished post-transfusion survival when stored at refrigerator temperature (1-6˚C). They can be stored for a maximum period of 5 days with continuous gentle agitation. A therapeutic dose for a typical adult patient is typically obtained by pooling five or more platelet concentrates. Platelet concentrates prepared by apheresis (single donor platelets) are stored and handled in the same manner as platelet concentrates prepared from whole blood. Each apheresis platelet unit should contain a minimum of $3.0x10^{11}$ platelets.[18,22]

Red blood cells

To prevent hemolytic transfusion reactions it is of primary consideration that the selected RBC is serologically compatible. It is desirable to select fresh (less than 10 days old) units in situations where it is particularly desirable to limit the number of transfusions, such as intrauterine and neonatal transfusion, since the post-transfusion survival of red cells is inversely related to the duration of storage.[16]

Platelets

Platelets express ABO antigens very weakly. So, Major ABO compatibility is of less importance in platelet transfusion. ABO incompatible platelet transfusions may result in lower post-transfusion survival, although this is usually not clinically

significant. An acute hemolytic reaction can occur because of transfusion of isohemagglutinins contained in the plasma of apheresis platelets from donors with high-titre of anti-A or anti-B. Therefore, more consideration is typically given to plasma compatibility with the recipient when non ABO-identical platelets are to be transfused.[16]

Transfusion associated reactions

These are adverse reactions to transfusion that occur during or shortly after transfusion. There are two broad categories of transfusion associated reactions based on time of initiation of the reaction viz. acute reactions and delayed reactions. The transfusion should be stopped immediately and I.V. line should be maintained with saline. All the suspected transfusion reactions should be reported to the blood bank. The work-up and treatment of a transfusion reactions must be predicted on the clinical picture.[19]

Acute Reactions:[19]

- Sepsis/Bacterial Contamination
- Mild Allergic Reactions
- Moderate (Anaphylactoid) and Severe (Anaphylactic) Allergic Reactions
- Acute Hemolytic Reactions
- Transfusion-Related Acute Lung Injury (TRALI)
- Transfusion-Associated Circulatory Overload (TACO)
- Hypotensive Transfusion Reactions
- Febrile Non-hemolytic Reactions

Delayed Reactions:[19]

- Transfusion-Associated Graft-vs.-Host Disease (TA-GvHD)
- Delayed Hemolytic Reactions (>24 Hours)

Storage related changes in whole blood:

Preservation and long term storage of Red Blood Cells (RBCs) are needed to ensure a readily available, safe blood supply for transfusion medicine.[23] During storage, preserved blood cells undergo progressive structural and functional changes that may reduce red cell function and viability after transfusion.[23] These alterations which are collectively termed as storage lesion include extensive biochemical, biomechanical and immunological changes involving cells of diverse origin.[24]

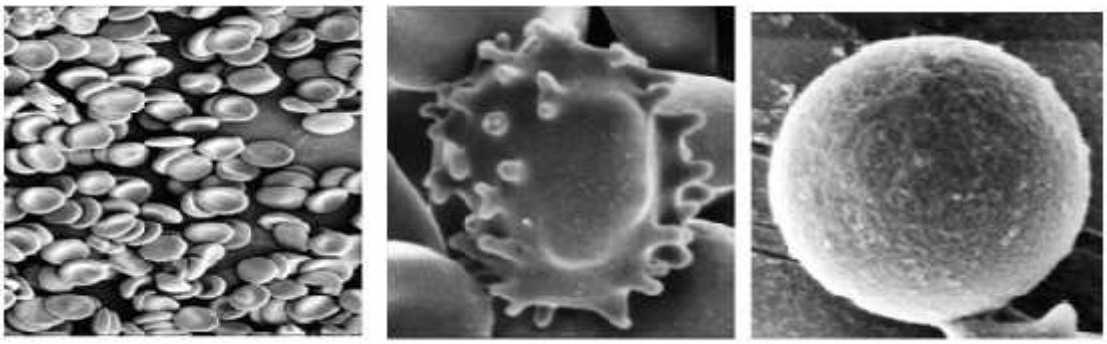
Hematological changes during rbc storage:

The properties of RBCs over several weeks of storage show a noticeable increase in Red cell distribution width (RDW), while other parameters including the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) remain same. In addition, the rate of morphological changes from discocyte to spherocyte gradually increases and becomes 50% at 2 weeks. Platelets will reduce to more than half of original with in first five days of storage and WBC will reduce as per their half life.[25]

Biomechanical changes during rbc storage:

The changes include alterations in corpuscle shape, deformability, osmotic fragility, and intracellular viscosity. These changes have been shown to affect RBC transit through the microcirculation with a resultant counterintuitive decrement in tissue oxygenation. Specific changes in RBC morphology include, a transition from a deformable biconcave disc to poorly deformable echinocytes with protrusions and ultimately non-deformable spheroechinocytes.[26] The most probable sites of damage are cytoskeletal proteins such as spectrin, ankyrin, protein 4.2 and band 3 in RBC membrane which leads to increased osmotic fragility and changes in electrolyte

imbalance of RBCs.[27,28] The osmotic fragility is used to determine the susceptibility of RBCs to osmotic stress.[26]



(a) (b) (c)

Fig.1: "Scanning electron micrographs of erythrocytes with signs of hemolysis."[26]

Micrograph (a) shows RBCs with normal biconcave shape without any sign of damage. Micrographs (b and c) shows spherocytes and echinocytes respectively.

In the case of leukoreduced packed RBC, the storage lesion is due to lipid peroxidation of RBC membrane and leads to morphological alterations in stored blood.[29]

Malondialdehyde (MDA) is one of the by-products resulting from lipid peroxidation of polyunsaturated fatty0 acids. Measuring MDA is a useful marker in assessing the extent of lipid peroxidation thus assessing the oxidative damage.[30]

A study done by Chaudary and katharia proved that MDA levels increased significantly from days 14 to day 28, which indicated lipid peroxidation due to oxidative stress.[31]

Collard *et al* [32] measured the MDA level in pediatric packed RBC bag and showed a significant increase in day 36 and day 42, which can be correlated with the

occurrence of oxidative stress and lipid peroxidation that resulted in membrane damage.

Immunological changes during RBC storage:

Allogenic blood transfusion results in the infusion into the recipient of large amounts of foreign antigens in both soluble and cell-associated forms. The persistence of these antigens in the circulation of the recipient may create conditions that allow the development of immune down-regulation. Evidence from a variety of sources indicates that allogenic blood transfusion enhances the survival of renal allografts and the incidence of postoperative bacterial infections, as well as reduce the recurrence rate of Crohn disease and/or activate infections with cytomegalovirus or human immunodeficiency virus. This clinical syndrome, the mechanisms of which remain to be defined, has been referred to in transfusion medicine literature as transfusion–associated immunomodulation (TRIM).[33]

Lee *et al* reported that donor WBCs can persist in humans for up to 1.5 years after blood transfusion and can elicit an immune response.[33]

A study conducted by Mane V *et al*, shows certain hematological changes do occur in stored whole blood over a duration of storage and rapid degeneration of leukocytes could lead to immunomodulation related to blood transfusion.[23]

Biochemical changes during rbc storage:

The biochemical changes within stored RBCs are principally related to alterations in energy metabolism with 2, 3-diphosphoglycerate (DPG) and ATP depletion.

2,3- DPG:

It is an allosteric modifier of hemoglobin which plays a critical role in the release of oxygen at the end-organ. Levels of 2,3-DPG have been shown to fall

quickly during the storage of RBC, becoming undetectable within 2 weeks. This observation has raised concern that despite improved oxygen delivery with transfusion, stored RBCs may not release sufficient oxygen to the tissues to the expected levels. Normalization of 2,3-DPG levels begins within hours of transfusion and is completely restored within 48to 72 hours.[13]

ATP:

Due to its central role in cellular metabolism, adequate levels of ATP are essential for innumerable cellular processes. Examples include the maintenance of Na+-K+ ATPase activity, RBC membrane stability, glucose transport, oxidative stress defense mechanisms, membrane phospholipid distribution and regional vasodilation under hypoxic conditions. While ATP depletion can result in the characteristic deformation changes seen with prolonged RBC storage. These morphologic changes are reversed with normalization of ATP levels.[13]

Potassium:

As a result of RBC damage, hemolysis occurs which leads to the release of potassium (K) into plasma. Normally the extracellular level of K is much lower than intracellular levels. The extracellular levels can be increased only if RBC's are hemolyzed or Na-K ATPase of RBC membrane is not functioning adequately. Sudden exposure of RBC's to additive solutions in blood storage bags may result in either lysis or damage of the RBC's due to osmotic stress.[34]

Baliarsingh S & Jaiswal M,[34] showed the association of an increase in extracellular baseline potassium levels in CPDA-1 whole blood bags with a greater increase of the same on further storage.

In a study by Hod E[35] *et al* provides evidence that transfusion of older stored RBCs produces a proinflammatory response that is associated with increased levels of

tissue iron in the liver, spleen, and kidney, and increased circulating levels of non transferring bound iron (NTBI). This suggests that the pro-oxidant effects of iron released after acute clearance of stored RBCs may be responsible for some of the harmful effects of RBC transfusion after prolonged storage.

A study conducted by Okrah C, Acquah B & Dogbe E showed that there was an increase in potassium level with an increase in storage time, due to this care is needed in determining age of the blood, volume transfused at a time and rate of transfusion to minimize hyperkalemia related blood transfusion complications.[36]

Mukherjee S *et al* [37] indicated that biochemical alterations do occur in stored RBC's and these changes are within acceptable limits of safety untill 21 days of storage.

Uvizl R *et al* [38] indicated that patients receiving multiple transfusions within a short span of time can show increased levels of potassium and lactate. This increase is proportional to the age of RBCs and Volume of RBCs transfused.

MATERIALS AND METHODS

SOURCE OF DATA

A cross-sectional study was carried out on blood donors who donated blood voluntarily in blood bank of BLDEU Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur.

Study period: 1st December 2014 to 30th June 2016.

INCLUSION CRITERIA: Prospective blood donors who fulfilled standard blood donor selection criteria (As per FDA guidelines) were included in the study.

1. Appearance: General healthy look
2. Age : 18-60 yrs.
3. Sex: Male or Female
4. Weight : Above 45kgs
5. Hemoglobin or PCV : > 12.5 gms or >38%
6. Blood Pressure: 100-160mm of HG/60-100 mm of HG
7. Temperature: Afebrile (Normal)
8. Pulse: 60-100/min
9. Donors not suffering or not having history of diseases like hepatitis B/C, HIV/AIDS, Sexually Transmitted Diseases, Malignancy, Malaria and any other major illness.
10. Donors not having any prior history of major surgery or blood transfusion with in past 6 months.
11. Female donors who are not pregnant, or not having a history of abortion with in last 3 months.
12. Three months interval, free from previous blood donations.

EXCLUSION CRITERIA:

Donors not fulfilling the above mentioned inclusion criteria.

METHODS OF COLLECTION OF DATA:

- The study was carried out on donors who came to donate blood voluntarily in the blood bank.
- All donors were required to fill voluntary donor selection/rejection form and then general physical examination was performed.
- Pre-donation hemoglobin assessment was assessed.
- Donors were selected based on routine donor selection criteria.
- After blood donation of 350/450 ml of whole blood, a blood sample of 15 ml from each blood bag was taken. Each sample was divided into 5 portions. Each portion consisting of 3 ml of blood was added into a plain test tube. One of these tubes was analyzed on the day of collection, which is regarded as control. The other tubes were kept in the blood bank refrigerator at $4\text{-}6^0$ C to be analyzed on day 7, day 14, day 21, day 28.
- Each sample was analyzed using the 5 part differentiated automated Hematoanalyzer for hematological parameters like RBC count, WBC count, Platelet count, Hemoglobin, Hematocrit (PCV) and Mean cellular hemoglobin (MCH).
- Samples were analyzed using the Cobas C 311 biochemical analyzer to measure Plasma Potassium.

FIG. 2: HEMATOLOGY ANALYSER SYSMEX XN-1000:

FIG. 3: BIOCHEMICAL ANALYSER COBAS C 311:

SAMPLE SIZE:

With the level of significance at 1%, Power of test of difference of mean as 90%, Anticipated Mean difference of potassium is 21.2 mmol/l and anticipated Standard difference of potassium is 25mmol/l.[5]

Statistical formula $n = \frac{(Z\alpha+Z\beta)^2 \times 2 \times SD^2}{MD^2}$

Where $Z\alpha = 2.33$ (One tail test)

$Z\beta = 1.28$ (One tail test)

Anticipated MD : 21.2

Anticipated SD : 25

Total calculated sample size is 50

Hence 50 cases were included in the study.

STATISTICAL ANALYSIS: Data was analysed by using statistical methods like,

1. Data will be presented as Mean+/- SD.
2. ANOVA
3. Tukey Post-Hoc test

RESULTS

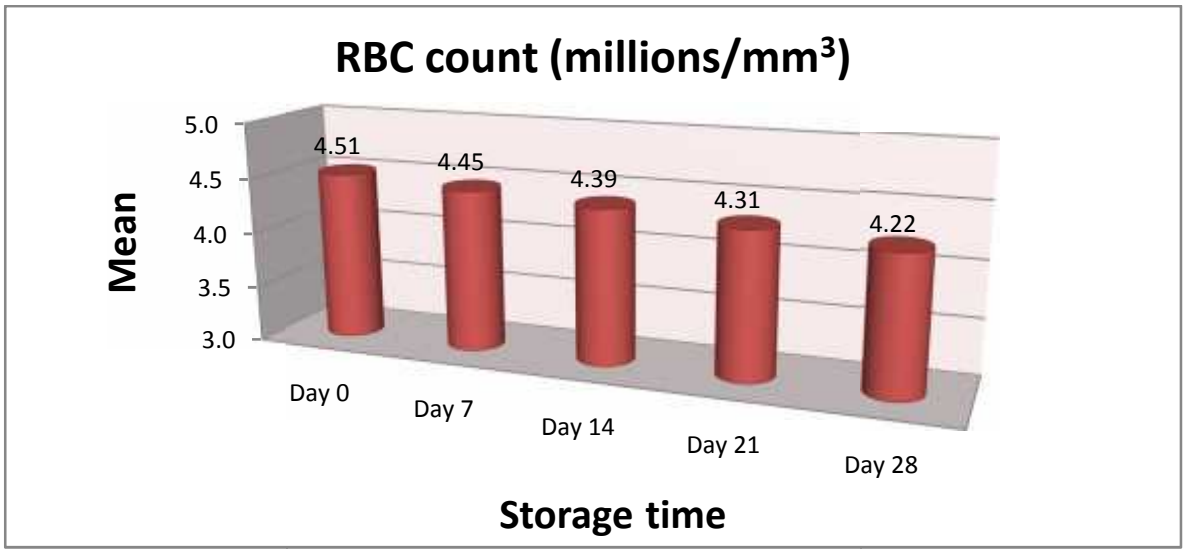
The purpose of this study was to analyse relationships between initial and final levels of hematological parameters (RBC count, WBC count, Platelet count, Hemoglobin, PCV, MCH) and concentration of potassium in the whole blood. The results were as follows:

Table 1: Mean distribution and ANOVA analysis of RBC count (millions/mm^3) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	3.4	6.1	4.5	0.5	4.36	4.67	1.9	0.104
Day **7**	3.1	6.1	4.5	0.6	4.29	4.62		
Day **14**	3.1	6.0	4.4	0.6	4.23	4.55		
Day **21**	2.5	6.2	4.3	0.6	4.14	4.49		
Day **28**	3.0	5.8	4.2	0.6	4.05	4.40		
Total	2.5	6.2	4.4	0.6	4.31	4.45		

*significant at 5% level of significance

Fig. 4 : Mean distribution of RBC count on various days of storage



The mean distribution and ANOVA analysis of RBC was slightly reduced from day 0 to day 28 but not as drastically as WBC count. The mean RBC count on day 0 was 4.5 and on day 28 was 4.2 with the p value of 0.104 which is not significant.

Table 2: Comparisons of Means of RBC count on various days of storage by Tukey Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	vs	Day 7	0.06	0.983	-0.26	0.38
Day 0	vs	Day 14	0.12	0.820	-0.19	0.44
Day 0	vs	Day 21	0.20	0.423	-0.12	0.52
Day 0	vs	Day 28	0.29	0.090	-0.03	0.61
Day 7	vs	Day 14	0.06	0.984	-0.26	0.38
Day 7	vs	Day 21	0.14	0.762	-0.18	0.46
Day 7	vs	Day 28	0.23	0.280	-0.09	0.55
Day 14	vs	Day 21	0.08	0.967	-0.24	0.39
Day 14	vs	Day 28	0.17	0.601	-0.15	0.49
Day 21	vs	Day 28	0.09	0.932	-0.23	0.41

*significant at 5% level of significance

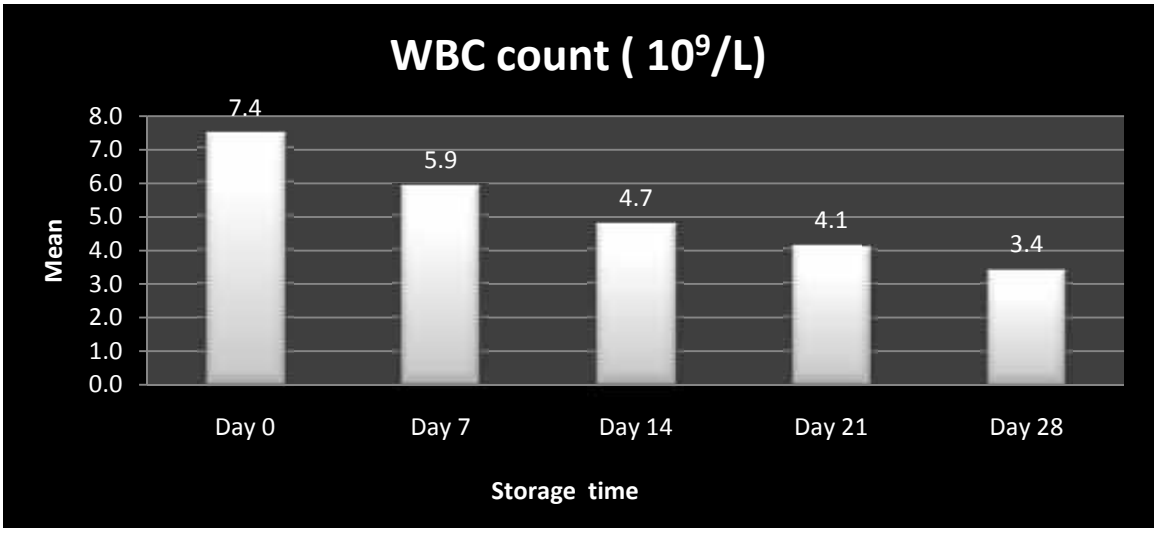
The RBC count shows no statistically significant change when compared between weeks of storage.

Table 3: Mean distribution and ANOVA analysis of WBC count (10^9/L) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	3.7	10.8	7.4	1.7	6.96	7.91	59.3	**<0.01***
Day **7**	3.1	10.5	5.9	1.6	5.43	6.32		
Day **14**	1.7	8.5	4.7	1.5	4.33	5.17		
Day **21**	1.5	8.9	4.1	1.5	3.68	4.51		
Day **28**	1.3	6.0	3.4	1.0	3.10	3.69		
Total	1.3	10.8	5.1	2.0	4.86	5.36		

*significant at 5% level of significance

Fig. 5 : Mean distribution of WBC count on various days of storage



The mean distribution and ANOVA analysis of WBC count showed reduction from day 0 to day 28. The mean on day 0 was 7.4 while on day 28 was 3.4 which shows a markedly reduced WBC count with the P value being <0.01.

Table 4: Comparisons of Means of WBC count on various days of storage by Tukey Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	vs	Day 7	1.56	<0.01*	0.76	2.37
Day 0	vs	Day 14	2.68	<0.01*	1.89	3.49
Day 0	vs	Day 21	3.34	<0.01*	2.54	4.15
Day 0	vs	Day 28	4.04	<0.01*	3.24	4.85
Day 7	vs	Day 14	1.13	<0.01*	0.32	1.93
Day 7	vs	Day 21	1.78	<0.01*	0.98	2.59
Day 7	vs	Day 28	2.48	<0.01*	1.68	3.28
Day 14	vs	Day 21	0.66	0.165	-0.15	1.46
Day 14	vs	Day 28	1.36	<0.01*	0.55	2.16
Day 21	vs	Day 28	0.70	0.121	-0.10	1.50

*significant at 5% level of significance

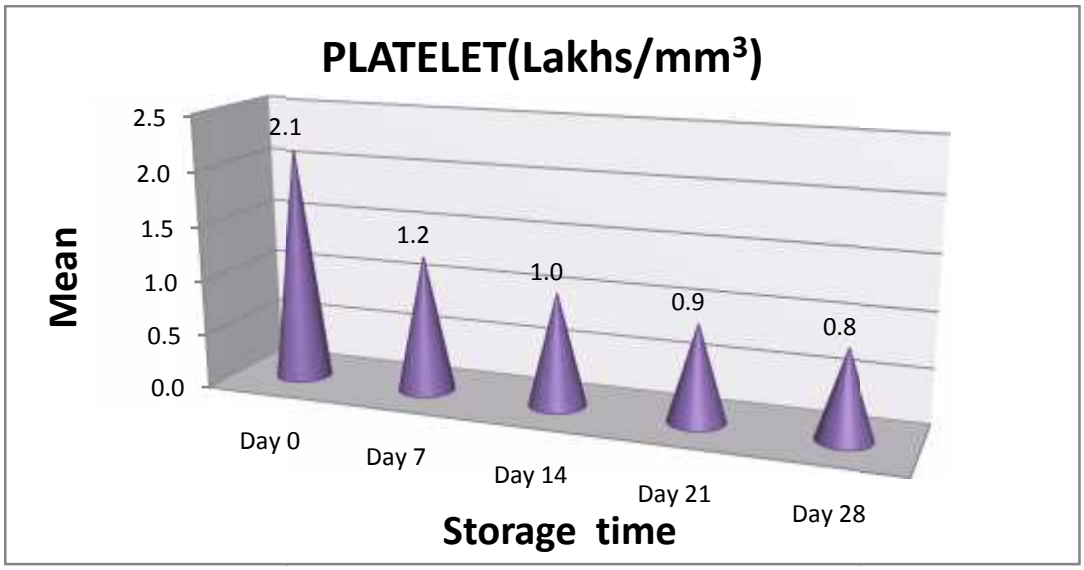
The mean value of WBC count on day 0 was 7.4 compared to mean value on day 7 being 5.9 which shows a maximum decrease of WBC count within the first week of storage with p value being <0.01.

Table 5: Mean distribution and ANOVA analysis of Platelet count (Lakhs/mm^3) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	0.7	3.3	2.1	0.5	1.97	2.28	88.8	**<0.01***
Day **7**	0.5	2.5	1.2	0.5	1.11	1.37		
Day **14**	0.4	2.1	1.0	0.4	0.92	1.12		
Day **21**	0.3	2.0	0.9	0.3	0.79	0.96		
Day **28**	0.2	1.4	0.8	0.3	0.71	0.88		
Total	0.2	3.3	1.2	0.6	1.13	1.29		

*significant at 5% level of significance

Fig. 6: Mean distribution of Platelet on various days of storage



The mean platelet count on day 0 was 2.1 and on day 28 was 1.2 which shows a markedly reduced platelet count with the p value being <0.01.

Table 6: Comparisons of Means of Platelet count on various days of storage by Tukey Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	Vs	Day 7	0.89	<0.01*	0.67	1.11
Day 0	Vs	Day 14	1.11	<0.01*	0.89	1.34
Day 0	Vs	Day 21	1.26	<0.01*	1.03	1.48
Day 0	Vs	Day 28	1.33	<0.01*	1.11	1.56
Day 7	Vs	Day 14	0.22	0.053	0.00	0.44
Day 7	Vs	Day 21	0.37	<0.01*	0.14	0.59
Day 7	Vs	Day 28	0.44	<0.01*	0.22	0.67
Day 14	Vs	Day 21	0.14	0.388	-0.08	0.37
Day 14	Vs	Day 28	0.22	0.053	0.00	0.44
Day 21	Vs	Day 28	0.08	0.878	-0.15	0.30

*significant at 5% level of significance

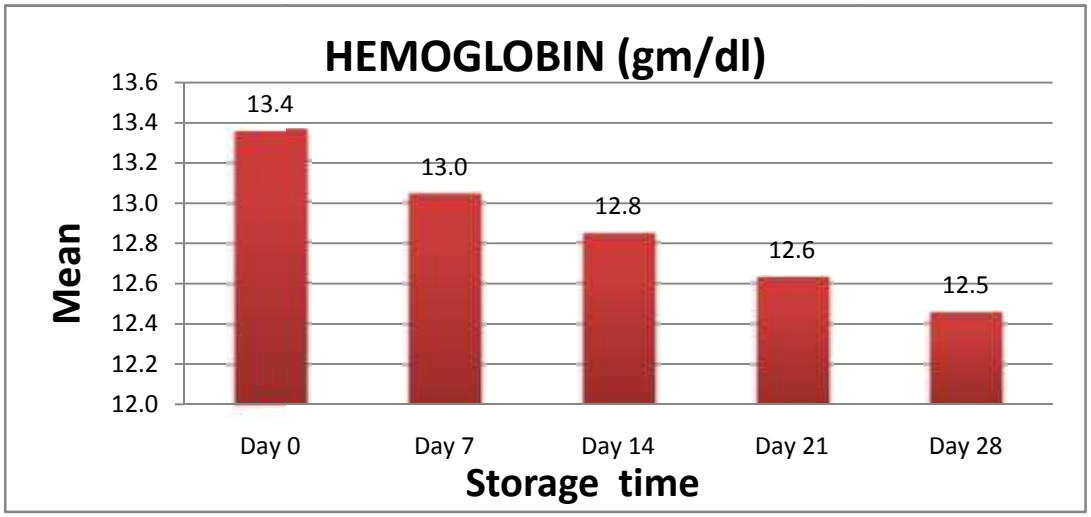
The mean value of platelet count on day 0 was 2.1 compared to mean value on day 7 being 1.2 which showed a maximum decrease of platelet count within the first week of storage with p value being <0.01.

Table 7: Mean distribution and ANOVA analysis of hemoglobin (gm/dl) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	10.1	19.5	13.4	1.5	12.94	13.77	2.5	0.041*
Day **7**	9.0	19.5	13.0	1.8	12.55	13.54		
Day **14**	8.5	16.6	12.8	1.6	12.38	13.31		
Day **21**	7.9	16.1	12.6	1.5	12.21	13.05		
Day **28**	9.5	16.5	12.5	1.5	12.03	12.88		
Total	7.9	19.5	12.9	1.6	12.67	13.07		

*significant at 5% level of significance

Fig, 7: Mean distribution of Hemoglobin (gm/dl) on various days of storage



The mean distribution and ANOVA analysis of hemoglobin was slightly reduced from day 0 to day 28 but not as drastically as WBC count. The mean of hemoglobin on day 0 was 13.4 and on day 28 was 12.9 with the p value of 0.041 which is not significant.

Table 8: Comparisons of Means of Hemoglobin on various days of storage by Tukey Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	vs	Day 7	0.31	0.857	-0.55	1.17
Day 0	vs	Day 14	0.51	0.481	-0.35	1.37
Day 0	vs	Day 21	0.73	0.141	-0.13	1.59
Day 0	vs	Day 28	0.90	0.036*	0.04	1.76
Day 7	vs	Day 14	0.20	0.970	-0.66	1.06
Day 7	vs	Day 21	0.42	0.674	-0.45	1.28
Day 7	vs	Day 28	0.59	0.333	-0.27	1.45
Day 14	vs	Day 21	0.22	0.957	-0.64	1.08
Day 14	vs	Day 28	0.39	0.725	-0.47	1.25
Day 21	vs	Day 28	0.17	0.982	-0.69	1.03

*significant at 5% level of significance

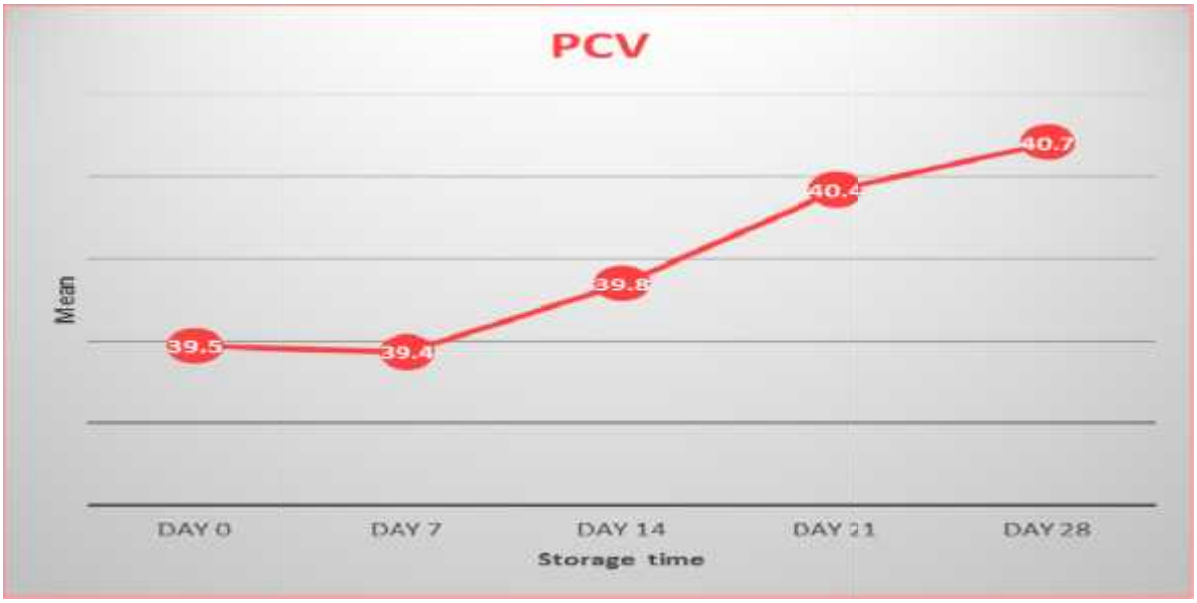
The hemoglobin shows no statistically significant change when compared between weeks of storage.

Table 9: Mean distribution and ANOVA analysis of PCV(%) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	29.7	55.4	39.5	4.5	38.18	40.75	0.7	0.612
Day **7**	26.2	55.3	39.4	5.2	37.96	40.91		
Day **14**	25.2	47.2	39.8	4.9	38.45	41.24		
Day **21**	23.2	48.6	40.4	4.9	39.02	41.81		
Day **28**	25.2	51.3	40.7	5.0	39.27	42.14		
Total	23.2	55.4	40.0	4.9	39.36	40.59		

*significant at 5% level of significance

Fig. 8 : Mean distribution of PCV(%) on various days of storage



The mean distribution and ANOVA analysis of PCV was slightly increased from day 0 to day 28. The mean of PCV on day 0 was 39.5 and on day 28 is 40.7 with the p value of 0.612 which is not significant.

Table 10: Comparisons of Means of PCV on various days of storage by Tukey-Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	vs	Day 7	0.04	1.000	-2.67	2.74
Day 0	vs	Day 14	-0.38	0.995	-3.08	2.32
Day 0	Vs	Day 21	-0.95	0.870	-3.65	1.75
Day 0	Vs	Day 28	-1.24	0.717	-3.94	1.46
Day 7	Vs	Day 14	-0.42	0.993	-3.12	2.29
Day 7	Vs	Day 21	-0.99	0.854	-3.69	1.72
Day 7	Vs	Day 28	-1.27	0.694	-3.98	1.43
Day 14	Vs	Day 21	-0.57	0.978	-3.27	2.13
Day 14	Vs	Day 28	-0.86	0.907	-3.56	1.84
Day 21	Vs	Day 28	-0.29	0.998	-2.99	2.41

*significant at 5% level of significance

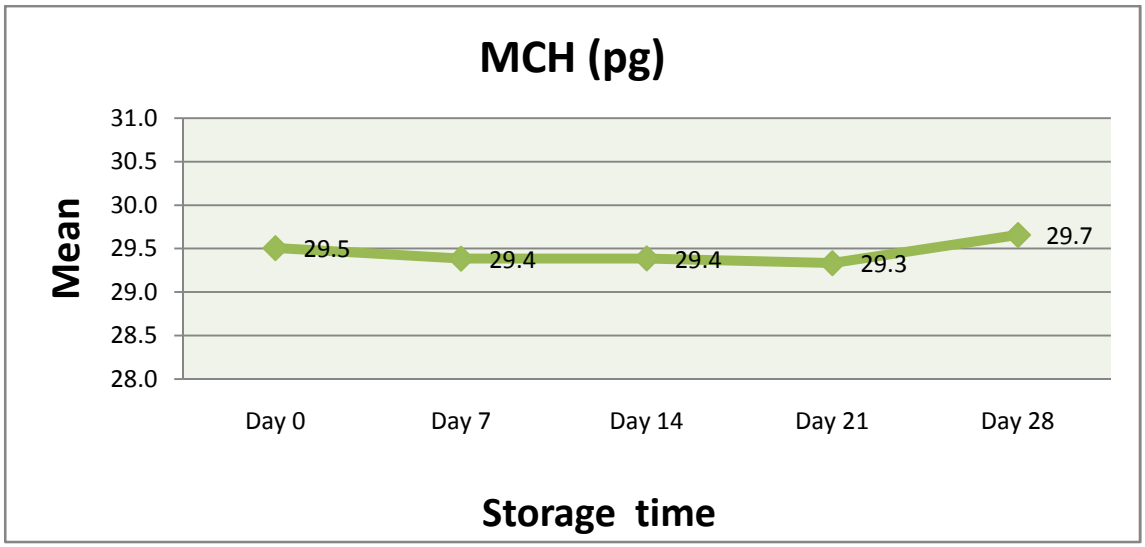
The PCV shows no statistically significant change when compared between weeks of storage.

Table 11: Mean distribution and ANOVA analysis of MCH(pg) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	20.5	36.7	29.5	3.0	28.66	30.36	0.1	0.984
Day **7**	20.3	36.5	29.4	2.9	28.57	30.20		
Day **14**	20.3	36.7	29.4	2.8	28.58	30.19		
Day **21**	20.2	36.7	29.3	3.0	28.47	30.20		
Day **28**	21.0	38.5	29.7	3.2	28.75	30.56		
Total	20.2	38.5	29.5	3.0	29.08	29.82		

*significant at 5% level of significance

Fig. 9 : Mean distribution of MCH on various days of storage



The mean distribution and ANOVA analysis of hematocrit (MCH) was constant from day 0 to day 28 with the p value of 0.984 which is not significant.

Table 12: Comparisons of Means of MCH on different storage points by Tukey Post Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	Vs	Day 7	0.12	0.999	-1.52	1.76
Day 0	Vs	Day 14	0.12	0.999	-1.52	1.76
Day 0	Vs	Day 21	0.17	0.998	-1.47	1.82
Day 0	Vs	Day 28	-0.15	0.999	-1.79	1.49
Day 7	Vs	Day 14	0.00	0.999	-1.64	1.64
Day 7	Vs	Day 21	0.05	0.999	-1.59	1.69
Day 7	Vs	Day 28	-0.27	0.991	-1.91	1.37
Day 14	Vs	Day 21	0.05	0.999	-1.59	1.70
Day 14	Vs	Day 28	-0.27	0.991	-1.91	1.37
Day 21	Vs	Day 28	-0.32	0.983	-1.97	1.32

*significant at 5% level of significance

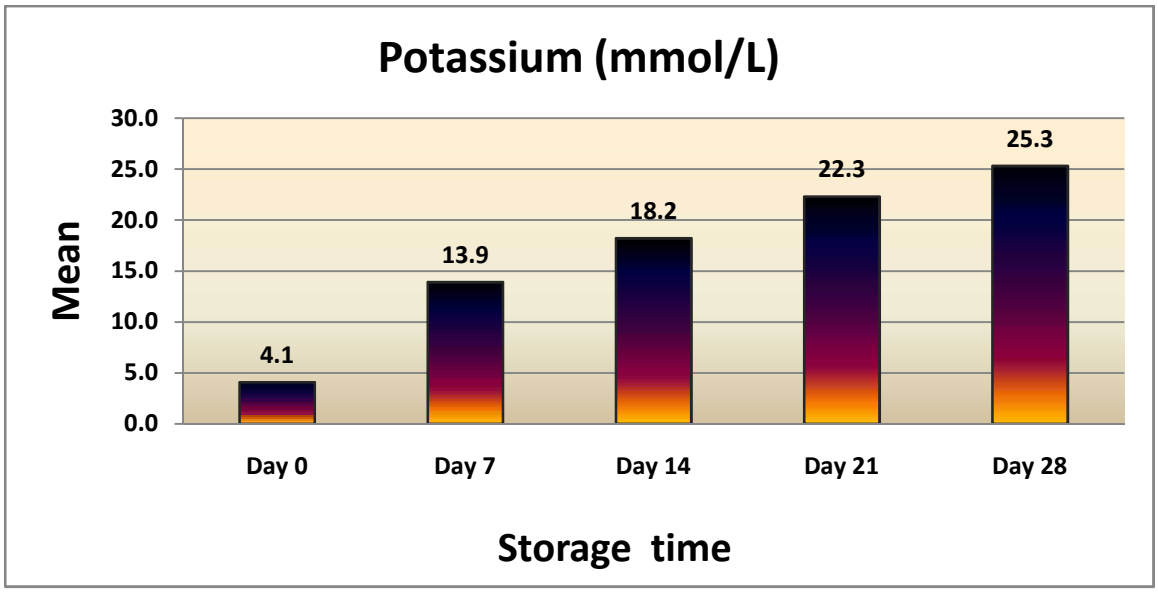
The PCV shows no statistically significant change when compared between weeks of storage.

Table 13: Mean distribution and ANOVA analysis of Potassium (mmol/L) on various days of storage

Storage	Minimum	Maximum	Mean	SD	95% Confidence Interval		F value	p value
Day **0**	2.7	5.4	4.1	0.6	3.91	4.26	304.4	**<0.01***
Day **7**	9.4	22.0	13.9	3.0	13.06	14.75		
Day **14**	11.2	27.0	18.2	3.5	17.23	19.19		
Day **21**	13.0	30.5	22.3	4.0	21.18	23.45		
Day **28**	18.3	36.6	25.3	4.4	24.06	26.55		
Total	2.7	36.6	16.8	8.1	15.75	17.78		

*significant at 5% level of significance

Fig. 10: Mean distribution of Potassium on various days of storage



The mean on day 0 was 4.1 mmol/L and on day 28 was 25.3 mmol/L which shows a significant increase with the p value being <0.01.

Table 14: Comparisons of Means of Potassium on different storage points by Tukey Post-Hoc test

Comparison			Mean Difference	p value	95% Confidence Interval	
Day 0	vs	Day 7	-9.83	<0.01*	-11.67	-7.98
Day 0	vs	Day 14	-14.13	<0.01*	-15.98	-12.28
Day 0	vs	Day 21	-18.23	<0.01*	-20.08	-16.39
Day 0	vs	Day 28	-21.22	<0.01*	-23.07	-19.38
Day 7	vs	Day 14	-4.30	<0.01*	-6.15	-2.46
Day 7	vs	Day 21	-8.41	<0.01*	-10.25	-6.56
Day 7	vs	Day 28	-11.40	<0.01*	-13.24	-9.55
Day 14	vs	Day 21	-4.10	<0.01*	-5.95	-2.26
Day 14	vs	Day 28	-7.10	<0.01*	-8.94	-5.25
Day 21	vs	Day 28	-2.99	<0.01*	-4.84	-1.14

*significant at 5% level of significance

The mean value of potassium on day 0 was 4.1 compared to mean value on day 7 being 13.9 which showed three fold increase within the first week of storage with p value being <0.01.

DISCUSSION

An estimated 90 million units of RBCs are transfused worldwide annually. Transfusion of RBCs saves lives and enables many medical therapies.[39] However, RBCs can be stored for a limited period of time. Blood is stored to achieve a good post-transfusion survival.[40] The practice of storing RBCs prior to transfusion has been in place for close to a century. Indeed, the storage and banking of RBCs is a necessity to provide a sufficient volume of RBCs for patient needs. However, banking blood is an unnatural state for RBCs to existing in the static environment of a refrigerator shelf, in a solution of glucose, citrate, and other additives, in the absence of a kidney or liver to detoxify products of ongoing metabolism and likewise with no reticuloendothelial system to remove senescent or damaged RBCs.[41]

Transfusion of stored RBCs, particularly those at the end of the approved shelf life, upon storage, undergo numerous biochemical, metabolic, hematological and molecular changes which result in irreversible damage with the concomitant release of potentially hazardous bioactive products referred to as RBC storage lesions.[42,43]

In recent years, the scope of clinical concern regarding transfusion of stored RBCs has widened from traditional issues of replacing lost RBCs with stored RBCs that could deliver oxygen to peripheral tissues, to concerns regarding the accumulation of toxicological entities in stored RBCs that could lead to medical sequelae upon transfusion.

Therefore the main focus of the present study is to analyze relationship between initial and final levels of RBC count, WBC count, Platelet count, Hemoglobin, PCV, MCH and plasma potassium which contribute for the storage lesions in stored whole blood.

Table 15: Means of RBC count in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
RBC Count (millions/mm^3)	Latham et al[44]	4.0±0.5	3.9±0.4	>0.01
	Mane et al[23]	4.8	-----	0.369
	Adias TC et al[6]	4.7	4.5	0.376
	Present study	4.5±0.5	4.2±0.6	0.104

p value <0.05 is significant

As mentioned in the table no:15, the studies done by Latham *et al,*[44] Mane *et al*[23] *and* Adias TC *et al*[6] Showed no significant decrease in the RBC count even at the end of 28 days of storage. The findings of the present study are consistent with the above mentioned findings.

Nevertheless, all the authors concluded that there is mild hemolysis during RBC storage, However it is not statistically significant. This proved that whole blood which is stored till day 28 contains more than 95% of viable RBCs and in that more than 70% of RBCs are expected to remain viable in circulation 24 hours after transfusion of stored blood.

In addition to the above findings, Antwi-Baffour S *et al*[24] and Mustafa I *et al*[26] also found that blood stored in CPDA-1 for a period of 35 days at 2-8^0 C produced significant changes in RBC osmotic fragility & morphological integrity. The present study is in concordance with these observations.

Mustafa I *et al*[26] also found that as the storage time increases cells starts transforming to spherocytes and echinocytes which indicate mild hemolysis during storage. This further explains why there is a need to review the guidelines of RBC storage in the blood bank.

Table 16: Means of WBC count in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
WBC Count (cells/mm^3)	Latham et al[44]	7.2±2.3	2.1±0.6	**<0.05**
	Ahmed S et al[25]	6.7	1.5	**----**
	Mane V et al[23]	7.59	2.57	**0.000**
	Adias TC et al[6]	5.43	2.77	**0.000**
	Ai-Nuaimy et al[5]	5.2±0.28	0.4±0.04 (Day 8)	**0.01**
	Oluyombo R et al[40]	5.1±1.9	0.2±0.12	**<0.01**
	Present study	7.4±1.7	3.4±1.0	**<0.01**

p value <0.05 is significant

As mentioned in the table no:16, In the present study, when the mean values of WBC on day 0 were compared with day 28, it was observed that there was a rapid reduction in WBC count (p value <0.01) . This was consistent with the other studies done by Latham *et al*,[44] Ahmed S *et al*,[25] Mane V *et al*,[23] Adias TC *et al*,[6] Ai-Nuaimy *et al*[5] and Oluyombo R *et al*[40] in which the mean WBC count showed a significant reduction from day 0 to day 28.

The cause of the rapid reduction in WBC count (Particularly granulocytes) is most likely due to the ATP depletion and moreover they are also consumed in the formation of microaggregates which are conglomerates of leucocytes, platelets, fibrin and cellular debris formed during storage.[45,46] The clinical significance of this observation is that stored whole blood would be particularly ineffective in management of aplastic anemia and leucopenia patients.[23]

It is believed that , these WBCs, upon exposure to the acidic conditions of storage and refrigeration, become activated and release cytokines, which can lead to

their delivery at high concentrations during transfusion.[42] In addition, during routine storage of RBCs, lipids accumulate in the plasma fraction that can prime neutrophils, causing neutrophil-mediated cytotoxicity of human pulmonary endothelial cells, which has been implicated as the mechanism of lung injury in transfusion-related acute lung injury (TRALI).[42]

Table 17: Means of Platelet count in different studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
Platelet count (Lakhs/mm^3)	Ahmed S et al[25]	2.53	0.36	----
	Mane V et al[23]	2.45	0.96	**<0.05**
	Adias TC et al[6]	112.70	105.20	0.195
	Ai-Nuaimy et al[5]	267±13.82	22.1±2.0 (Day 8)	**0.01**
	Present study	2.8±0.5	0.8±0.3	**<0.01**

p value <0.05 is significant

As mentioned in the table no:17, In the present study, when the mean values of platelet count on day 0 were compared with day 28, there was rapid deterioration in platelet count which was statistically significant (P value <0.01). This was consistent with the other studies done by Ahmed S *et al*,[25] Mane V *et al*[23] and Ai-Nuaimy *et al*[5] in which the mean platelet count showed a significant reduction from day 0 to day 28. But the study done by Adias TC *et al*[6] showed a reduction of platelet count from day 0 to day 28 which is not statistically significant.

The cause of this rapid decrease in platelet count is most likely due to their short life span, ATP depletion and consumption in microaggregates.[23] The clinical significance of this observation is, stored whole blood may be useful as a source of platelets within first five days of storage but the storage at 4^0C would make the platelets loose their hemostatic functions with in 48 hours.[25] This fact has to be kept in mind especially in the centres which still do not have the blood component services.

Table 18: Means of Hemoglobin level in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
Hemoglobin (gm/dl)	Latham et al[44]	12±1	12±1	>0.05
	Mane V et al[23]	13.6	14.0	0.945
	Adias TC et al[6]	11.85	12.14	0.952
	Ai-Nuaimy et al[5]	16.50±0.35	10.85±0.16	**0.01**
	Dallal bashi AY etal[10]	14.6±3.8	12.4±0.13	**<0.05**
	Present study	13.4±1.5	12.5±1.5	0.041

p value <0.05 is significant

As mentioned in the table no:18, In the present study, when the mean values of hemoglobin on day 0 were compared with that of day 28, it was observed that there is a slight decrease in hemoglobin values, however, it was not statistically significant. This was consistent with the studies done by Latham *et al,*[44] Mane V *et al*,[23] Adias TC *et al*.[6] However, the studies done by Ai-Nuaimy *et al*[5] and Dallal bashi AY *et al*[10] did show a significant reduction of hemoglobin values from day 0 to day 28. They have attributed this decrease to the hemolysis that occur during storage.

Table 19: Means of PCV level in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
PCV (%)	Antwi-Baffour S et al[24]	38.47±3.11	36.40±3.07	0.147
	Latham et al[44]	35±3	36±4	>0.01
	Ahmed S et al[25]	40.1	36	-----
	Mane V et al[23]	37.89	38.52	0.345
	Adias TC et al[6]	33.63	34.20	0.312
	Ai-Nuaimy et al[5]	44.3±0.72	38.2±0.94	**0.01**
	Dallal bashi AY et al[10]	43.8±0.1	38.1±0.13	**<0.05**
	Oluyombo R et al[40]	38.8±3.1	37.5±3.2	**<0.05**
	Present study	39.5±4.5	40.7±5.0	0.612

p value <0.05 is significant

As mentioned in the table no:19, In the present study, when the mean values of PCV on day 0 were compared with day 28, it was observed that there was no statistically significant change. This was consistent with the other studies done by Latham *et al,*[44] Mane V *et al,*[23] Adias TC *et al,*[6] Ahmed S *et al.*[25] However, the study done by Ai-Nuaimy *et al,*[5] Dallal bashi AY *et al,*[10] Oluyombo R *et al*[40] showed a statistically significant reduction of hematocrit values from day 0 to day 28 which correlates with the mild hemolysis of RBCs during storage.

Table 20: Means of MCH level in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
MCH (pg)	Mane V et al[23]	30.35	30.00	0.790
	Adias TC et al[6]	29.18	29.23	0.805
	Present study	29.5±3.0	29.7±3.2	0.984

p value <0.05 is significant

As mentioned in the table no:20, In the present study, when the mean values of MCH on day 0 were compared with day 28, it was observed that there was no change and MCH values are constant through out the period of storage. This is consistent with the other studies done by Mane V *et al*,[23] Adias TC *et al*.[6]

Table 21: Means of Potassium level in various studies from day 0 to day 28:

Parameter	Studies	DAY 0	DAY 28	P value
Potassium (mmol/L)	Opoku-Okrah et al[36]	3.31	**14.98 (Day 20)**	**<0.05**
	Mukherjee S et al[37]	6.4±1	37.8±5.7	**<0.001**
	Baliarsingh S et al[34]	2.9±0.5	**24.1**±4.2	**<0.0001**
	Adias TC et al[6]	2.64	**15.71**	**<0.000**
	Present study	4.1±0.6	25.3±4.4	**<0.01**

p value <0.05 is significant

As mentioned in the table no:21, In the present study, the parameter which showed a consistent and detrimental increase throughout the storage period is potassium, with the average level at the end of four weeks reaching six times that of baseline. This was consistent with the other studies done by Opoku-Okrah *et al*,[36] Mukherjee *et al*,[37] Baliarsingh *et al*[34] and Adias *TC et al*[6]. Our study also proved that one unit increase of baseline potassium is a good predictor of an increase in around two and half units of potassium on day 7, around four units on days 14 and 21, and six units on day 28 of storage and this is consistent with the study done by baliarsingh S *et al.*[34]

The raise in potassium level is mainly due to the sudden exposure of RBC's to additive solutions in blood storage bags resulting in either lysis or damage of the more fragile population of RBC's due to osmotic stress which continues even during further storage.[47] The supernatant potassium concentration in whole blood or RBC units is frequently much higher, especially in units nearing the end of their storage life. Clinical hyperkalemia has been recognized as a transfusion complication for decades,

and there have been reported cardiac arrests attributed to transfusion-associated hyperkalemia.[48]

CONCLUSION

The present study indicates that rapid degeneration of WBCs could lead to adverse immune effects, microbial risks, and non-hemolytic febrile transfusion reactions. Therefore, Whole blood or RBC concentrates should be leukodepleted before storage.

The present study indicates that there is a rapid and consistent increase of potassium during storage of whole blood. Therefore, Fresh whole blood or RBC concentrate should be used where ever they are indicated.

The present study suggests that blood banks should also check for baseline potassium level on the day of collection and blood bags should be selected for transfusion by harmonising low potassium with the first in first output (FIFO) system:

- To avoid the ill effects of hyperkalemia, blood units with pre-existing high potassium values should be considered for early release from blood banks
- Potassium absorption filter should be used during the transfusion to prevent transfusion related hyperkalemia.

The present study also indicates that stored whole blood can be used as a source of platelets within first five days of storage.

It is time to shift the attention back to study the prolonged storage effects on whole blood to improve the allogenic efficacy of blood transfusion.

"Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."—Winston S. Churchill, "The Bright Gleam of Victory".

SUMMARY

- Present study was conducted as a cross-sectional study from December 2014 to June 2016.
- Total 50 samples were studied.
- After blood donation of 350/450 ml of whole blood , a blood sample of 15 ml from each blood bag was taken. Each sample was divided into 5 portions. Each portion consisting of 3 ml of blood was added into a plain test tube. One of these tubes was analyzed on the day of collection, which is regarded as control. The other tubes were kept in the blood bank refrigerator at 4-6^0 C to be analyzed on day 7, day 14, day 21, day 28.
- Each sample was analyzed using the automated Hematoanalyzer for hematological parameters like RBC count, WBC count, Platelet count, Hemoglobin, PCV, MCH and Cobas C 311 biochemical analyzer to measure Plasma Potassium.
- All the study samples were analyzed for hematological and potassium changes on day 0 (Control), day 7, day 14, day 21 and day 28 of storage.
- The parameters like RBC count, hemoglobin, PCV, MCH did not show any significant change when compared with day 0 and day 28.
- The parameters like WBC count and Platelet count showed a significant reduction when compared with day 0 to day 28.
- The Plasma potassiumshowed a consistent and significant increase throughout the storage period.
- Whole blood should be leukodepleted before storage to prevent immunomodulation.
- Blood banks should use potassium absorption filters to prevent transfusion related hyperkalemia.

LIMITATIONS OF THE STUDY

The ATP and RBC membrane cytoskeletal proteins are important for the integrity of the RBC membrane. The decrease levels of these two parameters leads to leakage of potassium and leads to hyperkalemia. The determination of above parameters was not performed in the present study.

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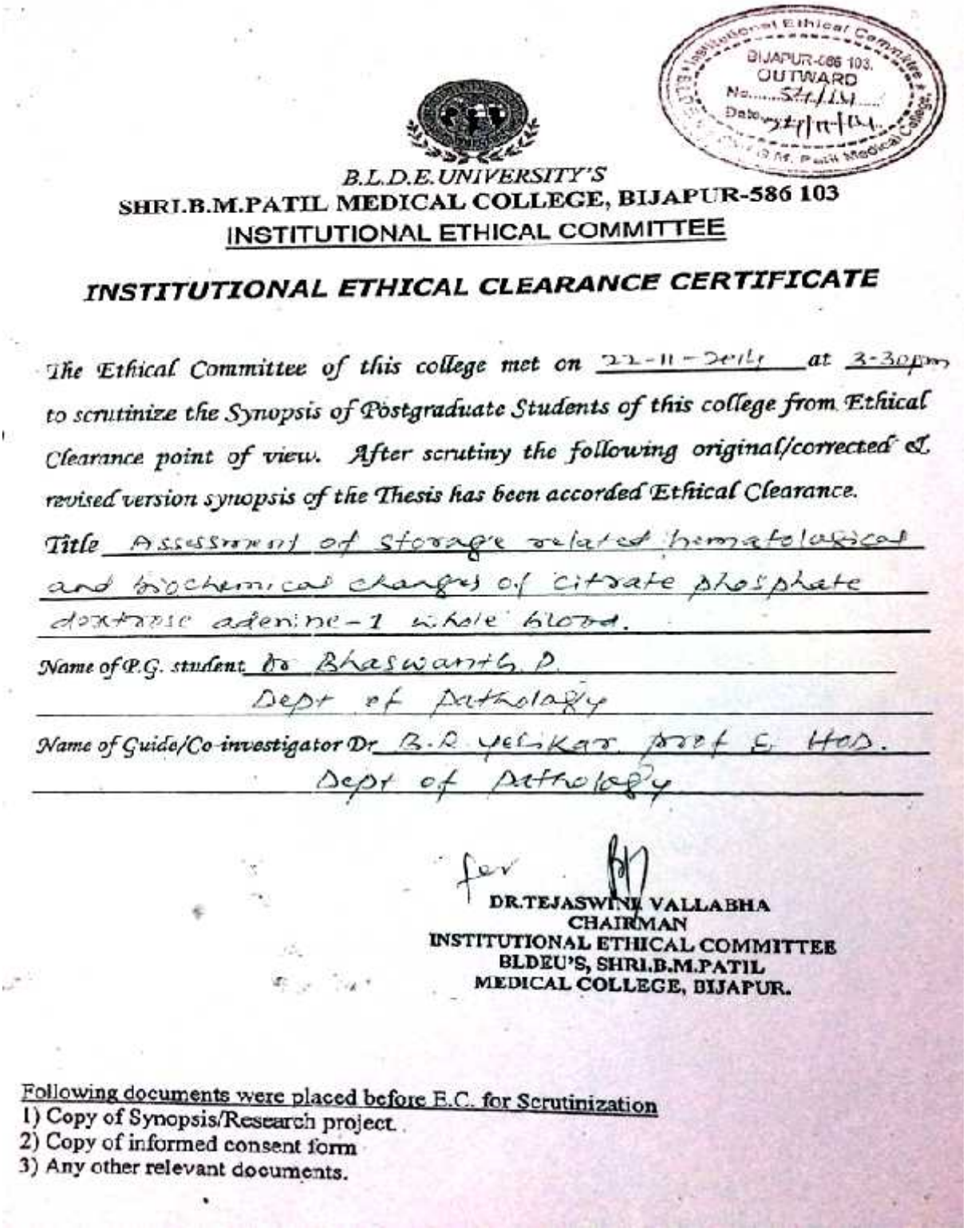
ANNEXURE-I

ETHICAL CLEARANCE



Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 571/14
Date 27/11/14.
S.B.M. Patil Medical College

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 22-11-2014 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title Assessment of storage related hematological and biochemical changes of citrate phosphate dextrose adenine-1 whole blood.

Name of P.G. student Dr Bhaswanth P.
Dept of Pathology

Name of Guide/Co-investigator Dr B.R Yerikar Prof & HOD.
Dept of Pathology

for
DR.TEJASWINI VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

B.L.D.E.UNIVERSITY , SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER ,BIJAPUR-586103

INFORMED CONSENT FORM FOR DISSERTATION/REASEARCH

I, the undersigned_____, S/O D/O W/O , aged _____ years, ordinarily resident of do hereby state/declare that Dr BHASWANTH.P of Hosptital has examined me thoroughly on _____ at (place) and it has been explained to me in my own language.

Further Doctor BHASWANTH.P informed me that he/she is conducting dissertation/research Titled "ASSESSMENT OF STORAGE RELATED HEMATOLOGICAL AND BIOCHEMICAL CHANGES OF CITRATE PHOSPHATE DEXTROSE ADENINE-1 WHOLE BLOOD" under the guidance of Dr B.R.YELIKAR requesting my participation in the study.

Further doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or

prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment,

I the undersigned Shri/ Smt _____ under my full conscious state of mind agree to participate in the said research/ dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

ANNEXURE-III

BLOOD DONOR QUESTIONNAIRE AND CONSENT FORM/VOLUNTARY DONOR

SELECTION/REJECTION FORM

LICENCE NO.:KTK/28C-56/97 BLOOD UNIT NO.: BLOOD GROUP AND Rh

TYPE :

DATE OF COLLECTION: EXPIRY DATE :

CONFIDENTIAL

Name: Age: Sex: D.O.B.:

Contact No.:

Occupation: Address for communication:

a.Have you donated previously: YES/NO

if yes then on how many occasions: When last donated:

b.Your blood group: Time of last meal:

Did you have discomfort during donation:

1.Do you feel well today?

YES/NO

2.Did you have something to eat in the last 4hrs?

YES/NO

3.Did you sleep well last night?

YES/NO

4. Have you any reason to believe that you may be infected by either hepatitis, malaria,HIV/AIDS, and/or veneral disease?

YES/NO

5. In the last 6months have you had any history of following:

Unexplained weight loss: Repeated diarrhea: Swollen glands:

6.In the last 6 months have you had any :

Tattooing: Ear Peircing: Dental extraction:

7. Do you suffer from or have suffered from any of the following diseases?

Heart disease: Lung disease: Kidney disease: STD: diabetes: Tuberculosis: Jaundice: Malaria: Hepatitis B/C: Cancer: Epilepsy: Fainting Spells:

Allergic Disease: Abnormal Bleeding Tendency: Typhoid:

Are you taking or have taken any of these in the past 72 hrs:

Antibiotics: Aspirin: Alcohol: Steroids: Vaccination: Dog Bite:

8. is there any history of surgery or blood transfusion in the past 6 months

Major surgery: Minor surgery: Blood transfusion:

9. Women Donors:

a. Are you pregnant?

YES/NO

b. Have you had an abortion in the last 3months?

YES/NO

c.Do you have a child less than 1yr old?

YES/NO

d. Are you having your periods today?

YES/NO

10. would you like to be informed about any abnormal test result at the address furnished by you.

YES/NO

11. Have you read and understood all the information presented and answered all the questions truthfully, as any incorrect statement or concealment may affect your health ao may harm the recipient.

YES/NO

I UNDERSTAND

A. Blood donation is a totally voluntary act and no inducement or remuneration has been offered.
B. Donation of blood /component is a medical procedure and that by donating voluntarly, I accept the risk associated with the procedure.
C. My blood will be tested for Hepatitis B, Hepatitis C, Malarial parasite, HIV/AIDS and veneral diseases in addition to any other screening tests required to ensure blood safety.

   I prohibit any information provided by me or about my donation to be disclosed to any individual or government agency without my prior permission.

DATE: TIME: DONOR SIGNATURE:

General physical examination:

Weight: Pulse: Hb: Temperature: BP:

Accept: Defer: Reason:

Signature of Medical officer

ANNEXURE IV

PROFORMA FOR STUDY :

Demographic Details:

1. Name: 2. Age : 3. Sex: M/F 4. OPD / IPD no.:

5. Present history:

6. Past history :

7. **History of intake of drugs:**

8. General physical examination**:**

Pallor : Icterus :

Built : Nourishment :

9. Vitals:-

PULSE : BP : RR :

Temp : Weight :

10. Investigations:

INVESTIGATIONS	**Day 0 (Control)**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
RBC COUNT					
WBC COUNT					
PLATELET COUNT					
HEMOGLOBIN					
PCV					
MCH					
PLASMA POTASSIUM					

Parameters studied and their normal reference values.

Parameters	Gender	Reference values
RBC count (millions/mm^3)	Males Females	4.4-5.7 4.0-5.2
WBC count (10^9/L)		4.0-11.0
Platelet count (Lakhs/mm^3)		1.5-4.5
Hemoglobin (g/dl)	Males Females	13.5- 18 12.5-16
Hematocrit (%)	Males Females	42- 52 37- 47
MCH (pg)		27- 31
Potassium (mmol/l)		2.8-5.2

KEY TO MASTER CHART

PCV	Packed Cell Volume
MCH	Mean Corpuscular Hemoglobin

MASTER CHART

Sl. no	WBC count (10^9/L)					RBC count (Millions/Cumm)					Hemoglobin (gm/dl)					PCV (%)					MCH (pg)					Platelet count (Lakhs/cumm)					Potassium (mmol/L)				
	DAYS					DAYS					DAYS					DAYS					DAYS					DAYS					DAYS				
	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
1	8	8	5	5	5	5	5	5	4	3	13	13	13	13	13	41	42	42	42	43	29	29	29	29	29	2	2	1	1	1	4	10	14	19	24
2	10	8	5	5	3	4	4	5	5	5	13	13	13	13	12	38	38	38	39	38	28	28	28	28	28	2	2	1	1	1	4	14	21	26	27
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***The following parameters have not been mentioned in the above table due to lack of space: age, sex, opd no**