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Comparative Analysis of Haemolysis Levels in Packed Red Blood Cells Prepared via Whole Blood Settling versus Centrifugation Methods: A Study at the Zonal Blood Bank, Mbeya, Tanzania

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BKM, AMA, CI, EIO, OBO were responsible for conceptualizing, designing, and conducting the study. Authors BKM, AMA, CI, EIO, OBO, OSU, TP, SHM, KJ, CFN managed data and sample collection, while Authors BKM, AMA, CI, CFN and TP carried out the laboratory analyses. Author BKM handled the data analysis. Authors BKM, CI and AMA wrote the initial draft of the manuscript, which all authors critically reviewed. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The term "blood transfusion" describes the safe administration of blood and blood products into a recipient's vein, including packed red blood cells, plasma, platelets, and whole blood. It is necessary for blood transfusion services to offer patients who need blood products safe and affordable blood and blood components for transfusion. Transfusions of red cell concentrate (RCC) are an essential part of contemporary medicine and are administered to hospitalized patients. When PRBC is stored, changes occur called red blood cell storage lesions which affect the quality of packed red blood cells.
Objective: To evaluate the haemolysis status of packed red blood cells prepared by the whole blood settling method and centrifugation method obtained in a zonal blood bank at -Mbeya, Tanzania.
Methods: A quasi-experimental study design was adopted, blood units were selected, and a complete blood count (CBC) was done at day zero. Blood units were divided into two groups of 16 units each for centrifugation method and sedimentation method for PRBC. Data were recorded, entered into Excel and analysed with SPSS version 23 for statistical interpretation. The analysed data were presented in the form of tables, charts and line graphs.
Results: In this study, from day 7th both methods resulted in minimal haemolysis, with mean hemolysis percentages of $0.00 \pm 0.03\%$ for centrifugation and $0.00 \pm 0.00\%$ for settling method, by the 28th day storage period, the hemolysis levels increased to $0.52 \pm 0.12\%$ in the centrifugation and $0.39 \pm 0.10\%$ in the settling.
Conclusion: Haemolysis in the settling method is lower than in the centrifugation method. The study found that centrifugation resulted in slightly higher hemolysis compared to settling, but the difference was not significant enough to affect the clinical efficacy of the transfusions.Highlight that differences were statistically insignificant.

Keywords: Packed red blood cells;whole blood;haemolysis;transfusion; blood storage lesion; blood components.

ABBREVIATIONS

ATP :Adenine Triphosphate
CBC :Complete Blood Cells Count
CPDA-1 :Citrate Phosphate Dextrose Adenine-1
DPG : Diphosphoglycerate
EDTA :Ethylenediaminetetraacetic Acid
FBP :Full Blood Picture
GDBS :Global Database on Blood Safety
HB :Hemoglobin
HCT :Hematocrit
KIU :Kampala International University
MMREC :Mbeya Medical Research and Ethics Committee in Tanzania
PRBC :Packed Red Blood Cell
RBC :Red Blood Cells
REC :Research and Ethics Committee
SHZBTS : Southern Highland Zone Blood Transfusion SERVICE
WB :Whole Blood
WHO :World Health Organization

1. INTRODUCTION

A blood transfusion refers to the safe process of intravenously delivering blood or its components, such as packed red blood cells, plasma, platelets, or whole blood, to a recipient (Booth et al., 2021).Safe blood transfusions rely on ensuring donor safety, addressing patient-specific needs, conducting accurate laboratory cross-matching, and maintaining the integrity of stored red blood cells (RBCs). According to the World Health Organization (WHO), countries should maintain a blood supply equivalent to at least 1% of their population to adequately meet clinical demands (Astutiningtiyas et al., 2022).Blood transfusion services must prioritize providing safe, accessible blood products tailored to patient requirements (Bhombo et al., 2022).These components, including packed red blood cells (PRBCs), are derived from whole blood using techniques like centrifugation or apheresis(Booth et al., 2021).Historically, whole blood was the primary source for platelets, plasma, and RBCs, but modern processing now emphasizes plasma removal to mitigate risks associated with leukoreduction—reducing white blood cells to lower transmission of viruses such as cytomegalovirus, HTLV I/II, Epstein-Barr virus, and herpes virus-8, as well as minimizing immune reactions in recipients (Barshtein et al., 2020).Red cell concentrate (RCC) transfusions are critical in modern healthcare, particularly in Sub-Saharan Africa, where anemia from malaria, malnutrition, sickle cell disease, and thalassemia drive demand. PRBCs are vital for restoring oxygen transport in such cases (Uyoga and Maitland, 2019). However, resource constraints in low-income regions often limit access to advanced blood-processing equipment (Sawadogo et al., 2016)**.**This research examines diverse techniques for processing blood and their influence on the quality of packed red blood cells (PRBCs). By analyzing levels of haemolysis in PRBCs generated through distinct methods, the study seeks to uncover variations in the integrity of the blood product and its subsequent effectiveness in transfusion scenario (Sawadogo et al., 2021). This study evaluates the storage stability of packed red blood cells (PRBCs) in comparison, analyzing aspects such as hemolytic characteristics to optimize transfusion protocols and guide the selection of blood products (Barshtein et al., 2021). The whole blood settling technique functions by utilizing gravitational force to enable the natural sedimentation process, during which red blood cells gradually descend and collect at the base of the storage container as time progresses(Sawadogo et al., 2016). Unlike other methods, centrifugation uses centrifugal force to quickly separate blood into its components, offering exact control over the parameters of the separation process(Ghanaati et al., 2019). When blood cells are refrigerated between 1°C and 6°C, they may undergo transformations termed storage lesions, encompassing both metabolic and physical alterations. Over time, metabolic shifts manifest as a decline in pH, reduced concentrations of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP), and a rise in extracellular potassium levels. Concurrently, physical degradation involves the breakdown of erythrocyte integrity and structural deformities. Collectively, these changes can result in hemolysis, shifts in hematocrit, pH instability, morphological abnormalities in red blood cells, and alterations in hemoglobin properties. Such effects underscore the progressive deterioration of stored blood components under these conditions (Syafrida et al., 2022). These modifications impact the characteristics and functionality of normal red blood cells, rendering them unfit for transfusion and potentially leading to adverse reactions linked to blood transfusions (Öhlinger et al., 2022).This research aims to determine whether red blood cell (RBC) concentrates prepared using a gravity-based whole blood settling technique exhibit comparable quality to those produced via conventional centrifugation. By analyzing the hematological stability of packed RBCs during storage, the study will specifically evaluate hemolysis levels in stored units to assess the effectiveness and equivalence of these two preparation methods.

2. METHODOLOGY

2.1 Study Design

The research employs a quasi-experimental design to evaluate and compare the storage stability of packed red blood cells derived from two preparation techniques: whole blood settling and centrifugation. Key parameters under investigation include hemolysis levels, red blood cell indices (such as hemoglobin concentration and cell volume), and the morphological integrity of erythrocytes during storage.

2.2 Study Area

The study was conducted at Southern Highland Blood Transfusion Service -Mbeya, Tanzania.

2.3 Sample Techniques

This was a purposive sample technique involving qualified whole blood unit.

2.4 Target Sample

All whole blood units which meet the criteria collected by SHZBTS –Mbeya

2.5 Data Collection and Procedures

Informed consent was obtained from the Zonal blood transfusion service manager after explaining the importance of the research to them. All precaution measures as stated in Good Clinical Laboratory Practice (GCLP).

3. DATA ANALYSIS

The data were initially input into spreadsheets using SPSS (Statistical Package for the Social Sciences) and subsequently analyzed via the analyst module in SPSS version 23. Day 0 whole blood samples were designated as the control group, and paired t-tests were performed to evaluate differences observed on days 7, 14, 21, and 28 relatives to this baseline. Additionally, the mean differences between day 7 and day 28 measurements for centrifuged packed red blood cells and settling packed red blood cells were statistically compared using a paired t-test, with a significance threshold set at $p < 0.05$.

4. RESULTS

4.1 Study Profile

In February 2025, a total of 32 qualified blood units were included in this study, all blood units were tested at day 0 for haemolysis. The blood unit was accepted due to free haemolysis, which was for confirmation that all units qualified for normal parameters before being included in our study. About 32 blood units were separated into two groups, 16 blood units for the settling method and 16 blood units for the centrifugation method.

Presents the mean haemolysis over the different storage periods. On Day 7, both methods resulted in minimal haemolysis, with mean hemolysis percentages of $0.00 \pm 0.03\%$ for centrifugation and $0.00 \pm 0.00\%$ for settling. By Day 14, hemolysis increased to $0.23 \pm 0.15\%$ in the centrifugation group and $0.06 \pm 0.07\%$ in the settling group. This upward trend continued on Day 21, with hemolysis reaching $0.37 \pm 0.12\%$ for centrifugation and $0.21 \pm 0.11\%$ for settling. At the end of the storage period (Day 28), hemolysis levels were $0.52 \pm 0.12\%$ for centrifugation and $0.39 \pm 0.10\%$ for settling.

The line graph depicting these trends illustrates a steady increase in hemolysis over time for both methods. Centrifugation consistently showed higher mean hemolysis percentages compared to the settling method at each time point. These observations suggest that while both methods are associated with increased hemolysis over time, the settling method may offer a slight advantage in preserving RBC integrity during storage.

When subjected to a paired sample t-test, no significant difference was observed at day 7 ($p > 0.05$), from day 14 onward, haemolysis was significantly higher in the centrifugation method. We explored the influence of storage time on haemolysis for blood samples processed by centrifugation and settling methods.

Table 1. Comparison of haemolysis between centrifugation and settling method

Time (Days)	Centrifugation Mean (±SD)	Settling Mean (±SD)
7	0.00 ± 0.03	0.00 ± 0.00
14	0.23 ± 0.15	0.06 ± 0.07
21	0.37 ± 0.12	0.21 ± 0.11
28	0.52 ± 0.12	0.39 ± 0.10

Table 2. Comparison of haemolysis between centrifugation and settling method (Paired t-test)

Time (Days)	Mean Haemolysis by Cent.	Mean Haemolysis by SM	t-test (p-value)
7	0.00	0.00	1
14	0.23	0.06	0.015
21	0.37	0.21	0.002
28	0.52	0.39	0.001

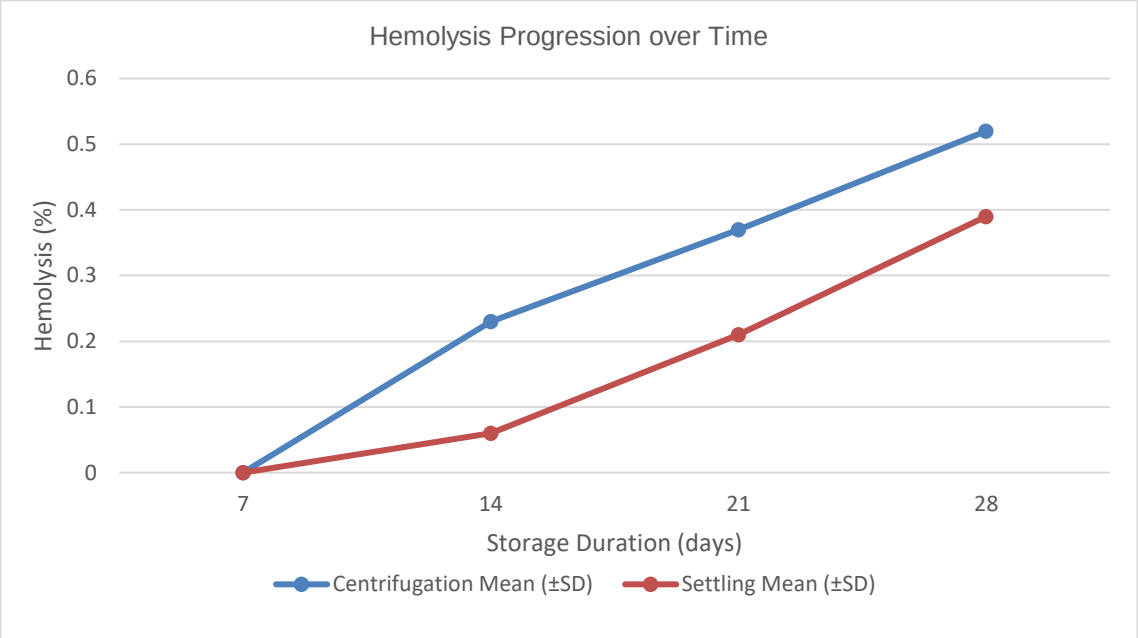

Fig. 1. Line graph of Comparison of haemolysis between centrifugation and settling method

Table 3. Comparison of correlation between haemolysis and storage time in centrifugation and settling method

Method	Pearson Correlation (r)	p-value
Centrifugation	0.83	<0.001
Settling	0.69	<0.001

Table 4.Comparison of storage time to predicts haemolysis progression between centrifugation and settling method

Variable	Coefficient (β)	p-value
Intercept	-0.01	0.82
Storage Time (Days)	0.021	<0.001
Centrifugation (vs. Settling)	0.12	0.002
Interaction (Time × Method)	0.015	0.005

Our analysis reveals that haemolysis strongly correlates with storage time, and blood samples processed by the centrifugation method tend to exhibit an increase in haemolysis over time. To predict the effect of each processing method on haemolysis progression, we conducted a multiple regression analysis incorporating storage time, processing method (centrifugation vs. settling), and their interaction (Storage time vs Method). Haemolysis strongly correlates with storage time. The centrifugation method tends to increase haemolysis over time.

The regression model revealed that storage time significantly predicts haemolysis progression ($\beta = 0.021$, $p < 0.001$), indicating that haemolysis increases over time regardless of the processing method. Furthermore, centrifugation is associated with a significant increase in haemolysis compared to the settling method ($\beta = 0.12$, $p = 0.002$). The significant interaction term ($\beta = 0.015$, $p = 0.005$) suggests that the rate of haemolysis progression over time is greater with centrifugation compared to settling.Storage time significantly predicts haemolysis progression. Centrifugation significantly increases haemolysis compared to settling. The interaction term is significant, indicating that the rate of haemolysis increase is higher in centrifugation.

5. DISCUSSION

This study assessed the hemolysis levels in red cell concentrates (RCCs) prepared using

centrifugation and sedimentation over a 28-day storage period. Our results showed a progressive increase in hemolysis for both methods, with centrifugation consistently resulting in slightly higher values compared to the sedimentation method. By Day 28, hemolysis was measured at 0.52 ± 0.12% for the centrifugation method and 0.39 ± 0.10% for the sedimentation method. Importantly, both values were within the internationally accepted threshold of 0.8% hemolysis, indicating that both methods are suitable for storage in terms of hemolysis levels. However, it is notable that centrifugation resulted in significantly higher hemolysis from Day 14 onward ($p < 0.05$), suggesting that the sedimentation method may better preserve RBC integrity during storage. Our findings are consistent with a study conducted in Burkina Faso (Sawadogo et al., 2021), which also found higher hemolysis in centrifuged samples compared to those prepared by sedimentation. However, the study in Burkina Faso reported hemolysis values ≥0.8% in 16.7% of centrifuged units, which were higher than our observations. This discrepancy could be attributed to differences in centrifugation conditions, such as centrifugation speed and duration. In our study, we used a centrifugation speed of 5000g for 7 minutes, whereas the Burkina Faso study employed 2490g for 20 minutes. The variations in centrifugation protocols, along with storage conditions (e.g., temperature, preservative solutions), may explain the higher hemolysis observed in their study. Our results suggest that when proper centrifugation speed and time are adhered to, hemolysis remains within acceptable limits, even with a higher centrifugation force. Similar findings were reported in Canada (Islamzada et al., 2022), where most packed red blood cells (PRBCs) did not exceed 0.8% hemolysis over a storage period of 42 days. This supports our findings that hemolysis remains within acceptable limits when proper storage protocols are followed, including appropriate centrifugation conditions and storage conditions. The Canadian study's longer storage period (42 days compared to our 28 days) further confirms that although hemolysis increases with time, it generally remains below the regulatory threshold when optimal processing methods are followed.

A study conducted in Malaysia (Sarijan et al., 2018) also found that hemolysis stayed within acceptable limits, echoing our results. The study in Malaysia similarly noted that prolonged storage can lead to increased hemolysis, but when proper handling and processing conditions are maintained, the hemolysis remains within the acceptable range. This finding reinforces the importance of controlled centrifugation protocols and storage practices to minimize the impact of storage lesions on RBC integrity. The increase in hemolysis observed in both methods over time can be attributed to the natural degradation of red blood cells (RBCs) during storage. Several factors contribute to this, including oxidative stress, ATP depletion, and membrane damage, all of which are exacerbated as storage time progresses (Haji et al., 2021). The slight increase in hemolysis in centrifuged samples, especially from Day 14 onward, may suggest that the centrifugation method induces greater mechanical stress on the RBCs, which could lead to more pronounced cell damage compared to the settling method, which is generally gentler on the cells. However, both methods demonstrated acceptable hemolysis levels by the end of the storage period (28 days), indicating that centrifugation and sedimentation both provide reliable methods for preparing red blood cell concentrates for transfusion. The slight advantage in RBC integrity offered by sedimentation in our study could suggest that for shorter-term storage or situations where RBC preservation is critical, the sedimentation method may be preferred. On the other hand, centrifugation remains a widely used and effective method for red cell separation, and its slight increase in hemolysis over time does not significantly affect the clinical efficacy of the PRBCs for transfusions, especially in contexts where short-term storage is common.

6. CONCLUSION

Our study provides valuable insights into the effects of centrifugation and sedimentation on the hemolysis and quality of red cell concentrates (RCCs) during 28 days of storage. Both methods-maintained hemolysis levels within acceptable regulatory thresholds (≤0.8%), with centrifugation resulting in slightly higher levels of hemolysis from Day 14 onwards. While the sedimentation method may offer a slight advantage in preserving RBC integrity, both methods appear equally suitable for short-term storage and transfusion purposes. Our findings are consistent with international studies that emphasize the importance of proper centrifugation protocols, storage conditions, and handling procedures in minimizing RBC damage during storage. Future studies exploring longer storage durations and evaluating other factors like storage media and temperature control may

provide further insights into optimizing RBC preservation for transfusion purposes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

The study obtained ethical approval from Kampala Internal University Research Ethical Committee (KIU-REC) with REC number 723-2024) and the Institute ofMbeya medical research and ethics committee in Tanzania (MMREC) grant the final acceptance letter for the study, with registration number, SZEC-2439/R.A/24/15.The Authors declared that human sample was used in this research and therefore it complies with the Helsinki declaration.The study methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from the Zonal blood transfusion service manager after explaining the importance of the research to them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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