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Research Article

BIOCHEMICAL CHANGES IN THE STORED WHOLE BLOOD ASSESSED AT PERIODIC INTERVALS IN CPDA-1 ANTICOAGULANT CONTAINING BLOOD BAGS: A STUDY AT THE BLOOD BANK OF TERTIARY CARE HOSPITAL OF SOUTHERN RAJASTHAN

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ABSTRACT

Objectives: The objectives of this study were to study the various biochemical changes occurring in a stored whole blood unit at day 0, day 17th, and the 35th day in full blood bag containing citrate phosphate dextrose adenine-1 anticoagulant.

Methods: A cross-sectional descriptive study had been carried out among 110 healthy volunteer donors in the Department of Transfusion Medicine, RNT Medical College, Udaipur, between August 2019 and July 2020. A 10 mL blood sample from each bag containing anti-coagulated blood being collected, and the level of sodium, potassium, chloride, total protein, and albumin were measured on days 0, 17th, and the 35th. Mean and standard deviation had been calculated for the parameters, a one-way analysis of variance test was applied to compare the differences, and a $p<0.05$ was considered statistically significant.

Results: A higher proportion of males in the age group 18–27 years, and a generally steady increase in serum potassium level with a steady decrease in serum sodium, chloride, total protein, and albumin levels over the 35-day storage period.

Conclusions: In this study, stored whole blood undergoing changes in its biochemical parameters had been studied and found to be significant. The study also recommends transfusion of fresh blood for high-risk patients.

Keywords: Blood, Citrate phosphate dextrose adenine, Potassium, Sodium, Chloride.

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INTRODUCTION

Blood is a body fluid composed of RBCs, WBCs, platelets, and plasma, performing distinct functions in the body. The primary role of RBCs is to transport blood gases, nutrients, hormones, and immune complexes [1,2]. WBCs provide immunity, platelets help prevent bleeding, and plasma provides different proteins and electrolytes. Blood is procured chiefly from voluntary donors and, then, separated into its components for therapeutic and prophylactic purposes in blood banks. The technique to store blood had been first started in 1915 with the discovery of sodium citrate as a blood anticoagulant. Since then, much progress having been made. At present, blood components could be stored for a prolonged time. Blood and its components are kept at their optimum temperature to increase its validity; however, specific alterations might occur proving to be detrimental to the patients [3,4].

Preserved blood cells undergo progressive structural and functional changes during storage that may reduce red cell function and viability after transfusion. Administration of prolonged preserved blood cells has been well-correlated with multiple studies suggesting a risk of severe complication or death in critically ill patients, patients undergoing cardiac surgery, and those having received a massive transfusion. As the time duration of whole blood storage containing CPDA-1 anticoagulant increases, the sodium/potassium pump gets immobilized, causing a decrease in intracellular potassium with an increase in cytoplasmic sodium levels, glucose levels decline, and acidosis occurs as a result of low pH levels [1,4]. The stored fragile erythrocytes eventually trigger the formation of hemoglobin-containing microparticles with release of cell-free hemoglobin, resulting in transfusion difficulties. Lipid peroxidation, oxidative stress to band three structures, and other morphological and structural molecular changes also occur, leading to spheroechinocytes formation and increased osmotic fragility. These changes that transpire in the red cells during the storage period are called "storage lesions" [5]. Levels of electrolytes, especially potassium, increase during the storage period. Hyperkalemia can cause severe complications in renal failure patients, also arrhythmias being a known complication. During storage, sodium level decreases below 135 mEq/L, causing hyponatremia, resulting in cellular edema and complications in the central nervous system such as osmotic demyelination and even depression [4,5]. To reduce the adverse effects of transfusion, it is essential to determine the changes that occur during the storage time. Therefore, this study had been conducted to find out differences in the biochemical parameters of blood during its storage.

METHODS

The study aimed to measure the biochemical changes occurring in a stored whole blood unit. The objectives of the study were to measure the changes in the level of sodium (Na^+), potassium (K^+), chloride (Cl^-), total protein, and albumin on the day of collection, considered as day 0, day 17th, and the 35th day in a stored whole blood bag containing CPDA-1 anticoagulant.

It was a cross-sectional descriptive prospective study conducted over a period of 12 months, that is, from July 2019 to August 2020. The study sample comprising 110 healthy volunteer blood donors reporting for blood donation (both male and female) and after having been tested for transfusion transmitted infections in the department of Transfusion Medicine, RNT Medical College (Udaipur). RNT Medical College being a tertiary care hospital and the department of Transfusion Medicine utilizing proper screening measures to prevent transmission of any blood-related infections.

An Institutional Ethics Committee approval was taken, and informed written consent had been obtained from every participant. A total of 350 mL blood had been obtained from each of the 110 healthy voluntary donors, into a citrate phosphate dextrose adenine (CPDA-1) anticoagulant containing blood bag. A 10 mL of the anti-coagulated blood sample from each blood bag was taken for study purposes and stored in a satellite bag on a quarantine shelf at 2–6°C temperature in proper sterile condition, with the rest of the blood being used for transfusion purposes. A blood sample (3 mL) in a plain tube/clot activator tube for testing parameters was transported to the biochemistry department in an insulated cold box on day 0 (day of collection), day 17^{th}, and 35^{th}. The biochemical parameters were measured using Roche Electrolyte 9180 Analyzer: sodium, potassium, chloride; likewise, total protein and albumin levels had been measured using Siemens Dimension RXL max Chemistry Analyzer.

Data were encoded and entered on a Microsoft Excel sheet (version 13), and analysis being done on SPSS version 16 (the Statistical Package for Social Sciences Version 20). The results had been presented in tables showing proportions of the differences in parameters on the first, 17^{th}, and 35^{th} day. Mean and standard deviation (SD) being calculated for the parameters on the first, 17^{th}, and 35^{th} day. A one-way analysis of variance test was applied to compare the differences, and analysis and inferences had been obtained. $p<0.05$ was considered statistically significant.

RESULTS

There was a higher proportion of males (86.18%; 92) compared to females (13.82%; 18) in my study group. The majority of the respondents (71.0%; 78) were aged 18–27 years, with a small proportion (1.8%; 2) aged 48 years or more (Table 1). Although the number of female respondents was much less than males, the age group's percent distribution was almost the same. Out of the 110 respondents, maximum of 57.28% had been educated up to the college level, 20% had an elementary education, 14.54% educated up to the university level, and 8.18% educated up to high school level (Graph 1). As per the previous study published in The Asian Journal of transfusion medicine, July 1, 2014, 8 (2):121–125, blood group O was the most common blood group in India, followed by blood group B, but here in my study, blood group B donors are more common in contrast to that study, as I have selected random samples to prevent the bias. The donors were 110 in number; they ranged from 19 to 55 years with corresponding blood groups of 18 A+, 1A-, 14 AB+, 1 AB-, 40 B+, 33 O+, and 3 O- (Table 2). The male and female donors had tested negative for HCV, Hbs-Ag, syphilis, and HIV 1 and 2.

The results showed a generally steady increase in serum potassium levels with a steady decrease in serum sodium, chloride, total protein, and albumin levels over the 35-day storage period as the whole blood aged in the storage. The plasma sodium variations in its mean value of 140.94 meq/L with a SD of ±2.27 on day 1 had shown reduction to 131.9 meq/L with a SD of ±2.61 on day 17^{th}. A decrease followed this mean value to 123.91 meq/L with a SD of ±2.54 on the past day, that is, day 35. Overall, summing up all mean values and SDs, the final mean value with SD for sodium is 132.2±0.17, and p-value for sodium is <0.001 suggesting significance. Hence, in this study, as the time of storage of whole blood increases, sodium level decreases significantly.

Contrary to sodium, the plasma potassium readings started with normal average mean value (3.99 meq/L) with a SD of ±0.41 on the day 1, followed by a sharp rise in mean value (10.46 meq/L) with a SD of ±0.72 on day 17^{th}. There was a similar increase in mean value to (18.0 meq/L)) with a SD of ±0.84 on day 35. The final mean value with SD for potassium is 10.8±0.22, and p-value for potassium is <0.001 suggest significant. Hence, in this study, as the time of storage of whole blood increases, the potassium level too significantly increases.

The results pertaining to plasma chloride had started with a mean value of 100.59 meq/L with a SD of ±2.56 on the day 1, subsequently reduced to 94.78 meq/L with a SD of ±2.62 on the day 17^{th}. There had been a decrease to 90.7 meq/L with a SD of ±2.75 on the past day, that is, day 35^{th}. The final mean value with SD for chloride is 95.3±0.09, and p-value for chloride is <0.001 suggesting significant. Hence, in this study, as the time of storage of whole blood increases, the chloride level decreases significantly.

On the other hand, the results of total protein started with a mean value of 7.37 g/dL with a SD of ±0.46 on the 1^{st} day and then reduced to 7.12 g/dl with a SD of ±0.48 on the 17^{th} day. A total decrease to 6.86 g/dL with a SD of ±0.47 on the past day, that is, day 3^{5th}. The final mean value with SD for total protein is 7.11±0.01, and p-value for complete protein is <0.001 suggesting significant. Hence, in this study, as the time of storage of whole blood increases, complete protein decreases significantly.

The results of albumin started with a value of 3.92 g/dL with a SD of ±0.36 on day 1 and then subsequently reduced to 3.52 g/dL with a SD of ±0.37 on the day 17^{th}. A decrease followed this to 3.09 meq/dL with a SD of ±0.35 on the past day, that is, day 35. The final mean value with SD for albumin is 3.51±0.01, and p-value for albumin is <0.001 suggesting significant. Hence, in this study, increased storage time of whole blood is associated with significantly decreasing albumin levels.

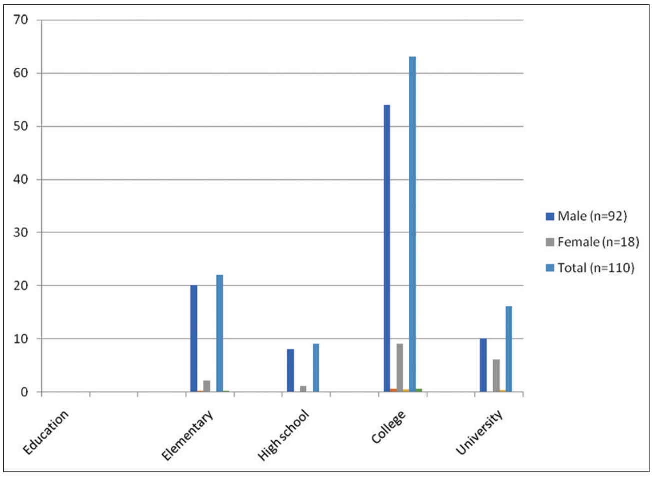
Details of these calculations are shown in Table 3.

Table 1: Distribution of respondents according to age and gender

Age	Male (n=92)		Female (n=18)		Total (n=110)	
	No.	%	No.	%	No.	%
Group (years)						
18–27	61	66.30%	17	94.44%	78	71.0%
28–37	18	19.56%	1	5.56%	19	17.2%
38–47	11	12.0%	0	0%	11	10.0%
48–57	2	2.17%	0	0%	2	1.8%

Table 2: Distribution of respondents according to blood group

	Male (n=92)		Female (n=18)		Total (n=110)	
	No.	%	No.	%	No.	%
A+ve	16	14.54%	2	1.81%	18	16.36%
A –ve	1	0.90%	0	0%	1	0.90%
AB+ve	10	9.09%	4	3.63%	14	12.72%
AB –ve	1	0.90%	0	0%	1	0.90%
B+ve	29	26.36%	11	10%	40	36.4%
B –ve	0	0%	0	0%	0	0%
O+ve	32	29.09%	1	0.90%	33	30%
O –ve	3	2.72%	0	0%	3	2.72%



Graph 1: Distribution of respondents according to education level

Table 3: Mean test for significance with standard deviation and *P* value for biochemical parameters for all the above mentioned days

Parameters	Day 0	Day 17	Day 35	Total mean	F value	**p value
Na+ (meq/L)	140.94±2.27	131.9±2.61	123.91±2.54	132.2±0.17	1293	<0.001
K+ (meq/L)	3.99±0.41	10.46±0.72	18±0.84	10.8±0.22	11556	<0.001
Cl- (meq/L)	100.59±2.56	94.78±2.62	90.7±2.75	95.3±0.09	387.0	<0.001
TP (g/dL)	7.37±0.46	7.12±0.48	6.86±0.47	7.11±0.01	31.65	<0.001
Albumin (g/dL)	3.92±0.36	3.520±0.37	3.09±0.35	3.51±0.01	147.70	<0.001

Mean test of significance for biochemical parameters for all the above mentioned days. *TP: Total protein

DISCUSSION

Blood is that magic potion that gives life to another person. Although we have made tremendous discoveries and inventions in science, we cannot yet make this magic potion called blood, reiterating the fact that human blood has no substitute [1].

In this study, we have tried to observe the electrolyte changes in stored whole blood units containing CPDA-1 as an anticoagulant at a periodic time interval. As per the previously done studies of stored whole blood, hemolysis starts to occur as the time of stored entire blood increases.

Here, in this study, whole blood had been stored in CPDA-1 bags. The anticoagulant present in the collection bag is composed of citrate (citrate chelates ionized calcium, which prevents coagulation), dextrose (dextrose being a source of energy for the red blood cells [RBCs]) and, phosphate containing anticoagulants (PCAs lowers the acidity in comparison to other anticoagulants without phosphate are also associated with a higher concentration of 2,3 DPG and red cell phosphate) and Adenine (adenine addition results in regeneration of ATP content and post-transfusion viability of red cells). Hemolysis is a prominent marker of the failure of the RBC storage system and bacterial and fungal contamination [6].

Hemolysis can be in the rupture or loss of microvesicles from the surface of intact cells. RBC breakdown causes hemolysis with release of hemoglobin and discoloration of the plasma. Abnormal hemolysis in a RBC unit is caused by several factors, including inappropriate handling during blood processing, improper storage conditions, bacterial hemolysins, and antibodies that cause complement lysis, defects in the RBC membrane, or any abnormality in the blood donor. The degree of hemolysis has been described as the percent of free hemoglobin to the total, with appropriate correction for the hematocrit. Blood transfusion as a therapeutic procedure might be more harmful than beneficial, and this is because every transfusion is associated with a potential risk to the recipient [7,8]. As the time of stored whole blood increases, the level of potassium increases and sodium decreases, which had been observed in certain previous studies as well as this study.

The transfusion of whole blood with relatively high potassium and low sodium concentrations had been associated with worse outcomes in several patients, including critically ill patients. The blood transfusion services have a duty of care toward blood recipients and must take steps to forestall these occurrences. By taking steps to determine what happens to sodium and potassium during blood storage, is in line with such efforts.

Results of this study are comparable to studies conducted by Mane *et al.* [1] and by Verma *et al.* [5]. Similar results have also been observed in overseas studies, like the study conducted in Nigeria by Adias *et al.* [2]. There have only been minor differences between my study and the other studies, mainly pertaining to the differences in the day of sampling and the types of machine or reagents used for measuring the biochemical levels of the aforementioned parameters.

Study limitations

Due to limited time and resources, the other critical biochemical parameters (pH, ATP, 2,3-DPG, calcium, lactate, urea, creatinine, phosphorus, AST, and ALT) and hematological parameters (MCV, MCHC, HGB, RBC, WBC, and lymphocytes) had not been evaluated. As the study had been done in collaboration with the biochemistry department, the technical factors affecting the instruments and reagents of the biochemistry department can also influence this study data. Although the appropriate study sample size had been calculated, we believe that it could be done with a larger sample size.

CONCLUSIONS

Significant changes are occurring in the stored whole blood due to various reasons. There should be a mandatory check for the electrolyte status of blood bags going to be issued for transfusion, especially transfusion for neonates, kidney disease patients, and high-risk patients who are unable to handle a high potassium load; stored blood having high potassium load may worsen the condition of these patients. Fresh blood after doing electrolyte status should provide to such patients. Addressing metabolic impairments by adjusting the additive composition and pH positively affects the maintenance of RBC energetics and levels of some antioxidant metabolites. Research into better additive solutions has to be promoted to maintain high 2,3-DPG and ATP levels, while minimizing hemolysis during storage.

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