

Storage Effect on Serum Electrolytes and pH in Whole Blood Stored in Traditional Refrigerator

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Abstract: *Whole blood storage is needed for the maintenance of readily and sufficient blood supply for transfusion. Survey has shown that most laboratories in Nigeria use traditional refrigerator instead of the standard blood bank for blood storage. Bizarre changes in the biochemistry of stored blood have been reported particularly when standard conditions are not adhered to strictly. This prompted the need for the investigation of probable changes in electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) and pH during whole blood storage using citrate phosphate dextrose adenine (CPDA-1) in traditional refrigerator (TR) and using values from samples stored in a standard blood bank (SBB) (approved blood bank refrigerator) as standard values. Thirty seven (37) apparently healthy voluntary adult male donors (aged 19 to 30 years) were recruited. Twenty (20) donors donated 450mls of whole blood each into CPDA-1 anticoagulant blood bag. These units were stored in SBB. While 17 subjects also donated 450mls of whole blood each into bags with the same anticoagulant. These latter units were instead stored in a TR and both refrigerators were allowed the same relatively stable power supply for 35 days. Samples were collected at Days 1, 7, 14, 21, 28 and 35. Serum Electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) and pH were analyzed using an Automated Biochemistry Analyzer (Olympus AU400 Automated Chemistry Analyzer). Mean sodium levels for TR refrigeration dropped from 136.6 ± 1.14 mmol/L (Day 1) to 132.0 ± 1.32 mmol/L (Day 35) as against 138 ± 0.22 mmol/L - 132.0 ± 1.32 mmol/L for SBB. This indicates that Na^+ levels declined as the number of days of storage increased. When mean values for SBB and TR for sodium were compared using an independent sample t-test, significant decreases were observed at days 7 ($F=1.684$, $t=2.305$, $p=0.027$) and 14 ($F=1.623$, $t=2.761$, $p=0.009$). Tremendous increase in K^+ levels from Day 1 to Day 35 was also observed. Mean K^+ values of 9.48 ± 0.94 mmol/L and 2.52 ± 1.72 mmol/L were respectively observed for the last and first days for TR refrigeration. Significant increase was observed when mean values of K^+ for SBB were compared with those of TR for all the days with the inferential statistics values: Day 7 ($F=17.256$, $t=9.283$, $p=0.000$), Day 14 ($F=10.358$, $t=-15.197$, $p=0.000$), Day 21 ($F=14.381$, $t=-20.285$, $p=0.000$), Day 28 ($F=4.810$, $t=-19.016$, $p=0.000$) and Day 35 ($F=0.499$, $t=-11.979$, $p=0.000$). Bicarbonate, pH and chloride levels for both groups were observed to decrease with storage time. Significant decreases were observed for Cl^- and HCO_3^- for Day 7 ($F=17.019$, $t=-6.496$, $p=0.000$ and $F=0.404$, $t=-2.177$, $p=0.035$ respectively), Cl^- only for Day 14 and 21 ($F=3.253$, $t=-5.553$, $p=0.000$ and $F=2.112$, $t=-4.964$, $p=0.000$) respectively. The results show that there were significant changes in the levels of all electrolytes and pH at different weeks of storage and the changes were more in the units stored in the traditional refrigerator and this could be clinically detrimental to the recipient on transfusion if adequate measures are not taken, particularly in massive transfusion and/or if the recipient already has an established clinical sequela that could exacerbate the condition.*

Keywords: Storage effect, serum electrolytes, pH, whole blood, traditional refrigerator

1. Introduction

Blood storage is currently a logistical necessity for the maintenance of a readily and adequate blood supply. In recent years, there has been an evolving and escalating debate regarding a functional issue in transfusion medicine: what is the effect of storing blood products on outcome in transfusion recipients? What is the effect of storage on biochemical parameters? The latter has been answered extensively by studies on storage induced changes in stored blood. However, in most developing countries, Nigeria for example, where the required facilities for proper blood storage are scarcely used in some settings, it is likely that whole blood or blood products that are collected and stored for future use may not be at its best for therapeutic or clinical use, or may be of clinical detriment to the recipient. Recent reports suggest that transfusion of blood not stored in the approved facilities and condition was associated with the risk of postoperative complications and higher mortality rate in surgical patients as well as post transfusion complications. Retrospective cohort studies have found a correlation between red blood cell (RBC) storage duration and morbidity and mortality rates after transfusion [1]-[3], suggesting progressive storage lesions may be responsible for adverse outcomes. The administration of whole blood or units containing cellular elements has been reported to pose many

risks and potential unfavorable effects. This is basically may not be unconnected with the gradual decomposition of blood components and as a result of the bioaccumulation of product of the cellular metabolism, that is, anaerobic glycolysis, particularly when the components are not stored at the approved temperature range. The biochemical composition of the stored whole blood are bound to undergo bizarre changes. The release of H_2O_2 and proteases by white blood cells present in unfiltered blood may also cause lysis of RBCs storage. The changes are however proportional to the storage time, temperature and other factors [4],[5] reported that during storage, there is an increase in K^+ and lactate levels and a simultaneous decrease in pH and Na^+ levels [5] Other changes include a reduction in RBC deformability, altered RBC adhesiveness and aggregability, and a reduction in 2, 3 - diphosphoglycerate (2, 3 - DPG) and adenosine triphosphate (ATP), bioactive compounds with proinflammatory effects also accumulate in the storage medium. These changes reduce post transfusion viability of red blood cells. A Normal blood sodium level is 135 - 145 millimoles/liter (mmol/L). Storage of anticoagulated whole blood for three weeks at 4°C under blood bank conditions may result in a rise in intracellular Na^+ and a fall in intracellular K^+ with concomitant opposite changes in Na^+ and K^+ levels in the suspending plasma. A decline in red blood cell ATP during the storage period did not appear to

be contributing to the changes. Increasing red blood cell ATP to levels 2 to 3 times normal did not prevent the cation changes from occurring. When assayed at 37°C in the presence of added Mg²⁺, ouabain-sensitive membrane ATPase activity and kinetics of activation by Na⁺ were unaffected by the three week period of cold storage. However, when assayed at 4°C without added Mg²⁺, simulating the conditions of storage, ATPase activity was negligible,[6].The clinical effects beyond transfusion are uncertain, but a growing body of evidence suggests that the storage lesion may reduce tissue oxygen availability, have proinflammatory and immunomodulatory effects and influence morbidity and mortality [6]. Red Blood Cell transfused patients had worse outcomes than non-transfused patients matched for clinical variables in several studies [7]. Large-volume RBC transfusion may contribute to changes in the patients' plasma biochemical parameters (hyperkalemia) and may therefore be related not only to the volume of RBC products but also to storage duration [8].Other changes include a reduction in red blood cell deformability, altered red blood cell adhesiveness and aggregability, and a reduction in 2,3-diphosphoglycerate and ATP. Bioactive compounds with proinflammatory effects also accumulate in the storage medium. These changes reduce post transfusion viability of red blood cells.

The bloodstream is the most critically buffered system of the entire body, far more sensitive than any other. Arterial and venous blood must maintain a slightly alkaline pH: arterial blood pH = 7.41 and venous blood pH = 7.36. Because the normal pH of arterial blood is 7.41, a person is considered to have acidosis when the pH of blood falls below this value and to have alkalosis when the pH rises above 7.41. Clinical problems of pH are all related to pH of the plasma of whole blood. pH in extracellular fluid is always close to that of blood. In the clinical situation if the actual pH of the blood is lowered, one can usually assume that the primary disturbance has been the addition to the blood of acid or the removal of base and vice versa. Blood pH may be changed if acids or bases are added to or removed from the blood. pH levels of stored blood have been reported to decrease due to anaerobic utilization of glucose through the glycolytic pathway to produce lactic acid which as a result reduces the pH of the stored blood which on transfusion, particularly massive transfusion within a short period of time as practiced in most settings where there is dearth of adequate facilities to arrest bleeding. It has been reported that a decrease in pH level and increases in lactate and potassium concentrations may occur within a few hours of storage while other changes may take weeks to appear [9]. Potassium is the major positive ion in the cells. The proper level of K⁺ is vital for normal cell function. In all of its functions, the most essential are regulation of the heartbeat and the function of the muscles. Hyperkalemic conditions can profoundly affect the nervous system and increases the chance of cardiac arrhythmias, which, when extreme, can be lethal. The normal blood potassium level is 3.5 - 5.0 millimoles/liter (mmol/L). In stored whole blood, it has been reported by Clement et al that there was relatively higher increment in potassium concentrations on day 10 and 15 by 21.4% and 60.7% respectively. The increase in potassium concentrations may in no doubt be due to leakage from cells to the plasma [9].Chloride is the major extracellular anion. It

has been reported that Sea water has almost the same concentration of chloride ion as human body fluids. Chloride also plays a role in helping the body maintain a normal fluid balance. The balance of chloride ion is closely regulated by the body. Significant increases or decreases in chloride can be deleterious or even fatal. The normal serum range for chloride is about 98 - 108 mmol/L. In CPDA-1 stored whole blood, chloride levels were observed to decrease after two days of storage[10].The bicarbonate ion acts as a buffer to maintain blood acidity and other fluids in the body. HCO₃⁻ levels are measured to monitor the acidity of the blood and body fluids. The acidity is affected by foods or medications that are ingested and the function of the kidneys and lungs. The normal serum range for bicarbonate is 22-30 mmol/L. The HCO₃⁻ test is usually performed alongside with tests for other blood electrolytes. A fall in bicarbonate was observed in stored blood using CPDA-1 anticoagulant preservative [10].

It is well documented that certain biochemical changes occur during the 35 to 42 days of blood cells storage at temperatures between 1°C and 6 °C. The biochemical structure of the red blood cell is being altered due to anaerobic glycolysis and these changes, as mentioned earlier, are relative to the storage period and temperature of storage. The accumulation of lactic acid and proteins appear in the red cells after 14 days of storage due to glycolytic metabolism. It has also been reported that a decrease in pH level and increases in lactate and potassium concentrations may occur within a few hours of storage while other changes may take weeks to appear [9].One of the most important electrolytic change in stored blood is that of potassium. During storage there is a slow but constant leakage of K⁺ from cells into surrounding plasma. In severe kidney disease even small amount of K⁺ fluctuations can be dangerous and relatively fresh or washed red cells are indicated. Due to a higher K⁺ content of stored blood <5 days old is recommended by [11] for neonatal exchange and top-up transfusion. Invariably, noncompliance with rules and regulations governing the storage of whole blood or any of its products could cause severe clinical consequences to the recipient on transfusion. A survey shows that most medical laboratories operating in Port Harcourt, Rivers State, Nigeria use traditional refrigerator instead of the convectional blood bank refrigerator for blood storage. Reasons may to a large extent be due to inability to procure blood bank refrigerators as they are considered expensive. The outcome of the survey prompted the need for this study. The survey depicted that out of 84 sampled laboratories in the city of Port Harcourt that operated blood banks, 61.9% (52 laboratories) used traditional refrigerator as their blood bank. This obviously is not in accordance with the basic requirements of The Blood Bank Society of Nigeria as well as American Association of Blood Bank (AABB) and other regulatory bodies for the operation of a blood bank. The result of the survey led to the evaluation of likely changes in electrolytes and pH that would occur in CPDA-1 stored whole blood using a traditional refrigerator and comparing values obtained with values that would be observed when units of blood contained in the same anticoagulant/preservative and subjected to the same power supply but instead stored in a standard blood bank (approved blood bank refrigerator).

2. Materials and Methods

This study was conducted in Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Rivers State, Nigeria. The study was stratified into two groups: Standard Blood Bank (SBB) refrigeration and Traditional Refrigerator (TR) refrigeration. Recruited subjects comprised of adult males (aged 19 to 30). They included a total of thirty seven (37) apparently healthy volunteer donor subjects that tested negative for: HCV, HbsAg, Syphilis and HIV 1 & 2 with corresponding blood groups of 10 A Rh "D" Positive subjects, 5 A Rh "D" Negative subjects, 10 O Rh "D" Positive subjects, 2 O Rh "D" Negative subjects, 10 B Rh "D" Positive subjects and 3 B Rh "D" Negative subjects. Twenty (20) of these donors donated 450mls of whole blood each into Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant blood bag. These units were stored in a Standard Blood Bank (SBB) at Braithwaite Memorial Specialist Hospital (BMSH). The remaining seventeen (17) subjects also donated 450mls of whole blood each into bags with the same anticoagulant. These latter units were instead stored in a Traditional Refrigerator (TR) and both refrigerators were allowed the same relatively stable power supply. The experimental phase of the study spanned through a period of thirty five (35) days. At the time of donation samples were collected for both groups and

analyzed to form the baseline values. Subsequent samples were taken at intervals of seven (7) days for the rest of the experiment: samples were taken at Day 1, Day 7, Day 14, Day 21, Day 28 and Day 35. Analysis of the biochemical parameters were done as given below: Plasma Electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) and pH were analyzed using an Automated Biochemistry Analyzer (Olympus AU400 Automated Chemistry Analyzer) and under good laboratory practices. Statistical tool used was Statistical Package for Social Sciences (SPSS).

3. Results

This study has shown changes in some biochemical parameters that occurred in CPDA-1 stored whole blood when subjected to both Standard Blood Bank and Traditional refrigeration, the latter being increasingly practiced in this part of the world (Port Harcourt City) according to the survey. It was observed that mean sodium levels dropped from $138 \pm 0.22 \text{ mmol/L}$ (first day) to the 35th day ($132.0 \pm 1.32 \text{ mmol/L}$) for SBB refrigeration. While mean sodium levels for TR refrigeration dropped from $136.6 \pm 1.14 \text{ mmol/L}$ (Day 1) to $132.0 \pm 1.32 \text{ mmol/L}$ (Day 35), (Table 1.0).

Table 1: Chart showing mean values for Na^+ for both SBB and TR refrigeration

Group/Days	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
SBB	138.2 ± 0.22	136.8 ± 0.97	136.2 ± 0.82	135.4 ± 1.18	134.7 ± 1.29	132.5 ± 1.04
TR	136.6 ± 1.14	136 ± 1.20	135.4 ± 1.05	134.8 ± 1.06	134.2 ± 1.09	132 ± 1.32

This indicates that Na^+ levels declined as the number of days of storage increased. When mean values for SBB and TR for sodium were compared, significant decreases were observed

at days 7 ($F= 1.684, t= 2.305, p= 0.027$) and 14 ($F= 1.623, t= 2.761, p= 0.009$) as shown in figure 1.0

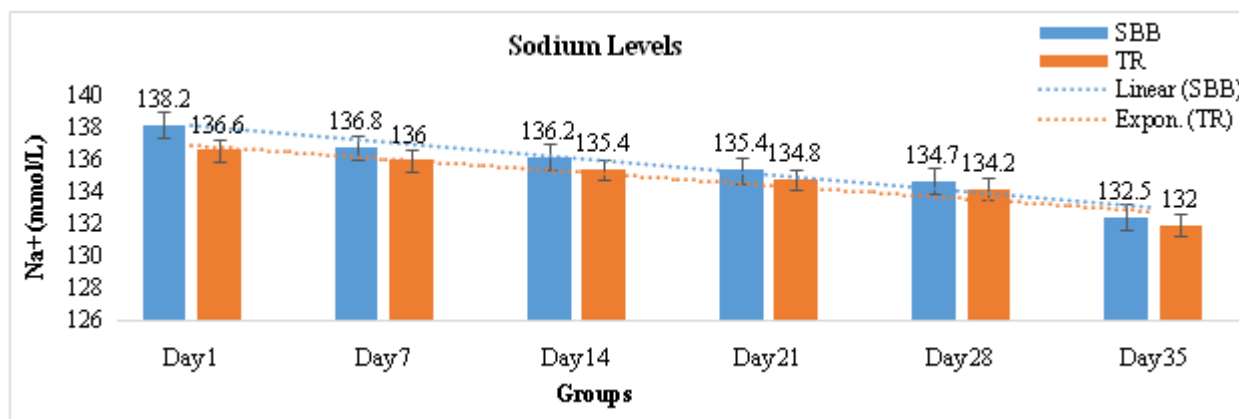


Figure 1.0: Chart showing mean values for Na^+ for both SBB and TR refrigeration

This study has also shown that there was a tremendous increase in K^+ levels from Day 1 to Day 35 for all groups. Mean K^+ value of $5.42 \pm 1.10 \text{ mmol/L}$ was recorded as the highest value and $2.89 \pm 0.11 \text{ mmol/L}$ recorded as the lowest

value for SBB refrigeration, while $9.48 \pm 0.94 \text{ mmol/L}$ and $2.52 \pm 1.72 \text{ mmol/L}$ were respectively observed for the last and first day for TR refrigeration, (Table 1.1 below)

Table 1.1: Table showing mean values for K^+ for both SBB and TR refrigeration

Group/Days	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
SBB	2.89 ± 0.11	3.1 ± 0.20	3.56 ± 0.32	3.92 ± 0.34	4.59 ± 0.48	5.42 ± 1.10
TR	2.52 ± 1.72	5.02 ± 0.90	7.4 ± 1.08	8.32 ± 0.90	8.91 ± 0.87	9.48 ± 0.94

Potassium values were observed to increase with the time of storage for both groups. Significant increase was observed when mean values of K^+ for SBB were compared with those of TR for all the days with the inferential statistics values, Day 7 ($F=17.256$, $t= 9.283$ $p= 0.000$), Day 14 ($F= 10.358$, $t=$

-15.197 , $p= 0.000$), Day 21 ($F= 14.381$, $t= -20.285$, $p= 0.000$), Day 28 ($F= 4.810$, $t= -19.016$, $p= 0.000$) and Day 35 ($F=0.499$, $t=-11.979$, $p=0.000$), shown in figure 1.1.

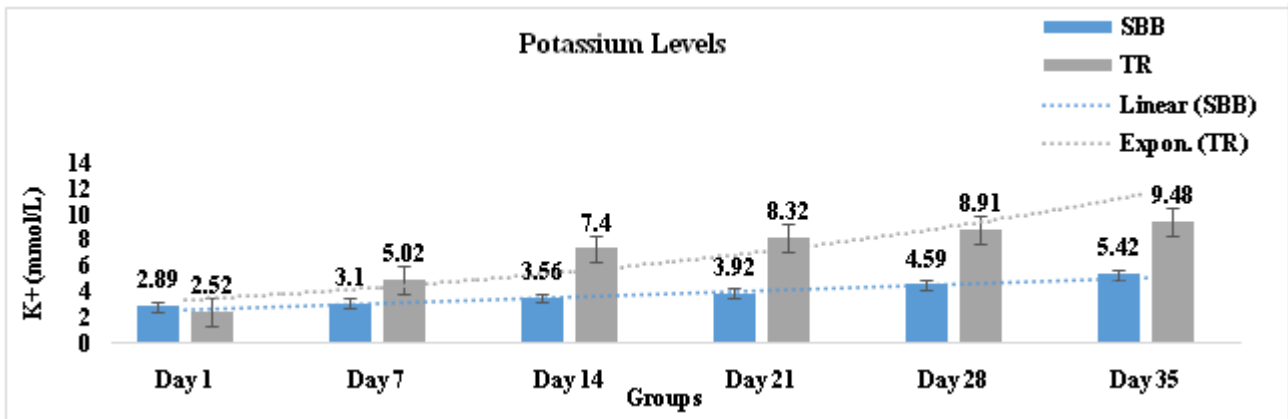


Figure1.1: Chart showing mean values for K^+ for both SBB and TR refrigeration

Bicarbonate, pH and chloride for both groups were observed to decrease with storage time. Mean Cl^- values for SBB and TR are shown in tables 1.2; 1.3 and figure 1.2; 1.3 below.

Table 1.2: Table showing mean values for Cl^- for both SBB and TR refrigeration

Group/Days	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
SBB	82.15±1.67	80.36±1.61	79.86±2.13	78.45±2.43	77.1±2.39	75.97±2.14
TR	89.94±3.42	86.14±3.59	85.15±3.59	83.72±3.96	78.16±4.05	76.82±3.80

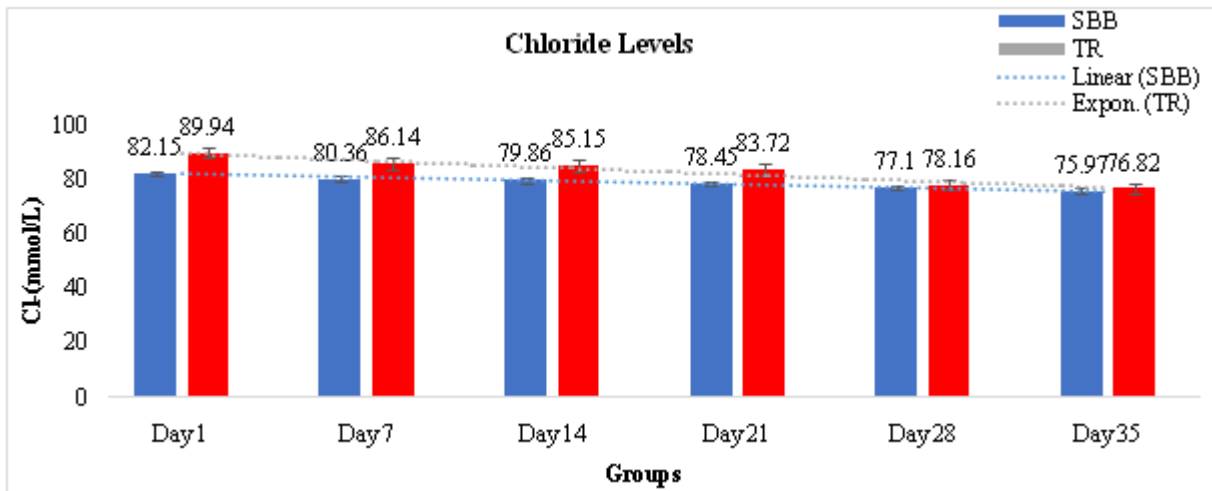


Figure1.2: Chart showing mean values for Cl^- for both SBB and TR refrigeration

Significant decreases were observed for Cl^- and HCO_3^- for Day 7 ($F= 17.019$, $t=-6.496$, $p= 0.000$ and $F= 0.404$, $t=-2.177$, $p= 0.035$ respectively), Cl^- only for Day 14 and 21 ($F= 3.253$, $t= -5.553$, $p= 0.000$ and $F= 2.112$, $t= -4.964$, $p=$

0.000 respectively) when mean values for SBB and TR were compared.

Table 1.2: Table showing mean values for HCO_3^- for both SBB and TR refrigeration

Group/Days	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
SBB	19.25±0.97	18.9±0.95	18.61±0.94	18.39±0.85	17.96±0.78	17.53±0.55
TR	19.86±0.96	19.55±0.84	19.01±0.7	18.86±0.88	17.79±0.74	17.38±0.60

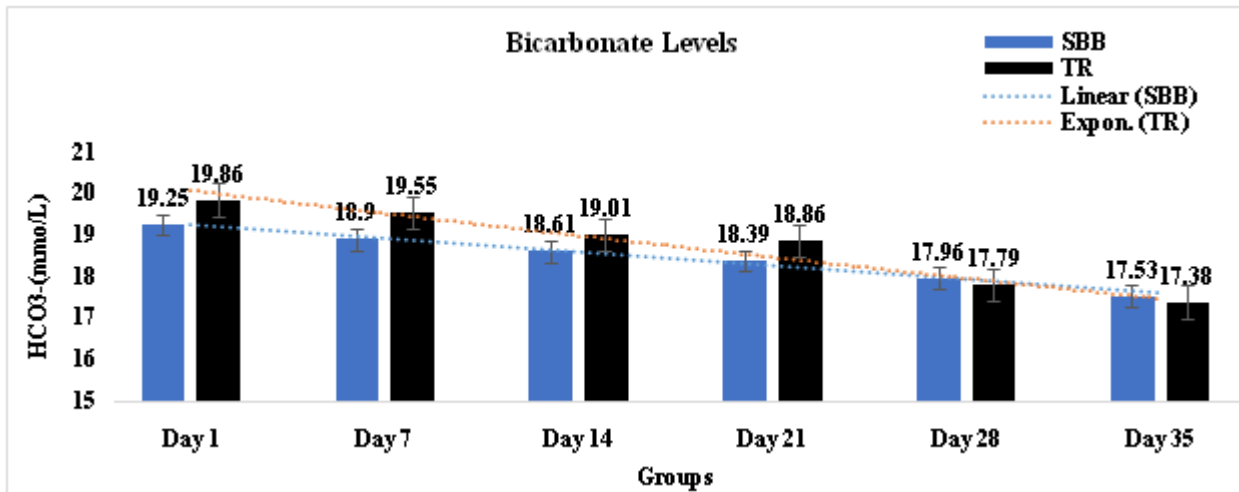


Figure 1.3: Chart showing mean values for HCO_3^- for both SBB and TR refrigeration

Table 1.4: Table showing mean values for pH for both SBB and TR refrigeration

Group/Days	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
SBB	6.92±0.21	6.49±0.34	6.38±0.35	6.25±0.30	6.16±0.23	6.07±0.59
TR	6.84±0.17	6.59±0.33	6.46±0.39	6.37±0.34	6.22±0.26	6.16±0.28

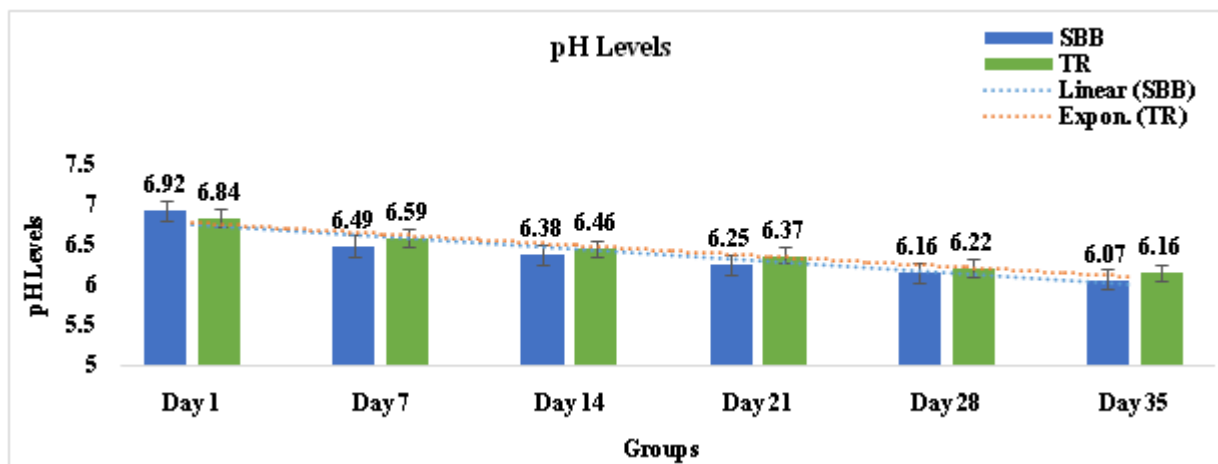


Figure 1.4: Chart showing mean values for pH for both SBB and TR refrigeration

4. Discussion

Na^+ level on the first day was observed to be $138 \pm 0.22 \text{ mmol/L}$ (table 1.0). This serves as the baseline value for Na^+ levels and was used to compare with Na^+ values of subsequent days (17, 14, 21, 28 and 35) to ascertain the level of statistical significance. The value is about one unit higher than the mean Na^+ (137.38 mmol/L) reported by [12]. Although they used ten samples and observed a non-significant relationship between the mean values for Day 1 and that of other days, [12]. During refrigerated storage, Na^+ and K^+ leak through the red cell membrane rapidly. The cells lose and gain Na^+ , however, the K^+ loss is greater than the Na^+ gain during storage.

The lowest mean Na^+ value ($132.0 \pm 1.32 \text{ mmol/L}$) was recorded on the last day (Day 35). This indicates that Na^+ levels declined as the number of days of storage increased. (Figure 1.0)[12] also recorded a decline in mean Na^+ levels ($137.38 - 129.44 \text{ mmol/L}$). However, in this study a significant level ($p < 0.001$) was observed when Na^+ values for Day 1 were compared with those of other days. Also,

mean Na^+ value for units that were stored in the traditional refrigerator was observed to be $136 \pm 1.14 \text{ mmol/L}$ for Day 1. This is one unit lower than that reported by [12]. Meanwhile, when Na^+ values for Day 1 were compared with those of other days for TR refrigeration, Day 7 was observed not to be significant, but all other days were significant at $p < 0.001$. Radovan et al also recorded a decrease in Na^+ levels when units that were stored for longer periods were transfused. They observed a mean *in vivo* Na^+ value of 137.0 mmol/L when whole blood collected on the first day were transfused [4]. It has been observed that following blood transfusion of stored blood, complications such as hyperkalemia, hyponatremia and citrate toxicity among other conditions do occur. Comparison between mean Na^+ values for SBB with those of TR refrigeration showed that there was a significant difference at ($p < 0.001$) for Day 1 and $p < 0.05$ for other days save Days 28 and 35 which both recorded no significant difference. The difference may probably be due to changes in the rheology of red blood cells due to the accumulation of waste products, particularly in the storage that had the temperature fluctuating between 2 to 9°C . No study has reported changes in Na^+ levels in CPDA-1 anticoagulant blood stored in a traditional refrigerator. However, [5]

reported extreme reduction in Na^+ levels when the temperature for RBC storage was allowed to fluctuate between 5°C above the AABB designated temperature ($2-6^\circ\text{C}$) for blood storage [5]. In severe kidney disease even small amount of K^+ fluctuation can be dangerous and relatively fresh or washed RBCs are indicated, [11].

This study has also shown that there was a tremendous increase in K^+ levels from Day 1 to Day 35 for all groups (figure 1.1). Mean K^+ value of $5.42 \pm 1.10\text{mmol/L}$ was recorded as the highest value and 2.89 ± 0.11 recorded as the lowest value for SBB refrigeration, while 9.48 ± 0.94 and $2.52 \pm 1.72\text{mmol/L}$ were respectively observed for the last and first day for TR refrigeration (table 1.1). K^+ values were observed to increase with the time of storage. This is in agreement with values obtained by [12]. In both groups, mean K^+ values were significant at all levels. It was also observed that increases in K^+ levels were more in the TR refrigeration. This may probably be due to excessive breakdown of RBC and leakage of K^+ into the plasma, and more leakage when the temperature increased. Electrolyte, particularly K^+ disturbances can be associated with a number of occurrences including drug usage [13] but the kidney is expected to manage it. Although, in cases of massive transfusion, the kidney becomes overpowered and the clinical consequences become inevitable. Hypokalaemia and hyperkalaemia have been seen as problem for some hospitalized patients [10] with hyperkalaemia being implicated for complications of massive blood transfusion [14]-[16]. Assessment of K^+ levels as an impact of transfused blood on biochemical parameters depending on the volume and age of administered product, as well as the biochemical changes occurring during the storage of these products *in vitro* were analyzed by [4]. According to them, K^+ values increased tremendously in both cases, recording a mean *in vivo* K^+ values greater than 5.5mmol/L [4].

This study shows an increase in plasma potassium levels which are also in accordance with those reported by [17]. Their study focused on hyperkalemia ($>5.5\text{mmol/L}$) in a group of 131 trauma patients undergoing cardiopulmonary regulation during the initial 12 hours after admission to a hospital. 96 (73.3%) of the patients received blood (a mean of 11.2 blood units/patient, range 1-55 whole blood units/patient). Interestingly, 38.5% of transfusion patients developed hyperkalemia, as compared with only 5.9% of patients without transfusion. The study documented a more dramatic rise in potassium levels in transfusion (from 3.7mmol/L to 5.3mmol/L) than in non-transfusion patients (from 3.6mmol/L to 4.0mmol/L). Blood stored at 1° to 6°C decreases the rate of cellular metabolism and energy demand which allows blood to be stored for 35 days. This makes the sodium-potassium pump inoperative and consequently allows potassium ions to exit the cell and sodium ions to enter via the semipermeable membrane. It was demonstrated in critically ill patients that the sodium levels will revert to their normal levels within 24 hours after transfusion, whereas the potassium levels take about 4 days to stabilize, but such is not the case if the patient has developed hyperkalemic or hypernatremic condition before transfusion. The condition is exacerbated when the patient receives large volume of RBCs of whole blood. [18], [9]. The plasma level of potassium may increase by $0.5-1.0\text{mmol/L}$ per day of

refrigerator storage [19]. There is a notion that the total amount of extracellular potassium in a unit of blood stored for 35 days falls within 7mmol/L to 25mmol/L [20].

Table 1.2 and figure 1.2 show mean chloride values for both SBB and TR refrigeration. Chloride values for the former decreased from $82.15 \pm 1.67\text{mmol/L}$ for the first day to $75.97 \pm 2.14\text{mmol/L}$ for Day 35. The latter group recorded a mean chloride level of $89.94 \pm 3.42\text{mmol/L}$ on the first day and values decreased gradually across the period, eventually 76.82 ± 3.80 was observed for the last day. In both cases, the chloride levels decreased from Day 1 to Day 35. Chloride values of both groups are in line with the work of [12]. Who reported 75.93mmol/L and 72.19mmol/L as highest and lowest values for Days 1 and 35 respectively. Chloride is the major anion (negatively charged ion) found in the fluid outside of cells and in the blood. In stored whole blood, chloride levels were observed to decrease after two days of storage [21]. Table 1.3 shows mean HCO_3^- values for both SBB and TR refrigeration. In the former, it was observed that the HCO_3^- values decreased from $19.86 + 0.96\text{mmol/L}$ to $17.38 + 0.60\text{mmol/L}$. Bicarbonate, pH and chloride levels for both groups were generally observed to decrease with storage time, (figures 1.3, 1.4 and 1.2 respectively). This is in line with the work of [20] who stated that a fall in bicarbonate was observed in stored blood using CPDA-1 anticoagulant preservative [20]. Decrease in HCO_3^- may be due to reduction in CO_2 levels due to leakage from the bag. CO_2 produced from metabolism of glucose accumulate and is expected to diffuse through the containing material. The plastic material should be sufficiently permeable to CO_2 in order to maintain higher pH during storage. Currently the blood is stored in plastic bags made of polyvinyl chloride (PVC) with plasticizer, di-(2-ethylhexyl) phthalate (DEHP). It is known that DEHP leaches from plastic into plasma and cell membrane during storage and may be harmful to the patient on transfusion.

5. Conclusion

The results show that there were significant changes in the levels of all electrolytes and pH at different weeks of storage and the changes were more in the units stored in the traditional refrigerator and this could be clinically detrimental to the recipient on transfusion if adequate measures are not taken, particularly in massive transfusion and/or if the recipient already has an established clinical sequela that could exacerbate the condition.

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