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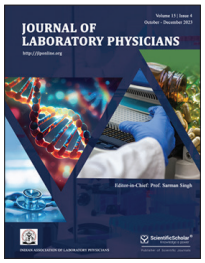

*Original Article*

# Changes in hematological and biochemical parameters and rate of hemolysis during the storage of packed RBC units: A prospective study

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## ABSTRACT

**Objectives:** Blood preservation is to provide viable and functional blood components for patients requiring blood transfusion. The blood is preserved in anticoagulants with or without additive solution. The structural or functional changes in red blood cells (RBCs) that occur during storage are known as storage lesions. The objective of the study was to compare hematological, biochemical, percentage of hemolysis, and effect of $_{P}$H among packed RBC (PRBC) stored in citrate-phosphate-dextrose-adenine (CPDA) anticoagulant and PRBC units stored in saline-adenine-glucose-mannitol (SAGM) preservative solution.

**Materials and Methods:** This was an observational study conducted for 2 years. A total 100 units of PRBC units were included. The hematological, biochemical, and hemolysis percentages were assessed from a day of preparation to day 35 of Storage. These parameters were compared among blood units stored in CPDA anticoagulant units and citrate-phosphate-dextrose with SAGM preservative units.

**Statistical analysis:** This study used both descriptive and inferential statistics for analyzing data. A $P < 0.05$ was taken as significant.

**Results:** The mean hemoglobin and hematocrit decreased continuously in both groups and were statistically significant. The mean corpuscular volume value was increased, mean corpuscular hemoglobin (MCH) decreased, and no significant change in mean corpuscular hemoglobin concentration. The mean percentage of red cell distribution width was nearly equal but red cell count showed variable results. The supernatant sodium level and PH level were decreased but the potassium level was increased continuously. The mean hemolysis percentage was slightly higher in CPDA units as compared to SAGM units.

**Conclusions:** The hematological and biochemical changes that were observed are of similar without any marked differences which suggest that both preservatives are useful. Therefore, PRBCs stored in CPDA and SAGM can be kept till the end of the storage period with a better blood bank inventory.

**Keywords:** Blood preservative, Hemolysis, Storage lesion

## INTRODUCTION

The preservation of human blood began during the First World War. The first anticoagulant preservative citrate-dextrose was introduced by Rous and Turner in 1916.[1] The next significant development was the introduction of acid citrate dextrose (ACD) solution by Loutit and Mollison

in 1943. This was followed by the development of citrate-phosphate dextrose (CPD) by Gibson in 1957 and CPD with adenine (CPDA) in 1978. The adenine content in CPDA is used for the synthesis of adenosine triphosphate (ATP) in stored blood, which helps enhance the viability of red blood cells (RBCs). Additive solutions are preservative solutions that are added to the RBC after removal of the plasma with or without platelet. The first RBC additive solution, saline-adenine-glucose (SAG) was developed by European researchers in the late 1970's. Soon after, in 1981, the same researchers added mannitol to help protect RBC membrane and reduce hemolysis, enabling refrigerated storage for up to 6 weeks. At present, RBCs stored at 2–6°C in ACD, CPD, or citrate -phosphate-double dextrose (CP2D) can be preserved for up to 21 days, while those stored in CPDA can last for 35 days. However, with additive solutions such as SAG-mannitol (SAGM), additive solution (AS)-1, AS-3, and AS-5, RBCs can be stored for up to 42 days.

The role of blood preservation is to maintain the viability and functional capacity of blood components under storage conditions. Because blood must be stored from the time of donation until the time of transfusion, the viability of RBCs must be maintained during storage time as well. The cold storage of packed RBCs (PRBCs) is associated with a gradual increase in the percentage of cells with impaired functionality.[2-4] Recent studies have demonstrated that there may be a correlation between the number of days of cold storage of PRBCs and adverse outcomes, including mortality among transfused patients, presumably due to structural or functional changes in RBCs that occur during storage. This phenomenon has been referred to as storage lesions. These alterations can be extensive and are primarily classified into two broad categories: Biochemical and HematologicalHematological. Biochemical parameters such as pH, lactic acid, glucose consumption, ATP level, 2,3-DPG level, sodium, and potassium level were affected in cold storage. The structural and functional change of RBC affects the hematologicalhematological parameters during storage. The hemoglobinhemoglobin and hematocrit parameters are mostly affected and a variation in other parameters such as mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) is also noticed in certain studies. Storage of red cells causes a progressive increase in hemolysis. Hemolysis during blood collection and storage is the most severe manifestation of the RBC storage lesion. The extent of hemolysishemolysis in blood components is an important indicator of cellular integrity and a quality parameter.

In India, the blood is stored in blood centers governed and regulated by the Central Drugs Standard Control Organization which is headed by the Drugs Controller General. The rules and regulations related to the storage of blood are followed as per the Drugs and Cosmetics Act of 1940 and its Rules of 1945, along with amendments made from time to time. The licensing and monitoring of blood banks are the responsibility of the Drug Controller General of India.[5]

The study aimed to evaluate the changes in hematological and biochemical parameters in PRBC units stored in Blood Bank refrigerator conditions. The objectives were as follows:

1. To compare the hematological and biochemical parameters among PRBC units stored in CPDA anticoagulant and PRBC units stored in SAGM preservative solution
2. To measure and compare the rate of hemolysis of blood units under successive days of storage
3. To measure and compare the effect of pH in storage conditions Objective.

## MATERIALS AND METHODS

This observational prospective study was conducted for a period of 2 years from October 2018 to September 2020 in the Department of Transfusion Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, a tertiary care center of Odisha, India. The study was approved by the Institutional Ethical Committee (444/dt. October 14, 2020).

### Sample size

A total of 100 units of PRBC were included in this study. Out of these, 50 units of PRBC were collected in CPDA anticoagulant and 50 units were collected in CPD anticoagulant with SAGM as the preservative solution.

### Inclusion criteria and exclusion criteria

Blood bags found to be transfusion-transmitted infection (TTI) reactive, under-collection, or over-collection blood units, hemolyzed, and clotted on visual inspection were excluded. The randomly selected PRBC units were included after excluding the blood units that did not meet the required criteria.

### Methodology

#### *Preparation of PRBC*

Each unit of triple blood bag contains 350 mL of whole blood with 49 ml of anticoagulant collected from eligible voluntary and replacement blood donors after fulfilling the eligibility criteria as per National Acquired Immunodeficiency Syndrome Control Organization guidelines. ABO grouping, RH typing, and TTI testing were as per our institutional standard operating procedure (SOP). PRBC was prepared using a refrigerated centrifuge (CRYOFUGE16, THERMOFISHER)

as per our institutional SOP. One hundred milliliters of blood was transferred into another single bag aseptically from each PRBC blood unit on the day of preparation by using a sterile connecting device and stored at 2–6°C in the blood bank refrigerator for 35 days for both CPDA and SAGM bag. Ten milliliters of blood samples were removed aseptically from each 100 mL blood unit on the day of preparation (day 0) and on storage days 7, 14, 21, 28, and 35.

Five milliliter blood sample was collected for analysis of hematological parameters such as hemoglobin, hematocrit, RBC count, MCV, mean corpuscular hemoglobin (MCH), MCHC, RDW at our department using Automated Hematological Analyzer (SYSMEX KX-21,3 PART) and another 5 mL blood sample was centrifuged at 2000 rpm for 10 min and the supernatant was separated. The supernatant was sent to measure the biochemical parameters such as sodium, potassium, pH, and hemolysis. The supernatant sodium and potassium levels were measured at the Department of Biochemistry in our institution. The $_{P}$H and Hemolysis were measured by $_{P}$H meter and HemoCue Plasma Hemoglobin meter in our department. The percentage hemolysis was calculated by measuring the total hemoglobin content, hematocrit, and plasma hemoglobin content of the unit using the formula:

Hemolysis rate (%) = Plasma hemoglobin (g/dL) × (100-Hematocrit)/Total hemoglobin (g/dL). At the end of our study period, all the blood units along with the remaining blood were discarded as per the biomedical waste management protocol of our institution.

### Statistical analysis

Data were entered and analyzed using IBM Statistical Package for the Social Sciences Statistics version 21 for Windows. Data were summarized using descriptive statistics such as percentages, means (standard deviation), and median. Inferential statistics such as the Chi-square test (for categorical data), Student's *t*-test (for data with normal distribution), Mann–Whitney U-test (for data with skewed distribution), and paired *t*-test were also used. A $P < 0.05$ was considered statistically significant.

## RESULTS

The packed red cell units were divided into two groups upon the anticoagulants used – CPDA and SAGM. The majority of donors were from the age group 25–34 years. The mean age group of donors of CPDA units and SAGM units collected from donors were 30.34 ± 6.12 and 29.28 ± 5.5, respectively. Among blood donors, 84 units were from male donors and 16 units from female donors. Among blood group distribution, the highest number of donors was found from Group O (48%) and the number of donors from other blood groups was B (29%), A (16%), and AB (7%). All donors were from Rh-positive groups.

The mean hemoglobin concentration levels were 17.0 ± 0.77 in CPDA units whereas 17.6 ± 0.92 (g/dL) in SAGM units on the day of preparation. There was a decrease in the mean hemoglobin levels in both groups which was statistically significant ($P < 0.05$) from day 0 to day 35**.** The observed mean hematocrit (%) value on different days of storage, i.e., on days 0, 7, 14, 21, 28, and 35 for CPDA units and SAGM units is shown in the Table 1. The decrease was statistically significant from day 7 to day 35 ($P < 0.05$).

Table 2 shows the mean value of MCV, mean concentration of hemoglobin (MCH), and MCHC. In SAGM units, the mean MCV showed a gradual increase from day 0 to day 35 but in CPDA units, the mean MCV increased from day 7 onward till day 35 and it was statistically significant from day 21 to day 35. There was a gradual decrease in mean MCH in both CPDA and SAGM units. In CPDA units, the mean MCV decreased from day 7 onward but in SAGM units, the mean MCV value decreased from day 0. The changes were statistically significant from day 0 to day 35 ($P < 0.05$). No significant change was observed in mean MCHC in both CPDA and SAGM units up to day 28 but there was a minimal decrease in mean MCHC level in CPDA units as compared to SAGM units in the 5$^{th}$ week of storage. From day 0 to day 35, the mean MCHC value was statistically significant between the two groups ($P < 0.05$).

Table 3 shows the comparison of the mean value of RBC count and percentage of RDW among CPDA and SAGM groups on the day of preparation (day 0) to Storage day 35. The mean RBC count in CPDA groups markedly decreased in the 1$^{st}$ week but increased in subsequent weeks till the end of storage but in SAGM groups, it increased continuously during the study period. At the end of storage, SAGM showed a higher RBC count in comparison to the CPDA group. The RDW percentage increased continuously in both in CPDA as well as SAGM groups during the study period. The mean percentage of RDW was nearly equal in both groups.

The biochemical parameters such as supernatant sodium and potassium are shown in Table 4. On the day of preparation, the mean supernatant sodium concentration was 143.98 ± 2.65 (mmol/L) in CPDA and 143.7 ± 2.13 in SAGM. During the progressive storage period, there was a continuous decrease in mean supernatant sodium levels in both CPDA and SAGM units. On day 35, SAGM units showed slightly higher value as compared to CPDA units. The changes were statistically significant from day 0 to day 35 ($P = 0.000$). In the CPDA group, the mean supernatant potassium on the day of preparation and the day 35 of storage were 4.33 ± 0.44 (mmol/L) and 33.05 ± 1.27 whereas in SAGM groups were 4.23 ± 0.49 and 32.57 ± 1.01, respectively. The mean supernatant potassium level increased markedly on day

**Table 1:** Comparison of hemoglobin and hematocrit between CPDA units and SAGM units from day 0 to 35 of storage.

| Days of storage | Hemoglobin (g/dL) | | | Hematocrit (%) | | |
|---|---|---|---|---|---|---|
| | CPDA Mean±SD | SAGM Mean±SD | *P*-value | CPDA Mean±SD | SAGM Mean±SD | *P*-value |
| Day 0 | 17.0±0.77 | 17.6±0.92 | *P*=0.000 | 63.6±2.11 | 55.71±1.11 | *P*=0.737 |
| Day 7 | 16.16±0.66 | 17.36±1.01 | *P*=0.000 | 51.5±1.15 | 53.01±1.37 | *P*=0.000 |
| Day 14 | 16.06±0.67 | 16.4±0.51 | *P*=0.001 | 50.6±1.02 | 51.58±0.99 | *P*=0.002 |
| Day 21 | 15.77±0.72 | 16.12±0.58 | *P*=0.001 | 49.5±1.24 | 50.83±0.91 | *P*=0.000 |
| Day 28 | 15.6±0.79 | 16.08±0.47 | *P*=0.000 | 48.6±1.54 | 49.94±0.74 | *P*=0.000 |
| Day 35 | 15.4±0.86 | 15.84±0.54 | *P*=0.000 | 47.4±2.11 | 49.4±1.17 | *P*=0.000 |

CPDA: Citrate-phosphate-dextrose-adenine, SAGM: Saline-adenine-glucose-mannitol, SD: Standard deviation

**Table 2:** Comparison of MCV, MCH, and MCHC between CPDA units and SAGM units from day 0 to 35 of storage.

| Days of storage | MCV (fl) | | | MCH (pg) | | | MCHC (g/dL) | | |
|---|---|---|---|---|---|---|---|---|---|
| | CPDA mean±SD | SAGM mean±SD | *P*-value | CPDA mean±SD | SAGM mean±SD | *P*-value | CPDA mean±SD | SAGM mean±SD | *P*-value |
| Day 0 | 88.17±2.37 | 88.4±2.43 | *P*=0.10 | 30.3±1.45 | 31±1.43 | *P*=0.000 | 31.86±1.98 | 31.72±1.58 | *P*=0.000 |
| Day 7 | 88.26±3.38 | 90.02±3.72 | *P*=0.51 | 30.6±1.47 | 30.8±1.52 | *P*=0.000 | 31.9±1.95 | 31.04±1.53 | *P*=0.000 |
| Day 14 | 90.3±2.6 | 91.5±2.48 | *P*=0.312 | 29.96±1.35 | 30.2±1.1 | *P*=0.000 | 31.48±1.84 | 31.81±1.63 | *P*=0.000 |
| Day 21 | 92.00±2.66 | 92.48±2.45 | *P*=0.041 | 29.5±1.33 | 29.8±1.2 | *P*=0.000 | 31.65±1.72 | 31.78±1.55 | *P*=0.000 |
| Day 28 | 93.1±3.4 | 93.3±2.84 | *P*=0.000 | 29.4±1.35 | 29.6±0.98 | *P*=0.000 | 31.53±2.04 | 31.7±1.62 | *P*=0.000 |
| Day 35 | 93.9±3.42 | 94.1±2.77 | *P*=0.002 | 28.99±1.28 | 29.4±1.16 | *P*=0.000 | 30.34±1.88 | 31.32±1.46 | *P*=0.000 |

fl: Femtoliter, pg: Picogram, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, CPDA: Citrate-phosphate-dextrose-adenine, SAGM: Saline-adenine-glucose-mannitol, SD: Standard deviation

**Table 3:** Comparison of RBC count and RDW between CPDA units and SAGM units from day 0 to 35 of storage.

| Days of storage | RBC count ($10^6$/$mm^3$) | | | RDW (%) | | |
|---|---|---|---|---|---|---|
| | CPDA mean±SD | SAGM mean±SD | *P*-value | CPDA mean±SD | SAGM mean±SD | *P*-value |
| Day 0 | 6.31±0.58 | 6.45±0.59 | *P*=0.000 | 13.1±0.48 | 13.37±0.47 | *P*=0.002 |
| Day 7 | 5.82±0.44 | 6.51±0.67 | *P*=0.000 | 13.8±0.51 | 13.8±0.37 | *P*=0.000 |
| Day 14 | 6.14±0.51 | 6.87±0.79 | *P*=0.000 | 14.3±0.55 | 14.18±0.31 | *P*=0.000 |
| Day 21 | 6.3±0.39 | 6.95±0.58 | *P*=0.002 | 14.7±0.77 | 14.62±0.32 | *P*=0.000 |
| Day 28 | 6.3±0.58 | 7.31±0.63 | *P*=0.000 | 15.5±0.78 | 15.59±0.75 | *P*=0.000 |
| Day 35 | 6.33±0.72 | 7.26±0.58 | *P*=0.000 | 16.1±0.99 | 15.9±0.71 | *P*=0.000 |

RDW: Red cell distribution width, CPDA: Citrate-phosphate-dextrose-adenine, SAGM: Saline-adenine-glucose-mannitol, RBC: Red blood cell, SD: Standard deviation

35 as compared to day 0 in both CPDA and SAGM units. The changes were statistically significant from day 7 to day 35 ($P$ = 0.000). The mean pH gradually declined but the hemolysis percentage increased progressive storage period as shown in Table 5**.**

## DISCUSSION

Hemoglobin and hematocrit were two important parameters in the storage of blood. In our study, the hemoglobin level decreased in both groups, which is statistically significant from day 0 to day 35 ($P < 0.05$). The decrement in hemoglobin was started from the 1st week onward in the CPDA group but well maintained in the SAGM group. Our study showed similar results to Aninagyei *et al*.[6] who studied and found the consecutive reduction of hemoglobin levels in all successive weekly estimations compared to baseline values. Our study was contradictory to the study of Shirvastava and Dutta[2] and Chhabra *et al*.,[7] where Shirvastava and Dutta[2] showed increased mean hemoglobin from day 1 to day 35, and Chhabra *et al*.[7] observed that their study was statistically non-significant in hemoglobin level. This indicated that RBC

**Table 4:** Relation of supernatant sodium and potassium level between the two groups at 0, 7, 14, 21, 28, and 35 days.

| Days of storage | Sodium (mmol/L) | | | Potassium (mmol/L) | | |
|---|---|---|---|---|---|---|
| | CPDA mean±SD | SAGM mean±SD | *P*-value | CPDA mean±SD | SAGM mean±SD | *P*-value |
| Day 0 | 143.98±2.65 | 143.7±2.13 | *P*=0.000 | 4.33±0.44 | 4.23±0.49 | *P*=0.110 |
| Day 7 | 137.18±2.33 | 140.08±2.72 | *P*=0.000 | 12.85±0.87 | 12.63±0.25 | *P*=0.000 |
| Day 14 | 130.85±1.67 | 133.83±2.96 | *P*=0.000 | 19.86±1.47 | 20.87±0.83 | *P*=0.000 |
| Day 21 | 127.1±1.95 | 129.34±1.33 | *P*=0.001 | 24.32±1.78 | 26.08±2.03 | *P*=0.000 |
| Day 28 | 122.92±1.75 | 125.25±1.07 | *P*=0.000 | 30.24±1.10 | 30.4±0.45 | *P*=0.000 |
| Day 35 | 119.11±1.81 | 121.12±0.99 | *P*=0.000 | 33.05±1.27 | 32.57±1.01 | *P*=0.000 |

CPDA: Citrate-phosphate-dextrose-adenine, SAGM: Saline-adenine-glucose-mannitol, SD: Standard deviation

**Table 5:** Relation of pH and hemolysis between the two groups at 0, 7, 14, 21, 28, and 35 days.

| Days of storage | pH | | | Percentage of hemolysis | | |
|---|---|---|---|---|---|---|
| | CPDA mean±SD | SAGM mean±SD | *P*-value | CPDA mean±SD | SAGM mean±SD | *P*-value |
| Day 0 | 7.2±0 | 7.19±0.02 | *P*=0.000 | 0.09±0.03 | 0.07±0.02 | *P*=0.001 |
| Day 7 | 6.9±0.1 | 6.96±0.05 | *P*=0.002 | 0.2±0.03 | 0.17±0.04 | *P*=0.210 |
| Day 14 | 6.87±0.05 | 6.88±0.03 | *P*=0.000 | 0.3±0.04 | 0.26±0.03 | *P*=0.000 |
| Day 21 | 6.76±0.04 | 6.76±0.04 | *P*=0.000 | 0.41±0.04 | 0.36±0.05 | *P*=0.000 |
| Day 28 | 6.6±0 | 6.66±0.06 | *P*=0.000 | 0.54±0.05 | 0.49±0.04 | *P*=0.000 |
| Day 35 | 6.54±0.06 | 6.51±0.04 | *P*=0.000 | 0.68±0.13 | 0.53±0.08 | *P*=0.000 |

CPDA: Citrate-phosphate-Dextrose-Adenine, SAGM: Saline-Adenine-Glucose-Mannitol, SD: Standard deviation

was well preserved in SAGM as compared to CPDA.

The mean hematocrit level showed a gradual and steady fall during the storage period in both groups. On the 1st week of storage, the hematocrit level fell more in both groups as compared to other weeks of storage. The results were similar to Bensinger *et al*.[8] The cause of the decrease in Hematocrit can be attributed to erythrocyte hemolysis as per Bailey and Bove.[9] At the end of storage, SAGM units showed higher hematocrit levels than CPDA units. This may be due to more adenine levels in SAGM units which generated more ATP in our study. The decrease in hematocrit level in our study was statistically insignificant on the day of preparation but significant on the rest of the days of storage. Our results were in accordance with the study by Shirvastava and Dutta[2] but contradicted the result of Chhabra *et al*.,[7] who observed that the result was statistically insignificant.

In complete blood count, MCV is an important parameter to identify the underlying cause of anemia. Although there was a gradual increase in mean MCV value in both groups throughout the study period, it was within the normal range. The result of our study was statistically insignificant. No correlation was observed between MCV and storage period or additive solution by Nakao *et al*.[10] but Batham and Nayak[11] observed that the mean MCV value was highly statistically significant. Although the mean MCH value decreased in both groups, it is maintained the normal range throughout the storage period and also statistically significant. Both MCH and MCHC values did not change in both groups during the storage period. In SAGM units, these were slightly better as compared to CPDA units in our study. Our study was similar to the results and statistical analysis of Adias *et al*.[12] but contradictory to the study by Shirvastava and Dutta,[2] who observed an increased MCH value at 35 days from baseline. The mean RBC count in CPDA units markedly decreased on day 7, but no significant difference was noted on day 14, 21, 28, and 35 as compared to day 0, whereas it increased continuously from day 0 to day 35 in SAGM units. The gradual increase of mean RBC count on storage was also found in other studies conducted by Tayer *et al*.[13] During the storage period, the mean RBC counts were within the normal range in both groups, and the differences between the two groups were found to be statistically significant from day 0 to day 35. The mean value of RDW (%) on day 0 for the CPDA blood bag was 13.10 ± 0.48 and for the SAGM blood bag was 13.37 ± 0.47, which increased gradually, and finally, at 35 days, the value was 16.10 ± 0.99 in CPDA units and 15.90 ± 0.71in SAGM units. The progressive increase in RDW was noticed in our study, which agreed with the study of Cohl *et al*.[14] The change in RDW between the two groups was statistically significant from day 0 to 35 ($P < 0.05$).

The present study showed that the percentage of hemolysis was 0.53 ± 0.08 in SAGM units and 0.68 ± 0.13 in CPDA

units during the 5th week of storage. This was statistically significant on days 0, 14, 21, 28, and 35 ($P < 0.05$). This result followed the guidelines dictated by the Council of Europe[15] and the US food and drugs administration (FDA).[16] As per the Council of Europe, the hemolysis percentage in all RBC units should be below 0.8%. Moreover, US FDA guidelines suggested that hemolysis concentration should be <1% at 95% of RBC units. The hemolysis percentage suggests that both units were safely transfused for up to 5 weeks. Compared to the methods of detection of hemolysis, our study was in accordance with the findings of Sawant *et al*.[17] studies, who evaluated hemolysis using the tetramethylbenzidine method. Again, SAGM units had a lower hemolysis percentage than CPDA units at 35 days of storage which may be due to higher concentrations of adenine, glucose, and mannitol that prevent hemolysis. This study was very similar to Zimmermann *et al*.[18] study, which studied the effect of adenine, mannitol, and glucose on hemolysis of stored blood and found lower hemolysis in ADSOL additives than those stored in SAGM.

The sodium concentration was nearly equal in both CPDA and SAGM units on day 1 of storage and gradually decreased up to 35 days. The loss of sodium was slightly higher in CPDA units as compared to SAGM units in the 5th week of storage. The differences in the value of mean sodium are found to be statistically significant. A study by Jobes *et al*.[19] showed a similar finding to the sodium value found in our study, where a declining level of sodium was reported during the storage of blood. The average weekly changes were likely to move sodium concentration in stored blood to a level outside the normal reference range. During storage, suppression of the Na-K ATPase pump caused leakage of potassium into the extracellular fluid and pulling of sodium into intracellular fluid, as a result of which sodium level decreases. The decreased sodium level caused possibly adverse clinical effects on recipients of such units, which increased the chance of edema in those recipients, as collaborated by Metheny,[20] especially in patients with low sodium intake and those with previous diarrhea.

Our study showed that the mean supernatant potassium level was equal in both groups (4.33 ± 0.44 in CPDA vs. 4.23 ± 0.49 in SAGM). There was nearly an eight-fold increase in the potassium level from the baseline value in both preservative groups. This indicates that the preservative had no role in preventing the leakage of potassium during storage. A similar result was observed by Adias *et al*.,[12] who found that the potassium level increased within seven days and continued subsequently. Mukherjee *et al*.[21] observed a significant rise in potassium from day 0 to day 21, which was in accordance with our study. A study by Opoku-Okrah *et al*.[22] and Murthy *et al*.[23] demonstrated that there was an increase in potassium concentration from day 10 onward. At the end of the study period, our study showed a lesser rise in the potassium level as compared to D'Alessandro *et al*.[24] where the potassium level rose to 50 meq/L. Our study was also similar to Verma *et al*.[25] study in statistical analysis, where the rise of potassium showed a statistically significant ($P < 0.05$) value from day 7 onward. This dramatic increase in potassium level may be due to the failure of the Na-K ATPase pump during the storage period, as hypothesized by Wallas *et al*.[1] This increase was dangerous for the recipient's body and more dangerous if transfused to a patient with severe renal disease. This can be prevented using potassium adsorption filters before transfusion or by transfusing blood with early days of storage only.[26]

The mean pH value dropped to 6.54 ± 0.06 in 35 days of storage from a baseline value of 7.2 ± 0 in CPDA units and 6.51 ± 0.04 from 7.19 ± 0.02 in SAGM units. The proper pH level is maintained in the blood units to maintain the physiological function of RBC units. A decrease in pH hampers the oxygen-carrying and oxygen-delivering capacity of hemoglobin at the tissue level. A similar result by Almoshary *et al*.[27] observed on day 0 and at the end of day 28. In standard blood bank refrigerators, the pH level declines in stored PRBC units due to a rise in lactate level from the anerobic metabolism of glucose. Gradually fall of pH of RBC units stored in both preservatives in our study period. This result was in accordance with Achor and Brown,[28] who compared pH in CPDA blood among traditional refrigerators and standard blood bank refrigerators and also similar to studies done by Huisman *et al*.[29] and Burger *et al*.[30]

## CONCLUSIONS

Of the two types of preservatives used, CPDA and SAGM, the hematological and biochemical changes that were observed are similar without any marked differences, which suggests that both preservatives are useful. Therefore, PRBCs stored in CPDA and SAGM can be kept till the end of the storage period with better blood bank inventory control and using special techniques such as leukofilter and potassium adsorption filter before transfusion.

### Key messages

The SAGM preservative is better than CPDA preservative in comparison of hemolysis percentage. High supernatant potassium level in progressive storage period suggests seven days old blood should be transfused to severe renal disease patients to avoid aggravating the diseases.

**Author contribution:** JS: Manuscript writing, data retrieving, data analysis; SM: Conceptualization, methodology framing, review of manuscript, BBS: Results validation, manuscript editing, review of literature, gave the final approval for publishing; NS: Manuscript editing and proof reading.

**Ethical approval:** The research/study was approved by the Institutional Review Board at Srirambhanja Medical College and Hospital, number 444, dated 14th October 2020.
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