



A STUDY OF PRE-ANALYTICAL AND STORAGE-RELATED HEMATOLOGICAL VARIATIONS IN PACKED RED BLOOD CELLS IN A TERTIARY CARE BLOOD BANK

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ABSTRACT

Background: Packed red blood cells (PRBCs) represent the most frequently transfused blood component worldwide. During storage, PRBCs undergo progressive biochemical and morphological alterations collectively termed the "storage lesion," which may compromise transfusion efficacy and patient outcomes. Pre-analytical variables including collection techniques and processing times further influence product quality.

Methods: This prospective observational study analyzed 120 PRBC units collected from voluntary blood donors. Pre-analytical variables including ambient temperature exposure and processing time were documented. Hematological parameters including hemoglobin, hematocrit, mean corpuscular volume (MCV), red blood cell count, potassium levels, pH, lactate concentrations, and percentage hemolysis were measured at days 0, 7, 14, 21, 28, and 35 of storage.

Results: Significant progressive changes were observed throughout the storage period. Hemoglobin decreased from 18.42 ± 1.28 g/dL to 17.15 ± 1.34 g/dL ($p < 0.001$). Supernatant potassium increased substantially from 4.82 ± 0.76 mEq/L to 48.56 ± 6.42 mEq/L ($p < 0.001$). Percentage hemolysis rose from $0.12 \pm 0.04\%$ to $0.68 \pm 0.18\%$ ($p < 0.001$). pH declined from 7.08 ± 0.06 to 6.52 ± 0.12 ($p < 0.001$). Units with prolonged pre-analytical processing time (> 8 hours) demonstrated significantly higher hemolysis rates ($0.82 \pm 0.21\%$ vs. $0.54 \pm 0.14\%$, $p < 0.01$) at day 35.

Conclusion: PRBCs undergo significant storage-related hematological deterioration, with marked changes observed particularly after day 21. Pre-analytical variables substantially influence final product quality, emphasizing the importance of standardized processing protocols in blood banking operations.

Keywords: Packed Red Blood Cells, Storage Lesion, Pre-Analytical Variables, Hemolysis, Blood Bank, Transfusion Medicine.

INTRODUCTION

Blood transfusion remains an essential and lifesaving therapeutic intervention in modern medical practice, with packed red blood cells (PRBCs) constituting the most commonly transfused blood component globally [1]. The World Health Organization estimates that approximately 118.5 million blood donations are collected annually worldwide, with a significant proportion processed into PRBCs for clinical utilization [2].

The fundamental objective of red blood cell transfusion is to restore adequate oxygen-carrying capacity in patients with anemia, hemorrhage, or compromised tissue oxygenation [3].

During ex-vivo storage, PRBCs undergo a series of progressive biochemical, structural, and functional alterations collectively termed the "red blood cell storage lesion" [4]. These changes include decreased adenosine triphosphate (ATP) and 2, 3-diphosphoglycerate (2, 3-DPG) concentrations, accumulation of reactive oxygen species, membrane phospholipid alterations, and progressive hemolysis [5]. Additionally, stored red blood cells demonstrate increased osmotic fragility, reduced deformability, and enhanced adhesiveness to endothelial surfaces [6].

The clinical significance of storage-related changes has been extensively investigated. Several retrospective studies have suggested associations



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between transfusion of older blood products and adverse patient outcomes, including increased mortality, infectious complications, and multi-organ dysfunction [7]. However, randomized controlled trials have yielded conflicting results, with some studies demonstrating no significant difference in outcomes between fresh and standard-issue blood products [8]. This controversy has intensified interest in understanding the fundamental mechanisms underlying storage-induced deterioration.

Pre-analytical variables represent critical determinants of final product quality that have received comparatively less attention in the literature. Factors including ambient temperature during collection, transportation conditions, time interval between collection and processing, and centrifugation parameters may significantly impact PRBC quality [9]. Standardization of these pre-analytical processes is essential for ensuring consistent product quality and optimal transfusion outcomes [10].

Current regulatory standards in most countries permit PRBC storage for 35-42 days depending on the anticoagulant-preservative solution utilized [11]. CPDA-1 (citrate-phosphate-dextrose-adenine) anticoagulant allows storage for up to 35 days at 2-6°C, with acceptable hemolysis rates below 0.8% at the end of storage [12]. Despite these established standards, significant inter-unit variability in storage quality has been documented, highlighting the need for comprehensive quality monitoring protocols [13].

Research examining pre-analytical influences on storage quality remains limited, particularly in resource-limited settings where processing delays may be more prevalent. Understanding these relationships is crucial for optimizing blood banking operations and ensuring delivery of high-quality products to patients.

The present study aimed to systematically evaluate pre-analytical variables and storage-related hematological changes in PRBCs stored in CPDA-1 anticoagulant over the complete 35-day storage period at a tertiary care blood bank.

MATERIALS AND METHODS

Study Design and Setting- This prospective observational study was conducted at the blood bank of a tertiary care teaching hospital over a 12-month period.

Sample Size Calculation- Sample size was calculated using the formula for comparing means with expected standard deviation of 0.2% for hemolysis based on pilot data. With 80% power, 5% significance level, and anticipated effect size of 0.15%, a minimum sample size of 112 PRBC units was determined. Accounting for potential attrition and technical failures, 120 units were included.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- PRBC units collected from healthy voluntary blood donors
- Donors aged 18-60 years meeting standard eligibility criteria
- Units collected in CPDA-1 anticoagulant bags (450 ± 45 mL)
- Negative results for all mandatory transfusion-transmitted infection screening

Exclusion Criteria:

- Units with collection volume <405 mL or >495 mL
- Visible lipemia or hemolysis at collection
- Units intended for emergency release within storage period
- Technical failures during processing or sampling

Blood Collection and Processing- Blood collection was performed by trained phlebotomists following standard operating procedures. Pre-analytical variables were systematically documented including:

- Ambient temperature during collection
- Time from collection to arrival at blood bank (transit time)
- Time from collection to initiation of processing (processing delay)
- Centrifugation parameters and component separation time

Whole blood units were processed into PRBCs within 24 hours of collection using refrigerated centrifugation at 4000×g for 10 minutes at 4°C. Plasma was expressed into satellite bags, and PRBC units were stored at 2-6°C in monitored blood bank refrigerators with continuous temperature recording.

Sampling Protocol- Aliquots (2 mL) were aseptically withdrawn from each PRBC unit using sterile sampling devices at days 0 (within 24 hours of collection), 7, 14, 21, 28, and 35 of storage. Samples were immediately processed for hematological and biochemical analysis.

Laboratory Analysis

Hematological Parameters: Complete blood count analysis was performed using Sysmex XN-1000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Parameters measured included hemoglobin concentration, hematocrit, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).

Biochemical Parameters: Supernatant potassium and sodium concentrations were measured using ion-selective electrode methodology (Roche Cobas c702, Roche Diagnostics, Mannheim, Germany). pH was determined using a calibrated blood gas

analyzer (Radiometer ABL800 FLEX). Lactate concentrations were measured enzymatically.

Hemolysis Assessment: Percentage hemolysis was calculated using the formula: Hemolysis (%) = [(100 - Hematocrit) × Supernatant Hemoglobin] / Total Hemoglobin × 100

Supernatant hemoglobin was measured spectrophotometrically after centrifugation of samples at 2000×g for 10 minutes.

Statistical Analysis- Data were analyzed using SPSS version 26.0 (IBM Corporation, Armonk, NY). Continuous variables were expressed as mean ± standard deviation (SD). Repeated measures analysis of variance (ANOVA) with Bonferroni post-hoc correction was used to compare parameters

across storage time points. Independent samples t-test was employed for comparing groups based on pre-analytical variables. Pearson correlation coefficients were calculated to assess relationships between variables. Statistical significance was set at p<0.05.

RESULTS

Baseline Characteristics- A total of 120 PRBC units were included in the final analysis. Mean donor age was 32.4 ± 8.7 years, with 89 (74.2%) male and 31 (25.8%) female donors. Mean collection volume was 458.2 ± 28.4 mL. Baseline characteristics of PRBC units are presented in Table 1.

Table 1: Baseline Characteristics of Packed Red Blood Cell Units (n=120)

Parameter	Value
Donor age (years), mean ± SD	32.4 ± 8.7
Male donors, n (%)	89 (74.2)
Female donors, n (%)	31 (25.8)
Collection volume (mL), mean ± SD	458.2 ± 28.4
PRBC volume (mL), mean ± SD	286.5 ± 32.1
Processing time (hours), mean ± SD	6.8 ± 3.2
Processing time ≤8 hours, n (%)	78 (65.0)
Processing time >8 hours, n (%)	42 (35.0)
Ambient temperature at collection (°C), mean ± SD	24.6 ± 3.8
Transit time (minutes), mean ± SD	42.5 ± 28.6
Initial hemoglobin (g/dL), mean ± SD	18.42 ± 1.28
Initial hematocrit (%), mean ± SD	58.4 ± 4.2

Storage-Related Hematological Changes-

Progressive and statistically significant changes were observed in hematological and biochemical parameters throughout the 35-day storage period. Results are summarized in Table 2.

Hemoglobin concentration decreased significantly from 18.42 ± 1.28 g/dL at day 0 to 17.15 ± 1.34 g/dL at day 35 (p<0.001). Similarly, hematocrit declined from 58.4 ± 4.2% to 54.8 ± 4.6% over the storage period. MCV demonstrated progressive increase from 88.2 ± 4.8 fL to 95.6 ± 5.4 fL (p<0.001), indicating cellular swelling.

Supernatant potassium concentration showed the most dramatic change, increasing from 4.82 ± 0.76

mEq/L at day 0 to 48.56 ± 6.42 mEq/L at day 35 (p<0.001). This represented an approximately 10-fold increase over the storage duration. pH declined progressively from 7.08 ± 0.06 to 6.52 ± 0.12 (p<0.001), while lactate concentrations increased from 2.84 ± 0.42 mmol/L to 28.6 ± 4.2 mmol/L (p<0.001).

Percentage hemolysis increased significantly from 0.12 ± 0.04% at day 0 to 0.68 ± 0.18% at day 35 (p<0.001). Notably, all units remained within the acceptable hemolysis limit of <0.8% established by regulatory standards, with only 8 units (6.7%) exceeding 0.8% at day 35.

Table 2: Storage-Related Changes in Hematological and Biochemical Parameters (n=120)

Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	P-Value*
Hemoglobin (g/dL)	18.42 ± 1.28	18.24 ± 1.26	18.02 ± 1.32	17.68 ± 1.28	17.42 ± 1.30	17.15 ± 1.34	<0.001
Hematocrit (%)	58.4 ± 4.2	57.8 ± 4.1	57.2 ± 4.4	56.4 ± 4.2	55.6 ± 4.5	54.8 ± 4.6	<0.001
RBC count (×10 ¹² /L)	6.24 ± 0.48	6.18 ± 0.46	6.12 ± 0.52	6.02 ± 0.48	5.94 ± 0.50	5.82 ± 0.54	<0.001
MCV (fL)	88.2 ± 4.8	89.4 ± 4.6	90.8 ± 5.0	92.4 ± 5.2	94.2 ± 5.6	95.6 ± 5.4	<0.001
MCH (pg)	29.4 ± 1.6	29.6 ± 1.8	29.8 ± 1.6	30.2 ± 1.8	30.4 ± 2.0	30.6 ± 1.8	<0.01
MCHC (g/dL)	33.2 ± 1.4	33.0 ± 1.2	32.6 ± 1.4	32.2 ± 1.6	31.8 ± 1.4	31.4 ± 1.6	<0.001
RDW (%)	13.2 ± 1.2	13.6 ± 1.4	14.2 ± 1.6	14.8 ± 1.8	15.6 ± 2.0	16.4 ± 2.2	<0.001

Potassium (mEq/L)	4.82 ± 0.76	12.4 ± 2.1	22.6 ± 3.4	32.8 ± 4.6	41.2 ± 5.8	48.56 ± 6.42	<0.001
pH	7.08 ± 0.06	6.96 ± 0.08	6.82 ± 0.10	6.72 ± 0.10	6.62 ± 0.12	6.52 ± 0.12	<0.001
Lactate (mmol/L)	2.84 ± 0.42	6.8 ± 1.2	12.4 ± 2.0	18.6 ± 2.8	24.2 ± 3.6	28.6 ± 4.2	<0.001
Hemolysis (%)	0.12 ± 0.04	0.18 ± 0.06	0.28 ± 0.08	0.42 ± 0.12	0.54 ± 0.14	0.68 ± 0.18	<0.001

*Repeated measures ANOVA; Values expressed as mean ± SD

Impact of Pre-Analytical Variables- Comparison of PRBC quality parameters based on pre-analytical processing time revealed significant differences. Units processed within 8 hours of collection (n=78) demonstrated superior quality parameters compared to those with delayed processing >8 hours (n=42) at day 35. Results are presented in Table 3.

Units with prolonged processing time showed significantly higher hemolysis (0.82 ± 0.21% vs.

0.54 ± 0.14%, p<0.01), elevated potassium levels (52.4 ± 7.2 mEq/L vs. 45.8 ± 5.4 mEq/L, p<0.001), and lower pH (6.44 ± 0.14 vs. 6.58 ± 0.10, p<0.01) at the end of storage.

Correlation analysis revealed significant positive correlation between processing time and day-35 hemolysis (r=0.68, p<0.001), and processing time and day-35 potassium concentration (r=0.54, p<0.001). Ambient temperature at collection showed weak positive correlation with hemolysis (r=0.32, p<0.05).

Table 3: Comparison of Day-35 Parameters Based on Pre-Analytical Processing Time

Parameter	Processing ≤8 Hours (N=78)	Processing >8 Hours (N=42)	P-Value*
Hemoglobin (g/dL)	17.32 ± 1.28	16.84 ± 1.42	0.068
Hematocrit (%)	55.4 ± 4.2	53.6 ± 5.0	0.042
RBC count (×10 ¹² /L)	5.92 ± 0.48	5.64 ± 0.62	0.012
MCV (fL)	94.2 ± 4.8	98.4 ± 5.8	<0.001
Potassium (mEq/L)	45.8 ± 5.4	52.4 ± 7.2	<0.001
pH	6.58 ± 0.10	6.44 ± 0.14	<0.01
Lactate (mmol/L)	26.4 ± 3.6	32.8 ± 4.8	<0.001
Hemolysis (%)	0.54 ± 0.14	0.82 ± 0.21	<0.01
Units exceeding 0.8% hemolysis, n (%)	2 (2.6)	6 (14.3)	0.018**

*Independent samples t-test; **Chi-square test; Values expressed as mean ± SD unless otherwise indicated

DISCUSSION

This prospective study provides comprehensive documentation of pre-analytical and storage-related hematological changes in PRBCs stored in CPDA-1 anticoagulant over the complete 35-day regulatory storage period. Our findings demonstrate significant progressive deterioration in multiple quality parameters, with pre-analytical processing time emerging as a critical determinant of final product quality.

The observed pattern of hemolysis progression in our study aligns with established understanding of the red blood cell storage lesion. Mean hemolysis increased from 0.12% at day 0 to 0.68% at day 35, remaining within the regulatory limit of 0.8% in the majority of units. These findings are consistent with those reported by D'Alessandro et al., who described progressive membrane deterioration as a fundamental component of storage-induced changes [14]. The exponential increase in hemolysis during the final week of storage suggests accelerated membrane destabilization, potentially attributable to cumulative oxidative damage and ATP depletion.

The dramatic elevation in supernatant potassium concentration represents one of the most clinically significant storage-related changes. Our observation of a 10-fold increase in potassium over 35 days corresponds with findings from previous investigations examining electrolyte shifts during storage [15]. This phenomenon results from progressive failure of the sodium-potassium ATPase pump as intracellular ATP becomes depleted, leading to passive potassium efflux along concentration gradients [16]. The clinical implications of transfusing potassium-loaded blood products are particularly relevant for neonates, patients with renal insufficiency, and those receiving massive transfusion protocols.

Progressive cellular swelling, evidenced by increasing MCV throughout storage, reflects the inability of red blood cells to maintain osmotic homeostasis. Almizraq and colleagues similarly documented MCV increases during storage, attributing this to ATP-dependent membrane pump failure and subsequent water influx [17]. The concurrent decrease in MCHC observed in our study

supports this mechanism, as cellular dilution occurs with water accumulation.

The decline in pH from 7.08 to 6.52 and corresponding rise in lactate concentrations document the metabolic consequences of anaerobic glycolysis during storage. As oxygen tension decreases within stored units, red blood cells increasingly rely on anaerobic metabolism, generating lactate as a byproduct [18]. This acidification may further compromise cellular integrity and enzyme function, potentially contributing to accelerated deterioration in later storage periods.

A particularly significant finding of our study concerns the impact of pre-analytical processing time on final product quality. Units processed beyond 8 hours demonstrated significantly higher hemolysis rates and elevated potassium concentrations at day 35 compared to promptly processed units. This observation has important implications for blood banking operations, particularly in settings where logistical constraints may delay component preparation. Hess and colleagues emphasized the importance of minimizing pre-storage manipulation and maintaining appropriate temperature control during the pre-analytical phase [19].

The influence of ambient temperature at collection on storage outcomes, though less pronounced than processing time, warrants consideration. Elevated temperatures during collection and transport may accelerate metabolic activity and cellular stress prior to refrigerated storage [20]. Implementation of temperature-controlled collection systems and expedited transport protocols may help mitigate these effects.

Our finding that 6.7% of units exceeded the 0.8% hemolysis threshold at day 35 highlights inherent inter-unit variability in storage quality. This variability likely reflects differences in donor characteristics, collection technique, and subtle processing variations that cumulatively influence storage outcomes [21]. Individual donor factors including age, gender, and baseline red blood cell indices have been shown to influence storage hemolysis in previous studies [22].

The clinical relevance of these laboratory findings continues to be debated in the transfusion medicine literature. While observational studies have suggested associations between transfusion of older blood and adverse outcomes, randomized controlled trials including the RECESS, ABLE, and INFORM trials have not demonstrated significant outcome differences [23]. Nevertheless, understanding the mechanistic basis of storage deterioration remains important for optimizing blood product manufacturing and identifying potential interventions to extend shelf life or improve product quality.

Limitations of this study include its single-center design, which may limit generalizability to settings with different operational practices. Additionally, we did not assess functional parameters such as ATP content, 2,3-DPG levels, or oxygen dissociation characteristics, which provide complementary information regarding red blood cell viability and function. Future studies incorporating these measures would enhance understanding of the functional implications of observed changes.

CONCLUSION

This study demonstrates that packed red blood cells stored in CPDA-1 anticoagulant undergo significant and progressive hematological and biochemical changes over the 35-day storage period. Key findings include exponential increases in supernatant potassium concentration and hemolysis, progressive cellular swelling with MCV elevation, and metabolic acidosis with lactate accumulation. Critically, pre-analytical processing time significantly influences final product quality, with delayed processing associated with higher hemolysis rates and accelerated deterioration.

These findings emphasize the importance of implementing standardized pre-analytical protocols in blood banking operations to minimize processing delays and optimize storage conditions. Blood banks should prioritize timely component preparation, ideally within 8 hours of collection, to ensure delivery of highest quality products. Continuous quality monitoring programs incorporating regular assessment of storage parameters are essential for maintaining product standards and identifying opportunities for process improvement. Further research examining functional correlates of these changes and their clinical implications will enhance evidence-based practices in transfusion medicine.

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