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# Haematological Changes, Lactate Dehydrogenase, and Red Blood Cell Antioxidant Enzyme Activity During Storage of Blood

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## Abstract

Background: Red blood cell (RBC) transfusion is widely used to correct anemia and restore oxygen transport. During storage at 4 ± 2°C, however, RBCs undergo progressive structural and biochemical alterations, which may affect their clinical effectiveness and safety.
Objective: This study aimed to investigate hematological changes and antioxidant enzyme activities—superoxide dismutase (SOD), glutathione peroxidase (GPX), and lactate dehydrogenase (LDH) in stored RBCs at different time points.
Methods: An exploratory study was conducted in the Departments of Hematology and Biochemistry, University of Health Sciences, Lahore, Pakistan. Whole blood (500 mL) from 25 healthy donors was collected into quadruple bags. About 90 mL from each mother bag was aseptically distributed into three satellite bags and stored at 4 ± 2°C. Samples were analyzed at baseline (day 0) and on days 14, 28, and 35. Hematological indices were measured using a fully automated hematology analyzer, while SOD, GPX, and LDH were assessed with a semi-automated chemistry analyzer. Data were analyzed using SPSS version 25.
Results: A significant reduction in SOD and GPX activity, accompanied by a rise in LDH levels, was detected by day 14 compared with baseline ($p \leq 0.05$). White blood cell (WBC) counts showed a steady decline during storage. In contrast, mean corpuscular volume (MCV) and red cell distribution width (RDW) significantly increased with longer storage duration ($p \leq 0.05$).
Conclusion: RBCs maintained better hematological stability and antioxidant enzyme activity within the first 14 days of storage. Shorter storage intervals may therefore be preferable, particularly for transfusion-dependent patients requiring repeated support.
Keywords: *Erythrocytes, Blood Preservation, Superoxide Dismutase, Glutathione Peroxidase, Lactate Dehydrogenase, Hematologic Tests*

## Introduction

Blood transfusion is a life-saving therapeutic procedure essential for the management of numerous medical conditions, including anemia associated with infections, hematopoietic disorders, cytotoxicity induced by certain medications, and acute blood loss due to trauma, childbirth, or surgery. Among the various blood components, red blood cell (RBC) transfusion is the most commonly practiced intervention, especially in developed healthcare systems.[1] Red blood cell transfusion has become a widely practiced medical procedure. Approximately 15 million units are transfused each year in the United States, with around 85 million units administered globally.[2, 3] To meet this high demand, RBCs are collected, processed, and stored under standard blood bank conditions, typically in citrate phosphate dextrose adenine (CPDA-1) anticoagulant solution at 4±2°C for up to 35–42 days. This practice supports the logistical requirement of maintaining a readily available and safe blood supply, contributing to the annual provision of over 110 million RBC units worldwide.[4]

However, during prolonged storage, RBCs undergo a series of biochemical and morphological alterations collectively termed “RBC storage lesions.[5] These changes include elevated lactate levels with accompanying pH reduction, increased hemolysis, potassium leakage, and significant declines in 2,3-bisphosphoglycerate (2,3-BPG) and adenosine triphosphate (ATP) levels. These alterations impair the functionality of transfused RBCs, increase the likelihood of their rapid clearance by macrophages, and are associated with adverse clinical outcomes such as transfusion-related acute lung injury

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(TRALI), prolonged hospitalization, and higher mortality rates.[6]

A major contributor to RBC storage lesions is oxidative stress, primarily driven by the accumulation of free radicals during storage. Free radicals attack membrane lipids and proteins, compromising membrane integrity and leading to hemolysis. Despite RBCs being particularly vulnerable to oxidative damage due to their high oxygen transport function, they are equipped with a robust antioxidant defense system comprising key enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GR). These enzymes play vital roles in neutralizing reactive oxygen species (ROS) and maintaining redox homeostasis. [7-9]

Under normal physiological conditions, there is a balance between oxidants and antioxidants in erythrocytes. However, during extended storage, this balance is disrupted, reducing the effectiveness of antioxidant defenses and increasing oxidative damage. SOD catalyzes the conversion of superoxide anions to hydrogen peroxide, which is further detoxified by catalase and GPx. GPx, a selenoenzyme, reduces hydrogen peroxide and lipid hydroperoxides, while GR is critical for regenerating reduced glutathione (GSH), a central molecule in oxidative stress resistance.[10, 11]

Given the role of oxidative damage in compromising stored RBCs, it becomes essential to evaluate not only the hematological integrity but also the activity levels of key antioxidant enzymes during storage. Therefore, the present study was designed to assess hematological changes and to evaluate the enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and lactate dehydrogenase (LDH) in blood bags preserved in CPDA-1 solution and stored at 4±2°C under standard blood bank conditions. LDH, a marker of hemolysis and cellular damage, was also included to correlate oxidative stress with membrane integrity and overall RBC viability during storage.

## Methodology

This exploratory study was carried out between 2016 and 2017 in the Departments of Haematology and Biochemistry, University of Health Sciences, Lahore, following approval from the Institutional Review Board. Blood samples were collected consecutively from healthy volunteer donors at the General Hospital Blood Bank, Lahore. The sample size was determined using an online statistical tool (Sample Size Determination in Health Studies, WHO, Version 2.0.21) with 90% power and a 5% level of significance. Donor eligibility was established in accordance with the American Association of Blood Banks (AABB) guidelines. Individuals with positive screening for Hepatitis B, Hepatitis C, Syphilis, or HIV, as well as those with significant medical history, were excluded. Blood bags exhibiting hemolysis, leakage, or discoloration were also excluded from analysis.

A total of 25 donors, each donated 500 mL of whole blood, collected into quadruple CPDA-1 blood bags (JMS Singapore Pvt. Ltd., Singapore). From each mother bag, approximately 90 mL of blood was aseptically separated into three satellite bags. The mother bags were analyzed at baseline (Day 0), and the satellite bags were stored at 4 ± 2°C for subsequent testing on Days 14, 28, and 35.

Antioxidant enzyme activity was determined by measuring Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Lactate Dehydrogenase (LDH) using Randox commercial kits (Randox Laboratories Ltd., UK) on the Rx Monza semi-automated chemistry analyzer (Randox Laboratories Ltd., UK). Hematological parameters, including red cell indices and platelet counts, were analyzed using the Sysmex XT-1800i automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

Statistical analyses were conducted using SPSS software version 25 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD). Changes in hematological and enzymatic parameters over the storage period were assessed using Repeated Measures Analysis of Variance (ANOVA), with Bonferroni post hoc correction applied where necessary. A p-value of $<0.05$ was considered statistically significant.

## Results

Comparison of complete blood count (CBC) values at baseline (day 0) with those obtained on days 14, 28, and 35 of storage revealed significant alterations in selected parameters. The mean white blood cell (WBC) count decreased progressively from 6.7 ± 1.4 × $10^3$/µL at baseline, with statistically significant reductions observed at each subsequent time point ($p < 0.05$). Mean corpuscular volume (MCV) showed a significant upward trend, rising from 82.2 ± 8.3 fL on day 0 to consistently higher values on later storage days ($p < 0.05$). Similarly,

mean corpuscular hemoglobin concentration (MCHC) demonstrated a modest but significant increase from its baseline of 32.4 ± 1.5 ($p < 0.05$).

Red cell distribution width (RDW) also increased markedly during storage, rising from 13.4 ± 1.5% at baseline to 16.2 ± 3.0%, 18.0 ± 2.9%, and 18.8 ± 2.3% on days 14, 28, and 35, respectively ($p < 0.05$), reflecting greater heterogeneity in red cell size with storage duration. In contrast, no significant differences were observed in red blood cell (RBC) count, hematocrit (Hct), or mean corpuscular hemoglobin (MCH) across the storage intervals ($p > 0.05$). These findings are summarized in Table I.

Superoxide dismutase (SOD) activity at baseline (194.6 ± 7.6 U/mL) demonstrated a significant decline by day 14 of storage ($p < 0.001$). This reduction persisted across later intervals, with further decreases observed on days 28 and 35, both remaining highly significant compared to baseline ($p < 0.001$). A similar trend was noted for glutathione peroxidase (GPX), where baseline levels (8524.8 ± 245.8 U/L) declined markedly by day 14 and continued to decrease on days 28 and 35 ($p < 0.001$ for all comparisons), reflecting a progressive loss of antioxidant capacity during storage. In contrast, plasma lactate dehydrogenase (LDH) activity increased substantially over time. Mean LDH concentration at baseline (291.4 ± 30.08 U/L) rose significantly to 692.8 ± 34.7 U/L on day 14,

Table I: Comparisons of Haematological Parameters in Stored Whole Blood on Different Storage Days.

| Parameters | Day 0 | Day 14 | P value | Day 28 | P value | Day 35 | P value |
|---|---|---|---|---|---|---|---|
| WBC $10^3$/µL | 6.7±1.4 | 5.5±1.3 | 0.003 | 5.9±1.3 | 0.043 | 5.7±1.4 | 0.027 |
| RBC $10^6$/µL | 4.5±0.81 | 4.8±0.68 | 0.224 | 4.8±0.65 | 0.212 | 4.7±0.49 | 0.272 |
| Haematocrit % | 45.2±5.3 | 45.2±7.9 | 0.997 | 44.8±5.8 | 0.734 | 45.4±5.3 | 0.887 |
| MCV fl | 82.2±8.3 | 88.2±9.3 | 0.021 | 92.0±10.6 | 0.001 | 94.6±10.5 | 0.001 |
| MCH pg | 27.5±3.3 | 27.6±3.6 | 0.874 | 28.0±3.6 | 0.592 | 28.5±3.4 | 0.350 |
| MCHC g/dl | 32.4±1.5 | 31.2±1.7 | 0.007 | 30.3±1.4 | 0.000 | 30.1±1.0 | 0.000 |
| RDW % | 13.4±1.5 | 16.2±3.0 | 0.000 | 18.0±2.9 | 0.000 | 18.8±2.3 | 0.000 |

Table II: Comparison of sod, GPX and LDH activity in stored whole blood on different storage days.

| Study Parameters | Normal Reference Values | Different Storage Days | Results (Mean ± SD) | *p*-value |
|---|---|---|---|---|
| SOD Activity U/mL | (164 – 240) | Day 0 | 194.6±7.6 | 0.000 |
| | | Day 14 | 159.4±6.2 | |
| | | Day 28 | 126.4±4.8 | |
| | | Day 35 | 95.0±4.6 | |
| GPX Activity U/L | (4171-10881) | Day 0 | 8524.8±245.8 | 0.000 |
| | | Day 14 | 6152.3±242.9 | |
| | | Day 28 | 4660.8±140.5 | |
| | | Day 35 | 3131.2±89.0 | |
| LDH Activity U/L | (230 - 460) | Day 0 | 291.4±30.0 | 0.000 |
| | | Day 14 | 692.8±34.7 | |
| | | Day 28 | 1161.6±33.1 | |
| | | Day 35 | 1451.3±32.4 | |

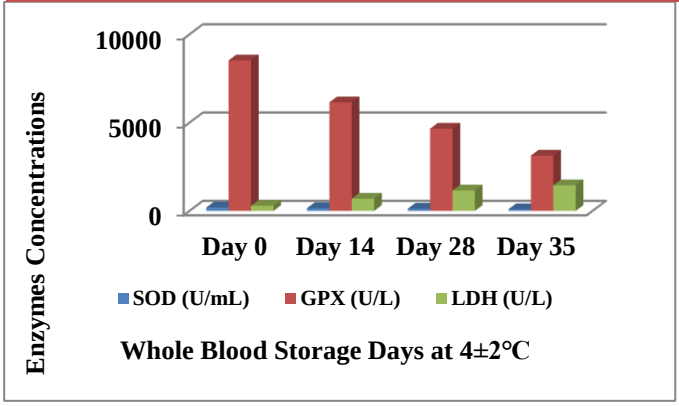


Figure 1. Trends in antioxidant enzyme (SOD, GPX) and LDH activity during whole blood storage at 4 ± 2°C.

1161.6 ± 33.1 U/L on day 28, and 1451.3 ± 32.4 U/L on day 35 ($p < 0.001$ for all comparisons). This consistent rise indicates cumulative cellular damage and hemolysis over prolonged storage. These findings collectively demonstrate a progressive decline in antioxidant enzyme activity (SOD, GPX) accompanied by a marked elevation in LDH, highlighting increased oxidative stress and reduced red cell integrity with advancing storage duration (Table II, Figure 1).

## Discussion

Blood transfusion is a term that refers to the therapeutic use of blood or blood components to ensure the safety and

security of blood transfusions.[12] The particular function of a blood bank is to store the blood in hypothermic condition. Despite the fact that hypothermia reduces alterations, blood variations do occur. The length of the storage time is proportional to the blood changes that occur during storage.[13] In order to properly utilize this precious limited blood supply, blood bankers use the strategy of "old blood out first".[14] According to experimental data, transfusion of older blood increases the chances of alloimmunization because stored blood contains high concentration of erythrocytes degradation products and pro-inflammatory cytokines.[15] A storage lesion is a morphological and metabolic change in blood that occurs during storage. These storage lesion reduces the survival of erythrocytes after transfusion and decreases the oxygen carrying capacity of erythrocytes.[16] In underdeveloped countries like Pakistan, whole blood preservation and transfusion are still prevalent. The current study looked at how haematological parameters, lactate dehydrogenase enzymes and anti-oxidant enzymes (Superoxide Dismutase and Glutathione Peroxidase) changed with time in whole blood stored at different intervals.

The changes in haematological parameters were examined in current study and we observed a gradual decrease in White blood cell (WBC) count during storage. In similar to our study, a drop in WBC count was observed by Latham Jr et al. who reported the reduction in WBC count from the 1st day of whole blood storage to 35 day of storage.[17] Our results are in agreement with Adias et al. who reported decrease in WBC count during storage. Decreased in WBC count is most likely be due to the degenerative changes that occur as the cells become aged.[18] During the storage of whole blood, we noticed an increase in red cell distribution width (RDW) from day 14 to day 35. When compared to day 0, there was a statistically significant rise in RDW on days 14, 28, and 35 (*P* value <0.05). Adias et al. published similar findings, reporting an increase in RDW during whole blood storage. An increase in RDW is most likely owing to greater variance in RBC size during blood storage, and it shows the degree of anisocytosis. We observed a consistent but statistically insignificant (p value >0.05) rise in RBC counts and mean cell volume throughout storage (MCV). Other parameters, such as mean cell Haemoglobin (MCH) and mean cell Haemoglobin concentration (MCHC), were comparable to Adias et al findings and stay constant throughout storage.[18]

Lactate dehydrogenase (LDH) is an intracellular enzyme that is released when tissue is damaged. LDH is abundant in erythrocytes and is utilized as a hemolysis marker (19). In present study, when comparing LDH activity on the first day of storage to LDH activity after 35 days of storage, we noted a steady rise in LDH activity. A statistically significant rise in LDH activity was observed on days 14, 28, and 35 respectively. Our findings matched those of Verma et al., who found an increase in LDH activity after 35 days of blood preservation and suggested a 7-day timeframe for blood storage.[20] Similarly, Ozment et al. showed a progressive rise in LDH activity during blood storage, which is consistent with our findings.[21] The increase in LDH activity in preserved blood could be related to free radical-induced membrane damage.[22] During storage, anaerobic glycolysis builds lactic acid in the blood, lowering the pH of the storage medium. The consequent drop in pH could be a factor in the gradual rise in LDH during storage. [20]

Superoxidase dismutase (SOD), glutathione peroxidase (GPX), and catalase make up the enzymatic antioxidant defense system that protects cells from oxidative stress (23). We measured the activity of superoxidase dismutase and glutathione peroxidase in whole blood on four different occasions in the current study, and we found a statistically significant reduction in superoxide dismutase and glutathione peroxidase on day 14 of storage. Our findings were comparable to those of Marjani et al., who found that superoxidase dismutase activity was reduced at day 11 and glutathione peroxidase activity was reduced at day 7 of storage. Another study conducted by Rajashe kharaiah et al. found a statistically significant decrease in superoxide dismutase activity and demonstrated that blood preservation for two weeks is safe. The study of Sekeroglu et al. also reported statistically significant reduction in SOD and GPX at 21 and 28 days of storage (24). The collapse of the antioxidant defense system is the primary cause of an increase in oxidative stress. The main contributing factor reducing the lifespan of erythrocytes during storage is an increase in oxidative stress and/or a decrease in antioxidant defense system (25). Oxidative damage is the most important factor causing RBC storage lesion which is caused by free radicals and can affect erythrocytes quality. In whole blood the bio-reactive

substances released by leukocytes in the storage medium such as histamine and cytokines, which may exert direct effects on recipients.

## Conclusion

The study concludes that whole blood storage beyond 14 days is associated with significant oxidative and hematological deterioration. Antioxidant enzyme depletion and rising LDH activity indicate progressive red cell damage, making early use of stored blood safer for transfusion-dependent patients.

LIMITATIONS: The study was limited by resource constraints, as only SOD and GPX were measured; catalase, another key antioxidant enzyme, was not evaluated.

RECOMMENDATIONS: Future studies should explore the role of additional antioxidants, either administered to donors before donation or supplemented in storage bags, to mitigate oxidative damage and extend red cell viability.

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