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Comparative Study on Some Biochemical Parameters in Stored Whole Blood in Standard Blood Bank and Traditional Refrigerator

Authors

Obisike Uchechukwu Achor*, Brown Holy

Medical Laboratory Science Department, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

*Corresponding Author

Obisike Uchechukwu Achor

Medical Laboratory Department, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Nigeria, Email: *obisike.uchechukwu@ust.edu.ng* +2348035594669

Abstract

Whole blood or any of its components is stored for the purpose of future transfusion. To ensure therapeutic relevance of the product, strict adherence to instructions and procedures on the operation of a blood bank is crucial. Storage outside the stipulated temperatures could lead to biochemical changes that may reduce the therapeutic value. This study aims at evaluating the in vitro effect of storage on selected biochemical parameters (Na^+, K^+, Cl^-, HCO_3^-, Total protein, Albumin, Ca^{2+}, pH, Glucose and Hemoglobin concentration) in citrate phosphate dextrose adenine (CPDA-1) whole blood stored in a Standard Blood Bank (SBB) and compare values with those stored in a Traditional Refrigerator (TR). A total of 37 apparently healthy volunteer donor subjects were used for the study. Twenty donors donated 450mL of whole blood each into CPDA-1 blood bags and were stored in a standard blood bank, while 17 units were collected from 17 donors into the same type of anticoagulant/preservatives but instead stored in a traditional refrigerator. Both refrigerators and standard blood bank were allowed the same relatively stable power supply for 35 days. Five milliliters of blood was taken at intervals of 7 days (1, 7, 14, 21, 28, and 35) from each of the bags for both SBB and TR methods of refrigeration and analyzed for ten parameters. It was observed that for both SBB and TR, K^+ levels increased from the 1^{st} to the 35^{th} day. K^+ levels for TR were significantly higher than those of SBB for days 7 ($F=17.256$, $p= 0.000$), 14 ($F= 10.358$, $p= 0.000$), 21 ($F= 14.381$, $p= 0.000$), 28 ($F= 4.810$, $p= 0.000$) and 35 ($F=0.499$, $p=0.000$). This statistically shows that the rise in K^+ level was more in TR refrigeration. Plasma Na^+ levels generally decreased for both groups. Comparison showed that Na^+ values for SBB were significantly higher than those of TR refrigeration for Days 7 ($F= 1.684$, $p= 0.027$) and 14 ($F= 1.623$, $p= 0.009$). Bicarbonate, albumin, pH, chloride and glucose levels for both groups were observed to decrease with storage time. Significant decreases were observed for Cl^- and HCO_3^- for Day 7 ($F= 17.019$, $p= 0.000$ and $F= 0.404$, $p= 0.035$ respectively), Cl^- only for Day 14 and 21 ($F= 3.253$, $p= 0.000$ and $F= 2.112$, $p= 0.000$ respectively), albumin and glucose for Day 35 ($F= 2.541$, $p= 0.036$ and $F= 1.272$, $p= 0.039$ respectively) when mean values for SBB and TR were compared. Other parameters for the different days were not significant. Mean total protein levels for SBB and TR were significant for Days 7 ($F= 13.136$, $p= 0.011$); 14 ($F= 9.842$, $p= 0.011$); 21 ($F= 6.344$, $p= 0.015$) and 28 ($F= 8.137$, $p= 0.035$), while no significant difference was observed on the last day. The results show that there were significant changes in the levels of almost all ten parameters at different weeks of storage and the changes were more in the units stored in the traditional refrigerator and this could pose a very high health risk to the recipient on transfusion.

Key words: *Blood, blood bank, biochemical parameters, transfusion, refrigerator, CPDA-1.*

Introduction

Preservation and long term storage of whole blood or any of its products is needed to ensure a readily available, safe blood supply for transfusion medicine. 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? Similarly, what is the effect of storage on biochemical parameters? The latter has been answered extensively by studies on storage induced changes in stored blood.[1] reported thatthe administration of whole blood or any of its units containing cellular elements may pose many risks and potential unfavorable effects. This is basically due to the gradual decomposition of blood components and as a result of the bioaccumulation of products of the cellular metabolism, that is, anaerobic glycolysis, particularly when the components are not stored at the temperature range as required by regulatory bodies, the biochemical composition of the stored whole blood are bound to undergo bizarre changes. The changes are proportional to the storage time, temperature and other factors [1]. During storage, Red Blood Cells are metabolically active without any waste disposal system (that is, no renal system or liver), so the stored cells are essentially marinated in a pool of ever-increasing waste products (example, lactate). The obvious fact is that storage is a distinctly unnatural state in which blood cells are exposed somewhat a non-physiological condition for various periods of time. The overall issue splits into two major questions: [2] to what extent can stored blood cells maintain their functional integrity and therefore constitute an efficacious product? [3], and are there untoward effects of storage based changes that may result in medical consequences due to the accumulation toxic substances in the blood? Large volume of blood transfusion may attribute to changes in the patients plasma biochemical parameters and may therefore be related not only to the volume of blood products but also to storage duration and temperature. Other changes include a reduction in Red Blood Cell (RBC) deformability, altered RBC adhesiveness and agreeability, and a reduction in 2,3–diphosphoglycerate (2, 3 – DPG) and adenosine triphosphate (ATP), bioactive compounds with proinflamatory effects also accumulate in the storage medium. These changes reduce post transfusion viability of RBCs. The clinical effect beyond transfusion are uncertain, but a growing body of evidence suggest that the storage lesion may reduce tissue oxygen availability, have proinflammatory and immunomodutatory effects and influence morbidity and mortality [4]. Noncompliance with rules and regulation governing the storage of whole blood or any of its products can cause severe clinical consequences to the recipient, [5].

In most developing countries, where the required facilities for proper blood storage are scarcely used, 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 useReasons may not be unconnected with their inability to procure blood bank refrigerators as they are considered expensive and lack steady power supply. This prompted the need for an evaluation of likely biochemical changes 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. 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. This study evaluated the *in vitro* effect of storage on ten biochemical parameters (pH, Total protein, Na^+, K^+, Cl^-, HCO_3^-, Glucose, Total Calcium, Albumin and Hb concentration) in citrate phosphate dextrose adenine (CPDA-1) whole blood stored in a standard blood bank and to compare values of same with those stored in a traditional refrigerator.

Materials and Methods

Study Subjects and Design

The study 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 bags. These units were stored in a Standard Blood Bank (SBB) at BMSH, while the remaining seventeen (17) subjects also donated 450mls of whole blood each into bags with the same anticoagulant and stored in Traditional Refrigerator (TR), both refrigerators were allowed the same relatively stable power supply. The standard blood bank in this case is a blood bank refrigerator with a temperature display and armed with a refrigerator alarm with model number WDB-220.

Whole blood collection

The donations were collected into a closed set of sterile blood packs with Citrate Phosphate Dextrose Adenine anticoagulant/preservative. Approximately 450 ± 10% mL of donor blood was collected via aseptic venipuncture into the CPDA-1 blood bag. The donation was collected over 5–10 minutes. The appointment took around 40 minutes for each donor including registration and interview time, as well as rest and refreshment time after the donation has finished.

Experimental Analysis

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. Invariably, samples were taken at Day 1, Day 7, Day 14, Day 21, Day 28 and Day 35. Sample collection was done by gently swirling the pint of blood, draining the traces of blood in the line then collection of samples into bottles for the estimation. Plasma Electrolytes (Na^+, K^+, Cl^-, HCO_3^-) and pH were analyzed using an Automated Biochemistry Analyzer (Olympus AU400 Automated Chemistry Analyzer) while plasma Total protein, Albumin, Glucose, Total Calcium and Hemoglobin concentration were analyzed spectrophotometrically based on methods modified by Randox Diagnostics UK and under good laboratory practices as guided by [6]. Statistical tool used was Statistical Package for Social Sciences (SPSS) version 22.0.

Results

Mean ± Standard deviation, p and F values as well as significance levels for the ten biochemical parameters for both SBB and TR refrigeration are shown in the Tables 4.1-4.6 below.Table 4.1 shows no significant difference between the SBB and TRvalues form the baseline. Table 4.2 shows values for day 7, depicting that plasma K^+, Cl^-, $HCO3^-$ values for SBB were found to be significantly lower than those of TR while Na^+ and TP values for SBB refrigeration were found to be statistically higher than those of TR ($p<0.05$). Mean values for albumin, pH, Ca^{2+}, glucose and Hb were not significant statistically when compared. From table 4.3, plasma K^+ and Cl^- values for SBB were found to be significantly lower than those for TR refrigeration ($p<0.05$). Whereas, TP and Na^+ values for SBB recorded significantly higher values when compared with those for TR refrigeration. However, mean HCO_3^-, albumin, pH, Ca^{2+}, Glucose and Hb concentration were not significant. Table 4.4 shows that K^+ and Cl^- levels for SBB were observed to be significantly lower while Na^+ and TP levels where significantly higher than those of TR refrigeration. Values for Na^+, HCO_3^-, albumin, pH, Ca^{2+}, glucose and Hb when compared were not significant ($p<0.05$). Table 4.5 shows that plasma K^+ levels for SBB were significantly lower than those of TR, whereas, rather significantly higher levels of TP were observed for SBB when compared with values for TR refrigeration. The remaining eight parameters were not significant. Table 4.6 shows that K^+ values for SBB were significantly lower than those for TR refrigeration. Whilst, albumin and Glucose levels for SBB had

significantly higher values than those of TR refrigeration. Mean values for Na^+, Cl^-, HCO_3^-, total protein, pH, Ca^{2+} and Hb concentration were observed to be non-significant.

Table 4.1 Comparing biochemical changes at day- 1 in SBB and TR Refrigeration.

	N	*Na+*	*K+*	*Cl-*	*HCO3-*	*TP*	*Alb*	*pH*	*Ca2+*	*Glu*	*Hb*
SBB	20	138.2	2.89	82.15	19.25	79.80	38.48	6.92	2.12	14.79	14.09
TR	17	136.6	2.52	89.94	19.86	77.24	38.27	6.84	2.08	15.11	13.77

Table 4.2 Comparing biochemical changes at day-7 in SBB and TR Refrigeration.

	N	*Na^+*	*K^+*	*Cl-*	*HCO3-*	*TP*	*Alb*	*pH*	*Ca^{2+}*	*Glu*	*Hb*
SBB	20	136.8	3.10	80.36	18.90	78.83	37.83	6.49	2.13	13.97	13.70
TR	17	136.0	5.02	86.14	19.55	76.29	37.61	6.59	2.17	14.38	13.74
p-value	-	0.027	0.000	0.000	0.036	0.011	0.647	0.387	0.701	0.232	0.893
F value	-	1.684	17.256	17.019	0.404	13.136	0.007	0.061	4.272	0.180	0.281
Remark	-	S	S	S	S	S	NS	NS	NS	NS	NS

Table 4.3 Comparing biochemical changes at day- 14 in SBB and TR Refrigeration.

	N	*Na^+*	*K^+*	*Cl^-*	*HCO_3^-*	*TP*	*Alb*	*pH*	*Ca^{2+}*	*Glu*	*Hb*
SBB	20	136.2	3.56	79.86	18.61	78.54	37.60	6.38	2.09	13.48	14.01
TR	17	135.4	7.40	85.15	19.01	76.01	37.15	6.46	2.11	13.56	13.84
p-value	-	0.009	0.000	0.000	0.098	0.011	0.325	0.491	0.809	0.778	0.480
F value	-	1.623	10.358	3.253	2.293	9.842	2.680	0.053	0.067	0.002	0.000
Remark	-	S	S	S	NS	S	NS	NS	NS	NS	NS

Table 4.4 Comparing biochemical changes at day-21 in SBB and TR Refrigeration.

	N	Na+	K+	Cl-	HCO3-	TP	Alb	pH	Ca2+	Glu	Hb
SBB	20	135.4	3.92	78.45	18.39	78.75	36.86	6.25	2.09	12.50	14.01
TR	17	134.8	8.32	83.72	18.86	76.29	36.29	6.37	2.07	12.35	13.96
p-value	-	0.080	0.000	0.000	0.104	0.015	0.272	0.266	0.798	0.704	0.344
F value	-	0.057	14.381	2.112	0.087	6.344	2.693	0.176	0.466	4.621	0.281
Remark	-	NS	S	S	NS	S	NS	NS	NS	NS	NS

Table 4.5 Comparing biochemical changes at day-28 in SBB and TR Refrigeration.

	N	Na+	K+	Cl-	HCO3-	TP	Alb	pH	Ca2+	Glu	Hb
SBB	20	134.7	4.59	77.10	17.96	78.98	36.18	6.16	2.33	11.39	14.35
TR	17	134.2	8.91	78.16	17.79	76.62	35.32	6.22	2.18	11.51	14.13
p-value	-	0.259	0.000	0.331	0.499	0.035	0.156	0.466	0.288	0.758	0.355
F value	-	0.899	0.499	3.119	0.044	6.964	2.541	2.727	0.085	1.272	0.049
Remark	-	NS	S	NS	NS	S	NS	NS	NS	NS	NS

Table 4.6 Comparing biochemical changes at day-35 in SBB and TR Refrigeration

	N	Na^+	K^+	Cl^-	HCO_3^-	TP	Alb	pH	Ca^{2+}	Glu	Hb
SBB	20	132.5	5.42	75.97	17.53	79.27	35.70	6.07	2.31	11.06	14.51
TR	17	132.0	9.48	76.82	17.38	77.55	34.42	6.16	2.21	9.88	14.65
p-value	-	0.161	0.000	0.394	0.441	0.051	0.036	0.179	0.320	0.039	0.537
F value	-	0.700	0.499	3.119	0.044	6.694	2.541	2.727	0.085	1.272	0.049
Remark	-	NS	S	NS	NS	NS	S	NS	NS	S	NS

SBB = Standard Blood Bank S = Significant
TR = Traditional Refrigerator NS = Not Significant

Discussion

Evaluation of changes in plasma levels of sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), total protein, albumin, pH, calcium (Ca^{2+}), glucose and hemoglobin (Hb) levels in Citrate Phosphate Dextrose Adenine stored whole blood, comparing values from units stored in a Standard Blood Bank (SBB) and Traditional Refrigerator (TR) had not been reported.

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. In the 20 units of CPDA-1 anticoagulant/preservative packaged whole blood stored in a standard blood bank, the mean Na^+ level on the first day was observed to be 138 ± 0.22mmol/L. This serves as the baseline value for Na^+ levels and was used to compare with Na^+ values of subsequent days (7, 14, 21, 28 and 35) to ascertain the level of statistical significance. The value is about one unit higher than the mean Na^+ (137.38mmo/L) reported by [7]. Although they used ten samples and observed a non-significant relationship between the mean values for Day 1 and that of other days. 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 ± 1.32 mmol/L) was recorded on the last day (Day 35). This indicates that Na^+ levels declined as the number of days of storage increased. [7] also recorded a decline in mean Na^+ levels (137.38 - 129.44mmol/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 ± 1.14mmo/L for Day 1. This is one unit lower than that reported by [7]. 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 significantly decreased at $p<0.001$. Radovan et al 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 137.0mmo/L when whole blood collected on the first day was transfused [1]. It has been observed that following blood transfusion of stored blood, complications such as hyperkalemia, hyponatremia and citrate toxicity among other conditions do occur. When mean values for SBB and TR for sodium were compared, significant decreases were observed at days 7 (F= 1.684, p= 0.027) and 14 (F= 1.623, p= 0.009). 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^{o}C. No study has reported changes in Na^+ levels in CPDA-1 anticoagulant blood stored in a traditional refrigerator. However, Lee et al reported extreme reduction in Na^+ levels when the temperature for RBC storage was allowed to fluctuate between 5^{o}C above the AABB designated temperature (2-6^{o}C) for blood storage [8].

This study has also shown that there was a tremendous increase in K^+ levels from Day 1 to Day 35 for both groups. Mean K^+ value of 5.42 ± 1.10mmo/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 ± 1.72mmo/L were respectively observed for the last and first day for TR refrigeration. K^+ values were observed to increase with the time of storage. This is in agreement with values obtained by [7]. In both groups, mean K^+ values were significant at all levels. 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, p= 0.000), Day 14 (F= 10.358, p= 0.000), Day 21 (F= 14.381, p= 0.000), Day 28 (F= 4.810, p= 0.000) and Day 35 (F=0.499, p=0.000).This shows 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 [9] but the kidney is expected to manage it.). However, in severe kidney disease even small amount of K^+ fluctuation can be dangerous and relatively fresh or washed RBCs are indicated.Hypokalaemia and hyperkalaemia has been seen as problem for some hospitalized patients [10] with hyperkalaemia being implicated for complications of massive blood transfusion [11]-[13]. 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 Radovan et al. According to them, K^+ values increased

tremendously in both cases, recording a mean *in vivo* K^+ values greater than 5.5mmol/l [1].

The increase in plasma potassium levels which are also in accordance with those reported by [14]. Their study focused on hyperkalemia (>5.5mmol/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 [15]. The plasma level of potassium may increase by 0.5-1.0mmol/L per day of refrigerator storage [16]. There is a notion that the total amount of extracellular potassium in a unit of blood stored for 35days falls within 7mmol/L to 25mmol/L [17].

The tables above also show mean chloride values for both SBB and TR refrigeration. Chloride values for the former decreased from 82.15 ± 1.67mmol/L for the first day to 75.97 ± 2.14mmol/L for Day 35. The latter group recorded a mean chloride level of 89.94 ± 3.42mmol/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 [7] 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 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 [17].

Also shown in the table are 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 + 0.96mmol/L to 17.38 + 0.60mmol/L. This is in line with the work of Letham et al who stated that a fall in bicarbonate was observed in stored blood using CPDA-1 anticoagulant preservative [17]. Decrease in HCO_3^- may be due to reduction in CO_2 levels due to leakage from the bag. CO_2 produced form 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.

Mean total protein values for Day 1 were observed to be 79.30 ± 0.86g/dl and 79.27 ± 1.76g/dl for Day 35. These values were for the SBB refrigeration, while for TR refrigeration, mean total protein levels of 77.24 ± 4.17g/dl and 77.55 ± 3.28g/dl were observed for Day 1 and Day 35 respectively. Slight increase in total protein levels may be due to lysis resulting to leaking of haemoglobin into the plasma. The release of hydrogen peroxidase and proteases by the leucocytes present in unfiltered blood may cause lysis of red blood cells during the storage period. Signs of haemolysis in the plasma or suspending fluid may suggest that the red blood cells have been either ruptured or it may be due to the loss of membrane-bound haemoglobin in microvesicles found on the cell's surface of intact cells.

Comparison between SBB and TR for total protein level for Days 1 and 35 were not significant, while at Days 7 (F= 13.136, p= 0.011);, 14 (F= 9.842, p= 0.011);, 21 (F= 6.344, p= 0.015) and 28 (F= 8.137,

p= 0.035) TP values for SBB were significantly higher than those for TR refrigeration.

Mean albumin levels for Day 1 was observed to be 38.48 ± 1.37g/dl and 35.70 ± 1.99g/dl for Day 35 in SBB refrigeration, while TR refrigeration recorded a mean albumin levels of 38.27 ± 1.65g/dl and 34.42 ± 1.47g/dl for the first and last days respectively. Also, for SBB, and TR refrigeration, there was significant decrease between both groups in Day 35 while other days recorded no statistical significance.

Mean pH levels for SBB in the first day was 6.92 ± 0.21, as against 6.84 ± 0.17 for TR. The last day recorded a mean pH value of 6.07 ± 059 and 6.16 ± 0.28 for SBB and TR respectively. pH levels decreased in both groups from the first day to the last day. Comparison between SBB and TR for pH shows no significant difference at all levels (Day 1 to Day 35).

Mean Ca^{2+} levels for Day 1 and Day 35 in the SBB refrigeration recorded values of 2.12 ± 0.33mmol/L and 2.31 ± 0.31mmol/L as against 2.08 ± 0.26 and 2.21 ± 0.28 for TR refrigeration in the first and last days respectively. No significant difference was observed throughout when Ca^{2+} values for both SBB and TR refrigeration were compared. This means that storage in both groups probably does not have effect on the levels of Ca^{2+}.

The tables also show that mean glucose levels for Day 1 and 35 for SBB are 14.79 ± 1.27mmol/L and 11.06 ± 2.16mmol/L, while TR refrigeration recorded mean glucose values of 15.11 ± 1.26mmol/L and 9.88 ± 0.76mmol/L for Day 1 and Day 35. It was observed that the glucose levels for both SBB and TR in all the days decreased as the days of storage increased. Comparison between glucose levels for SBB and TR showed that the glucose level for SBB were significantly higher than those of TR refrigeration on Day 35 (F= 1.272, p= 0.039), while in other days no significant difference was observed. The decrease of ATP concentration during storage causes the cellular reactions requiring energy, for example, phospholipid membrane distribution, active transport, and antioxidant reactions, to also decrease. The decrease in glucose level in this study is in line with the work of [18] that stated that it has been indicated that there is a 60% decrease in ATP levels after more than 5 weeks of storage [18]. The continuous reduction in ATP concentrations and acidification results in irreversible shape alteration of the RBC as echinocytic surface protrusions appear. The phospholipid bilayer loses its asymmetry and the shedding of microvesicles occur [19].

Mean Haemoglobin (Hb) levels for the first day was observed to be 14.09 ± 0.73g/dl as against 13.77 ± 0.45g/dl for TR refrigeration. Day 35 recorded mean Hb levels for both SBB and TR refrigeration as 14.5 ± 0.77g/dl and 14.65 + 065g/dl respectively. When compared, Hb values for SBB and TR for all days were not significant. This shows that there is probably no significant storage effect on the levels of Hb in the two groups in CPDA-1 stored whole blood.

Conclusion

This study has recorded increase in *plasma* potassium levels in whole blood as the days of storage increased. The rate of increase being significantly higher in units stored in the traditional refrigerator. Transfusion of such blood into a patient who already has complications due to an underlying hyperkalemic condition may exacerbate the clinical condition. Death may result due to hyperkalemic shock, particularly if large volume of whole blood is transfused as. This study has also shown that there is a decrease in plasma sodium, chloride, bicarbonate, and albumin and pH levels in both SBB and TR methods of refrigeration. As stated earlier, the decrease in plasma sodium levels were observed to be more in the TR refrigeration. Decreases in plasma bicarbonate, total protein, glucose and albumin were also higher in the TR refrigeration, but at different weeks of storage. Transfusion of whole blood with storage induced increase/decrease in biochemical components could lead to severe clinical consequences. In all, the lower temperature at standard condition keeps the rate of glycolysis and other cellular activities at lower limit and minimizes the proliferation of bacteria that might have entered the blood unit during venipuncture or from the atmosphere. The

rate of diffusion of electrolytes (particularly sodium and potassium) across the cell membrane is also less at lower temperature. Increases in storage temperature can lead to rise in many metabolic activities leading to extreme leakage of substances from cells.

Following the outcome of this study, it is recommended that regulatory agencies should as a matter of urgency perform a thorough assessment of all medical laboratories blood banking services to ensure strict adherence to the code of practice of the professional body, particularly in the operation of blood bank. Disciplinary measures should be taken on defaulters.

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