ORIGINAL RESEARCH PAPER

Pathology

A STUDY ON BIOCHEMICAL CHANGES OF LACTATE, POTASSIUM, AND SODIUM IN STORED BLOOD UNITS

KEY WORDS: Blood Transfusion, Whole Blood storage, Potassium, Sodium, Lactate

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ABSTRACT

Introduction: Blood is a bodily fluid that comprises platelets, plasma, WBCs, and RBCs, each of which has a particular function in the body. Blood transfusion is an important life-saving intervention that is performed in a wide range of pathological circumstances. **Objectives:** To evaluate the cellular and biochemical changes in stored whole blood from Day 0 to Day 28. **Materials and Methods:** The present study was a hospital-based prospective simple cross-sectional study. After blood donation, a blood sample of 30 ml was collected in a satellite bag from each blood bag. Each sample was divided into 5 portions in a K2 EDTA test tube, plain test tube, and lithium heparin tube. Tubes were kept in the refrigerator at 4-6 degrees and analyzed for plasma lactate, potassium, and sodium on days 7,14, 21, and day 28. statistical analyses were performed using SPSS Version 20. **Results:** The present study included a total of 62 donors' blood. The mean age of the study cohort was 35 years. Among the studied individuals, 41 were male and 21 were females. The cellular level analysis showed a decrease in white blood cells after day 7 from 6.0 x109/ L to 3.5 x109/ L on day 28. The Red blood cell count was 5.6x1012/L on Day 0 and a significant decrease was observed on Day 28. Plasma potassium and lactate levels were significantly increased from Day 0 storage to Day 28. Contrary to Potassium Levels, Plasma Sodium level was significantly decreased from Day 0 storage to Day 28. **Conclusion:** The present study recorded significant variation in Hematological and Biochemical levels in the CPDA-stored whole blood from Day 0 to Day 28. The Long duration of storage has a high negative impact on the transfusion. Therefore, it is advised to transfuse whole blood before the seventh day of donation for effective transfusion.

INTRODUCTION

Blood is a bodily fluid that comprises platelets, plasma, WBCs, and RBCs, each of which has a particular function in the body. RBCs are primarily responsible for the transportation of immune complexes, hormones, nutrients, and blood gases. WBCs protect against infection, platelets aid in stopping bleeding, and plasma supplies various proteins and electrolytes (1). Blood transfusion is an important life-saving intervention that is performed in a wide range of pathological circumstances. The Food and Drug Administration (FDA) of the United States set the maximum 35-day storage period for whole blood anticoagulated in citrate phosphate dextrose adenine-1 (CPDA-1)(2).

Clinical studies have suggested that blood units kept in reserve for long periods (typically described as 14–21 days) may be harmful to patients, increasing their risk of morbidity and mortality (3). However, it has been suggested that an extended period of blood storage before a transfusion increases the possibility of complications. Because red blood cells (RBCs) experience structural and functional alterations that may impact their overall viability and function after transfusion. Furthermore, RBC storage media damage is linked to bioactive substances including histamine, cytokines, and lipids, generated by "passenger leucocytes that may exert a direct effect on metabolic and physical changes associated with the deterioration in cells." (4). There is additional evidence to support the hypothesis that preserving red blood cells in a hypothermic environment may lower metabolism and energy requirements, which in turn may cause the ATP-dependent sodium-potassium pump to malfunction and enable sodium and potassium to freely circulate within and out of cells (5).

Red blood cells from stored blood may be altered by cytokines, enzymes, and ions from white blood cells, such as potassium and calcium, which could hurt transfusion recipients. Multiple membrane molecules involved in adhesion, oxygen delivery, and complement regulation are impacted by these alterations (6). Therefore, the current study assessed the biochemical changes of lactate, potassium, and sodium in stored blood units. Determining the alterations taking place during the storage time is vital to reducing the unfavorable consequences of transfusion.

MATERIALS AND METHODS

Ethical considerations:

Institutional ethical committee clearence was obtained from Shri B.M Patil Medical College, Hospital and Research Center, BLDE (Deemed to be University), Vijayapura. Written informed consent was taken from the participants before the collection of Blood.

Study area:

The present study was carried out on individuals who donated blood voluntarily in the blood bank of the pathology department of Shri B.M Patil Medical College, Hospital and Research Center, BLDE (Deemed to be University), Vijayapura.

Study design:

Hospital-based prospective simple cross-sectional study

Study Period: September 2022 to August 2024

Inclusion criteria:

Healthy blood donors who fulfilled standard blood donor selection criteria (As per FDA guidelines) were included in the study

Exclusion criteria:

Clotted blood samples and Blood bags were found to be positive for transfusion-transmitted infection and donors who did not fulfil the mentioned inclusion criteria were excluded from the study.

Sample collection and analysis:

After blood donation of 350/450 ml of whole blood, a blood sample of 30 ml is collected in a satellite bag from each blood bag. Each sample was divided into 5 portions. Each portion

consisting of 6 ml of blood was added into a K2 EDTA test tube, plain test tube and lithium heparin tube. Each of these tubes was analyzed on the day of collection, which is regarded as a control. The other tubes were kept in the blood bank refrigerator at 4-6 degrees to be analyzed on days 7,14,21 and day 28. Each sample was analysed using VITROS 5.1/FS fully automated biochemistry analyser to measure plasma lactate, potassium and sodium and hematology analysed used SYSMEX XN 1000.

Statistical Analysis:

A statistical package for the social sciences (SPSS) (Version 20) was used to perform statistical analyses after the data was entered into a Microsoft Excel sheet. Results were presented as Mean, SD, counts, and percentages. ANOVA was used to examine continuous variables. Categorical variables were analyzed using the Chi-square test/Fisher's exact test. If $p<0.05$ will be considered statistically significant.

RESULTS

The present hospital-based study included a total of 62 donors' blood. The mean age of the study cohort was 35 years. The Majority of the individuals belonged to 31-40 years of age followed by 21- 30 years, 41- 50 years, and 11-20 years. Among the studied individuals, 42 were male and 20 were females. The study showed maximum of males and females i.e. 45.2% of males and 47.6% of females belonged to 31-40 years of age. (Figure 1).

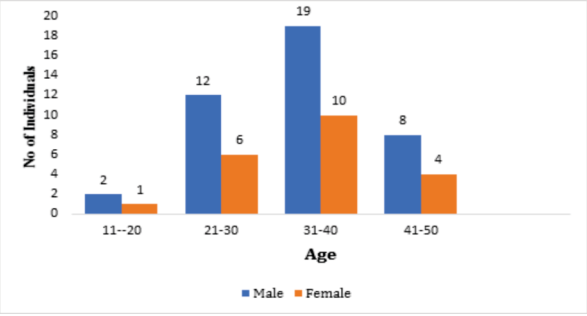

Figure 1. Age and gender-wise distribution of the study cohort

The cellular level analysis showed a decrease of white blood cells after day 7 from 6.0 $x10^9$/ L to 3.5 $x10^9$/ L on day 28 the mean count of WBC was reduced significantly during this storage from to day 28 (Table 1).

Table 1. Mean count of White blood cells in the storage period from Day 0 to Day 28

Storage Days	Mean Count (109/ L)	SD	95% CI	F-value	P value
Day-0	7.6	1.5	7.2-7.9	74.8838	0.0001
Day-7	6.0	1.6	5.6-6.3		
Day-14	4.6	1.3	4.2-4.9		
Day-21	4.2	1.4	4.5-3.8		
Day-28	3.5	1.6	3.9-3.1		

The Red blood cell count was 5.6x10^{12}/L on Day 0 and showed a slight decrease after 21 days of storage; however, a significant decrease was observed from Day 0 to Day 28 (Table 2).

Table 2. Mean count of Red blood cells in the storage period from Day 0 to Day 28

Storage Days	Mean Count (1012/L)	SD	95% CI	F-value	P value
Day-0	5.6	1.2	5.3-5.8	9.69	0.0002
Day-7	5.3	1.0	5.03-5.5		
Day-14	5.1	1.1	4.8-5.3		
Day-21	4.9	1.0	4.6-5.1		
Day-28	4.5	0.8	4.2-4.7		

The haemoglobin estimation showed a significant reduction in Hb level from Day 0 to Day 28. Hemoglobin level was 14.2 mg/dl on Day 0 and Day 28, hemoglobin level was 12.1 mg/dl in our study (Table 3).

Table 3. Mean Range of Hemoglobin in the storage period from Day 0 to Day 28

Storage Days	Mean Range mg/dl	SD	95% CI	F-value	P value
Day-0	14.2	1.7	13.3-15.1	3.88	0.004
Day-7	13.6	1.6	12.7-14.4		
Day-14	12.8	1.4	11.9-13.6		
Day-21	12.5	1.5	11.6-13.3		
Day-28	12.1	1.4	11.2-12.9		

In the present study, the mean potassium level was 3.5± 1.1 mEq/L followed significant increase in means level 7.6 ±0.9 Meg/L on Day 7. As the number of storage days increased, a significant rise in potassium levels was observed. On Day 28 mean potassium level was 18.2 ± 1.2 mEg/L. The mean potassium levels in different days of storage groups are shown in Table 4.

Table 4. Potassium level in the storage period from Day 0 to Day 28

Storage Days	Mean Potassium Level (mEq/L)	SD	95% CI	F-value	P value
Day-0	3.5	1.1	3.2-3.7	1688.7	0.00001
Day-7	7.6	0.9	7.3-7.8		
Day-14	11.0	1.1	10.7-11.2		
Day-21	13.1	1.0	12.8-13.3		
Day-28	18.2	1.2	17.9-18.4		

Contrary to Potassium Levels, Plasma Sodium level significantly decreased from Day 0 storage to Day 28 (P value- 0.0001). The mean sodium level was 146.4±1.4 mEg/L on Day 0 followed by 138±1.3 mEq/L on Day 14. This decrease was 128.5±1.1 mEq/L on Day 28 of storage. The mean sodium levels in different days of storage groups are shown in Table 5.

Table 5. The sodium level in the storage period from Day 0 to Day 28

Storage Days	Mean Sodium Level (mEq/L)	SD	95% CI	F-value	P value
Day-0	146.4	1.4	146.1-146.9	2708.0	0.0001
Day-7	142.7	1.0	142.2-143.0		
Day-14	138.7	1.3	138.4-139.0		
Day-21	131.3	0.8	131.0-131.6		
Day-28	128.5	1.1	128.2-128.8		

The mean lactate levels in different days of storage groups are shown in Table 6. In our study plasma lactate level was significantly increased from Day 0 to Day 28 (P value - 0.00001). The mean level of lactate was 14.1±0.8 mEq/L on Day 0 followed by 14.2±0.9 mEq/L, 23.6±1.0 mEq/L, 28.5±1.3 mEq/L and 32.4±1.1 mEq/L on Day7, Day 14, Day 21, and day 28 respectively.

Table 6. Lactate level in stored blood for the period

Storage Days	Mean Lactate Level (mEq/L)	SD	95% CI	F-value	P value
Day-0	14.1	0.8	13.8-14.3	3057.3	0.00001
Day-7	19.2	0.9	18.9-19.4		
Day-14	23.6	1.0	23.3-23.8		
Day-21	28.5	1.3	28.3-28.7		
Day-28	32.4	1.1	32.2-32.6		

DISCUSSION

The availability of blood for transfusions is improved by blood reservations. However, concerns are raised about the significance and toxicity of such blood that is stored for long periods. several medical retrospective studies suggest that transfusion of stored blood can be adverse, especially to

individuals who are seriously ill such as severe myocardial infarction etc. When compared to patients who received fresh blood, they found that patients who received extensively preserved blood had a higher rate of death, morbidity, infections, renal and lung collapse, oedema, and thrombosis (7).

In the current study, CPDA-1 bags were used for preserving whole blood. The anticoagulant contained in the collecting bag is made of citrate, which inhibits coagulation by chelating ionized calcium. Our study evaluated the Biochemical elevation of potassium, sodium, and lactate in stored blood from Day 0 to Day 28.

Cellular changes that occur during the storage of whole blood in the CPDA-1 bag were highlighted in our study. A significant decrease of WBC was observed from Day 0 to Day 28 i.e. 7.6 $x10^9$/ L to 3.5 $x10^9$/ L. Several studies have supported these findings by recording significant reductions in mean WBC count ranging from 7.0 $x10^9$/ L to 3.0 $x10^9$/ L in Day 0 to day 35 storage (8). Moreover, Cohl et al. (1981) stated that these modifications in white blood cells are certainly the result of the cumulative impacts of the loss of distinct cell properties, particularly degeneration, which is thought to occur as the cell ages. (9).

The present study also showed a decrease in mean RBC count from 1st day to the 28th day which is to a study done by Marabi PM et al., 2020; Chaurasiya AK et al., 2022; Shrivastava P, Dutta S 2020; (7, 8,10). In contrast to the findings of our study, Batham N. et al. recorded no significant elevation in RBC count during the storage of 35 days (11). A decrease in RBC count on storage affects oxygen delivery, reduced RBC stability, changes in different metabolites, and the cell's metabolic state. Research indicates that even certain recipients might be affected by allogenic RBC infusion (12).

Haemoglobin level estimation demonstrated a slight decrease from day 0 to Day 28 (from 14.2±1.7 to 12.1±1.4) similar to several previous studies (7,8). A study recorded a slight increase in Hemoglobin levels. This may be due to the accumulation of lactic acid and proteins, byproducts of glycolytic metabolism, which in vivo are easily eliminated from the circulation. These substances stay in the body during storage and cause physical alterations as well as cell lysis, which releases unbound haemoglobin into plasma (13).

The most common intracellular cation is potassium, while the most common external cation is sodium. A few homeostasis mechanisms are required to maintain the movement of potassium and sodium between the extracellular and intracellular environments. According to recent studies, potassium efflux, a well-known consequence of extended red blood cell reserve causes vesicle formation and could have a big impact on the outcome of the transfusion (7). As the storage time of whole blood increases, the level of potassium increases and the level of sodium decreases in our study. The results of our study are comparable to studies conducted by Mane et al.,2015; Verma et al.,2015; Adias et al., 2012; Rewal D et al., 2023; and Opoku-Okrah C et al., 2015 (14-18). This study's significant increase in potassium may be caused by the slow and continuous release of potassium ions from cells into the surrounding plasma during storage. Our study also recorded a significant increase of plasma lactate from 14.1±0.8 mEq/L on Day 0 to 32.4±1.1 mEq/L on Day 28. This could be because red blood cells' (RBCs) anaerobic metabolism increases during storage, causing an accumulation of plasma lactate and a subsequent reduction in pH (19).

CONCLUSION

The present study recorded significant variation in Hematological and Biochemical levels in the CPDA-stored whole blood from Day 0 to Day 28. Whole blood stored for > 7 days has decreased WBC and hemoglobin and reduced efficacy with elevated markers of red cell storage lesion such as increased lactate, potassium, and reduced sodium level. The Long duration of storage has a high negative impact on the transfusion. Therefore, it is advised to transfuse whole blood before the seventh day of donation for effective transfusion.

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