Original Research Article

Serial assessment of storage induced changes in electrolytes, pH, glucose and cell counts of CPDA-1 stored whole blood.

Anbuselvi Mattuvar Kuzhali S[1], Balasubramanian Kabali[2], AnjanaR[3], Manigandan S[4], Karan S[5], Rajkumar[6]

[1]Department of Physiology, [2]Retd. Director, Institute of Physiology and Experimental Medicine, Madras Medical College, [3,4,5]Final year MBBS student, [6]Professor and HOD, Department of Blood bank, Stanley Medical College, All affiliated to The Tamil Nadu Dr. MGR Medical University, Chennai, India

Abstract

Background: Most donor blood units for transfusion purposes are stored in Citrate Phosphate Dextrose Adenine-1 (CPDA-1) at blood-banks until used. Despite improvements made in blood storage attempts, CPDA-1 may still cause morphological and degenerative changes in red blood cells (RBCs) if storage is prolonged. The alteration in pH and electrolyte levels will alter the hemodynamics and cause detrimental effects after transfusion. **Aim:** To estimate the serial changes in the biochemical and haematological parameters of CPDA-1 stored whole blood for 7 days. **Materials and Methods:** After donor screening, 20 units of CPDA-1 stored whole blood was tested for the biochemical parameters like pH, electrolytes, glucose and blood cell counts. pH meter and fully automated analyzer was used. Data are evaluated and analyzed using SPSS 24.0. **Results:** A significant decrease in plasma concentrations of sodium (consistent), glucose (>day5), pH (consistent) and increase in potassium concentration (>day3) was observed ($p<0.000$). A significant positive correlation was found between the mean pH levels with mean potassium and glucose concentrations ($p<0.0001$). A significant concomitant decrease in both RBC and WBC was observed. **Conclusion:** Our study revealed that the more acidic pH and rapid rise in K^+ in stored whole blood after 3 days. The biochemical changes were within acceptable limits of safety and can provide more utility if used within 5days of storage.

Keywords: CPDA-1 stored whole blood, potassium, pH, duration

Corresponding author
Dr. Anbuselvi Mattuvar Kuzhali S, Assistant Professor, Department of Physiology, Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai
Telephone: + 9940804760, Email: dranbuselvimk@gmail.com

Introduction

Blood collected for transfusion is routinely stored for 33 to 41 days, depending on the preservation solution, yet the Food and Drug Administration allow red cells to be stored up to 42 days.[1] This time limit is determined mainly by the shelf life of viable RBCs in blood bags and by changes ATP and 2,3-DPG during storage.[1a] In 4°C to 6°C liquid storage, the blood cells undergo progressive structural and functional changes reducing its viability over time.[2] Blood banks in any tertiary health care setup have to ensure a readily available, safe blood supply for transfusion.

In recent years, the efficacy of stored blood and blood products is an escalating debate. However, these had been well documented in several invitro studies.[3-6]In resource limited settings, where the facilities necessary to maintain a proper storage conditions are scarce, whole blood or blood products that are collected and stored under such settings

may not meet its therapeutic or clinical purpose and might be of clinical detriment to the recipient.

Studies had well documented that concentration of (purine nucleoside precursor) present in the CPDA-1solution is upto the Food and Drug Administration (FDA) minimum standard of 75% and it helps to prevent the loss of red cell viability to some extent.[7,8] Whether it is whole blood or packed RBCs stored in various preservative solutions, the post-transfusion loss of red cell viability is mainly related to excessive glucose utilization, anaerobic glycolysis and subsequent decrease in erythrocyte's ATP (adenosine-5'-tnphosphate) levels.[7,9-11]The concomitant biochemical and hematologic changes resulting from this energy depletion is referred to as RBC storage lesions.

Over time, the membrane of RBCs become less deformable and sensitive to osmotic changes.[12-15]The loss of phospholipids and cholesterol from membrane of the blood cells in stored blood is mainly due to the process of vesicular budding from the surface of cell membrane.[16,17] Lactic acid accumulates intracellularly and potassium accumulates extracellularly.[15,18-21] The utility of oxygen decreases due to loss of 2,3-diphosphoglycerate (2,3-DPG).[20] Except for the membrane deformability and the cell structural changes, most of the storage lesions are reversible after transfusion. However, it is important to identify the time point of the reversible changes since their correction may improve the efficacy/functional utility of stored blood in an already metabolically compromised patients.[22]

Our work is intended to identify the time point at which the changes in cell counts and biochemical parameters of CPDA-1 stored whole blood is drastically varied.

The objectives of this study were

1. To estimate the changes in glucose, pH, sodium, potassium and blood cell counts over a period of 7 days in CPDA-1 stored whole blood.
2. To correlate the changes in pH with the changes in glucose and potassium over a period of 7 days in CPDA-1 stored whole blood.

Materials and Methods

(i) Place of study and subjects: The study protocol was cleared by the Ethics committee of the Institute. The study was conducted in the Department of Blood bank, Stanley Medical College in collaboration with the Department of Physiology, Stanley Medical College. After taking consent from donors, they were screened as per regulations of Drugs and Cosmetics Rules, Govt. of India.[6a] The donors were 20 healthy male volunteers of mean age of 28.2 ±8.4 with their corresponding blood groups noted to be 6O+, 1O-, 4A+, 2B-, 5B+ and 2AB+. Prior to collection, all the donors were tested negative for blood transmissible pathogens: HCV, HbsAg, Syphilis and HIV 1 & 2.

(ii) Blood collection and storage: Procedure followed for blood collection and storage is described.

Blood bags: Blood bags (for adults) with capacity of 450 ml ± 10% (Agarry) that contains CPDA-1 anticoagulant was used. Citrate phosphate dextrose adenine solution (developed in 1968) has been proved to help storing whole-blood for 5 weeks.[23] Most of the blood collection bags (adult) contain 63 ml CPDA anticoagulant to ensure the viability of blood cells stored for 28-35 days at 2-8°C.[23a]

Collection procedure Phlebotomy: Blood was collected from each donor by phlebotomy as per standard procedure with adequate safety precautions to avoid infections and contamination.[23a] Protective gloves were worn during collection. Under sterile dry conditions, about 350ml of fresh whole blood was drawn into plastic blood bags (J. Mitra Industries Pvt. Ltd. Haryana, India) with Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant.6b Safety measures were followed to discard lancets, needles and syringes to avoid injury. The collected blood bags were carefully placed on the quarantine shelf in the blood bank refrigerator that maintains temperature at 2-6°C.

Quality control parameters: The initial quality check of fresh whole blood was performed (visual examination of bag, volume and haematocrit) as per Directorate General Health Services criteria (DGHS) as described in Table.I.6c

(iii) Biochemical parameters: Twenty units of whole blood collected with anticoagulant CPDA1 (citrate phosphate dextrose adenine) were tested serially for the supernatant plasma sodium, potassium, pH,

glucose and blood cell count for 7 days. The supernatant plasma sodium, plasma potassium, plasma glucose level and cell counts were estimated by fully automated analyzer(Cosmos Biomedical Ltd, Japan) and the analyzer enumerates 20 parameters with 3-part differentiation of WBC. pH was measured by using digital pH meter (spectrophotometer, Indian Laboratory and Scientific Instrument Ltd., Delhi). The procedures were followed as per the laboratory standard operating manual. All the above mentioned biochemical parameters were estimated daily from day 1 till day 7 of storage.

Statistical analysis: The correlations between levels of the grouping variable is tested using Repeated measure ANOVA and was measured over serial time points within subjects. The extra assumption of within-subjects effects was tested by Mauchly's test of Sphericity and corrected with Greenhouse-Geisser or Huyhn-Feldt. To assess the correlation between biochemical parameters during storage, Pearson's correlations coefficient (r) was applied. $p<0.05$ was considered statistically significant. All analyses were performed with the software package SPSS Version 24.0 for windows (USA).

Results

Twenty units of CPDA-1 stored fresh whole blood was collected and serially tested at 24hour time intervals for 7days to identify the exact time point at which the changes in pH, glucose, electrolytes and RBC/WBC count becomes compromised due to storage. The quality control parameters (blood volume and haematocrit) of all the twenty units of collected CPDA-1 whole blood included in the study was confirmed to the standards established by Directorate General Health Services as mentioned in Table 1.[6c]

(i) Effect of storage on biochemical parameters: While analyzing the changes that occurred in the biochemical parameters of CPDA-1 stored whole blood from Day 1 to Day 7, we found a significant decrease in the mean sodium concentration, mean glucose concentration and the mean pH values (Table 2) whereas a consistent and significant rise was observed in the mean potassium (K+) concentration ($p<0.00$). There was a steady decrease in sodium concentration. But, the rise in K+ concentration was within normal limits till day3, after which significantly high levels was observed till day7. This implies that fresh whole blood stored for ≤3 days must for transfusion to critically ill patients or patients with chronic renal failure to prevent detrimental effect in the recipients.

The decrease in glucose concentration doubled on day6 and afterwards as shown in Table.2. However, the mean pH of the CPDA-1 stored whole blood showed a significant acidic value ($p<0.000$). A significant positive correlation was found between the mean pH and mean plasma glucose levels (r^2= 0.968; p=0.002) and between the mean pH and mean potassium levels (r^2= 0.844; p=0.0001). The variances of the difference between successive variable levels was found to be equal by Mauchly's test and Greenhouse-Geisser or Huyhn-Feldt corrections were adjusted as mentioned in Table 3

(ii) Effect of storage on blood cell counts: At the end of day 7, the mean values of the RBCs and WBCs showed a highly significant decrease as depicted in Figure 1 & Figure 2. While observing the daily proportion of decrease in cell counts between RBC:WBC, it was found to be 1.6%: 1.1% on day2, 1.8%: 1.4% on day3, 2.5%: 2.1% on day4, 3.7%: 2.7% on day5, 4.1%: 3.5% on day6 and 5.4% : 4.4 % on day7. Here we could see that the reduction% (calculated by [(d1-d2/d2)*100] for day2, same formula used till day7] doubled on day 5 and the cell count after 5th day indicates that their destruction is faster. Focussing the amount of decrease in WBCs, the amount of destruction of RBCs for the same day seems to be more. The probable reason could be the granulolysis of WBCs release few bioreactive substances like cytokine, histamine etc., which cause lysis of RBCs and other cells.

Discussion

Serial assessment of the changes in the levels of sodium, potassium, glucose, pH, RBC count and WBC count was done in twenty units of CPDA-1 stored whole blood collected from the blood bank. The collected whole blood was stored in blood bags with CPDA-1 anticoagulant. This anticoagulant contains citrate that prevents coagulation (by chelating or binding to ionized calcium), dextrose the predominant energy substrate for the blood cells, phosphate lowers acidity to maintain the pH of blood (acts as buffer, have a higher concentration of 2,3 DPG and red cell phosphate) and adenine that

maintains a high ATP level in RBCs (post-transfusion viability of RBCs maintained by adenine).[23-25]

(i) Effect of storage on glucose: Our study reported a significant decrease in glucose levels from day1 to day7. In ex-vivo conditions of CPDA-1 stored whole blood, the actual glucose concentration available in stored blood bags is less. Glucose is the main source of energy for red cell metabolism via glycolytic pathway.[7] However, in blood bags the energy is utilized from dextrose a component of CPDA-1.[8] When the storage duration increases, there happens a shift in RBC metabolism to anaerobic that induce changes in ATP generation. Over a period of time, there is a concomitant ATP (adenosine triphosphate) depletion and decrease in red cell viability.[9-11] Studies quote that when RBCs were suspended in SAGM (additive solution composed of saline, adenine, glucose and mannitol), the shelf life of RBCs prolonged mainly because of the fact that an adequate concentration of glucose was maintained by the 900 mg dextrose added per 100 ml of SAGM solution.[26]

(ii) Effect of storage on pH: During storage, increased concentration of lactic acid due to anaerobic glycolysis in red cells may have caused a decrease in pH as observed in this study. pH, a major determinant of RBC metabolism during storage. The threshold level or the lower limit of pH below which the ATP generation by red cells decreases subsequently was 6.2 as estimated by ploting the pH curve of many samples of stored blood in previous studies.[27] Though ATP measurement was not done in the present study, at day 1 pH was within normal range which decreased to 6.76 at the end of 7th day which was above the lower limit of pH (threshold of 6.2). This satisfies the fact that ATP generation would most likely be persisting in all the CPDA-1 stored whole blood preparations till day7. The possible explanation for the rapid reduction in ATP of CPDA-1 stored whole blood may be due to the lesser fractions of adenine and other nutrients present in CPDA-1 for ATP generation.[8-11]

(iii) Effect of storage on potassium and sodium: Our study has reported an increased K+ concentration within 3 days and continued subsequently showing a significant rise in K+ concentration (11.7±1.76) on day 7 of storage which was similar to other studies.[28,29] The only factor which affects the post transfusion very intensely is the serum potassium levels where the change in potassium levels were independent of the recipient's conditions of metabolic acidosis or renal failure or blood volume. This might be due to slow leakage of K+ from cells influenced by the temperature, alterations in cellular characteristics, pH and changes in the storage medium.

The leakiness of the cell membrane to K^+ in CPDA-1 stored blood might be due to:

1. inhibition or reduced function of sodium-potassium ATPase pump at less than 5°C with resultant increase in extracellular release of K+(hyperkalemia) and entry of Na+ ions into RBCs (hyponatremia) as observed in the present study.[30,31] Adias *et al.* also observed hyperkalemia in their study without any significant change in Na+.[17]To stabilize the pump with more ATP and the pump enhancers has no effect.[30]
2. As a counter-exchanger process, acidosis results in hyperkalemia and alkalosis results in hypokalemia.[32]For every 0.1 unit of pH change, the concomitant change in the serum potassium level was 0.4 mmol/L. This can be prevented by using potassium adsorption filters prior to transfusion or by transfusing blood within 0-3 days of storage.[31]
3. It was also stated that gamma irradiation of RBCs in blood bags for sterilization purpose can also damage the membrane integrity.[32]
4. The acidic pH, depletion of ATP, reduced glycolytic activity and increase in cellular density may be the potential reasons for the alterations in membrane permeability.

In a study by Strauss, the supernatant plasma level of potassium increased to 50 meq/litre at the 42nd day of stored red blood cells.[33]The bioavailability of K+ (ionic K+ in the volume of extracellular fluid) is very low for small volume(neonatal) transfusions.[33-36] However, the permitted or assumed the K+ concentration of CPDA-1 RBC with 70% haematocrit at 35th day of storage has been estimated to be 70-80 meq/litre which is higher than in stored CPDA-1 whole blood.[33] This brings the choice of CPDA-1 stored whole blood as a better clinical utility for neonatal transfusions. Due to a higher K content of stored blood, blood <5 days old is recommended by Ono et al. for neonatal exchange and top-up transfusion.[33-36]

The measured pre and post transfusion potassium levels mostly affect patients in critical care unit,

those who require more transfusions as in cardio pulmonary bypass and neonates who require exchange transfusions.[29,37]These complications from hyperkalemia may be prevented by (i)washing stored blood RBCs before transfusion, (ii) using freshly stored blood of <24hrs for massive transfusions; (iii) pretreatment of stored blood with insulin to redistribute the extracellular potassium and (iv) use of leucodepleted stored blood for any transfusion. Post transfusion potassium value above 5.5meq/l may be associated with following symptoms such as malaise, palpitations, muscle weakness and mild hyperventilation.

Latham *et al*. and Bailey *et al.* also observed a decline in concentration of plasma glucose, pH and increase in potassium concentration with storage of whole blood.[34]There exists a positive correlation between haemoglobin and potassium levels as well as between glucose and pH.[37]

(iv) Effect of storage on red cell and white cell count: A steady significant decrease in RBC and WBC counts was observed when their mean values were compared from day 1 to day 7. In conditions of storage, the granulolysis of WBCs release few bioreactive substances (histamine, hydrolases, cytokines and proteins) that might alter or induce physical/metabolic changes in other blood cells such as early senescence, membrane reticulation, vesiculation, alteration of cytoskeleton, loss of 2,3-DPG, enzymatic desilylation, attachment of phosphatidyl serine to cell surface, decrease in cell size and increased cellular density.[33,34,38,39]In response to these changes, hemolysis (major causative factor for hyperkalemia) and lysis of other blood cells occurs as observed in our study.[38]However, it can be acceptable for clinical utility provided when other hematological parameters appear to be stable during a specific time period.

LDH levels best reflects the degree of hemolysis.[40] In our study the levels of LDH were not tested. The degree of haemolysis was observed to be <0.8% in leukoreduced SAGM RBC due to the presence of mannitol (membrane stabilizer).[40,41] This emphasize the need for membrane stabilizers in the additive solutions of stored whole blood or red cells. Castro et al. has also identified total hemoglobin concentration, bilirubin level, lactate dehydrogenase, and the arginine:ornithine ratio to be markers of hemolysis.[42]

Limitations of the study: The levels of adenine concentrations, ATP, 2,3,-DPG, lactate and other hematological indices would have been a supportive evidence for this study. Another limitation is the sample size. These results will be extrapolated in future studies with larger samples and assessment of parameters till 42days

Conclusion

Inspite of storing blood with CPDA, the storage duration has a negative impact on the biochemical composition of the stored whole blood. The present study substantiates a fall in pH, glucose, sodium, red cell and white cell count except potassium in CPDA-1 stored whole blood. A drastic increase in potassium levels after day3 and faster reduction% of red cell and white cell counts after day5 evidenced in this study suggests to use CPDA-1 stored whole blood less than 3-4days for critically ill patients whereas CPDA-1 stored whole blood less than 7days can be used for normal transfusions.

While transfusion of blood with such characteristics should not pose any major problems, we believe that clinicians, transfusion services, and staff in blood banks should be aware of the nature of the storage lesions to aid in the selection of appropriate blood for transfusion. An in-vivo pilot study needs to be carried out in all tertiary health care setup to confirm the post-transfusion utility as well as safety.

Acknowledgement: We thank Dr. Rajkumar, Professor and HOD of blood bank for helping and guiding us to do this study. Our thanks to the volunteers and all the Staff in blood bank for helping us to take samples at definite time points. Our sincere thanks to Dr. M. Saravanan, Professor and HOD of Biochemistry for guiding us in testing the samples.

Conflicts of interest: Nil.

References

1. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med. 2008; 358:1229-39.

2. Bonaventura J. Clinical implications of the loss of vasoactive nitric oxide during red blood cell storage. ProcNatlAcadSci U S A. 2007; 104:19165-66.
3. Bennett–Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, et al. Evolution of adverse changes in stored RBCs. PNAS. 2007; 104:7063-68.
4. Purdy FR,Tweeddale MG, Merrick PM. RBC storage duration. Can J Anaesth. 1997; 44:1256–61.
5. Zallen G, Offner PJ, Moore EE. Age of transfused blood is an independent risk factor for post injury multiple organ failure. Am J Surg. 1999; 178:570–72
6. Leal-Noval SR, Jara-Lopez I, Garcia-Garmendia JL, Marin-Niebla A, Herruzo-Aviles A, Camacho- Larana P, Loscertales J. RBC storage duration and morbidity and mortality rates after transfusion. Anesthesiology.2003; 98:815–22.

6a. Malik V. Drugs and Cosmetic Act, 1940. 16th ed. New Delhi: Eastern Book Company; 2003.p. 279-303.

6b. Brecher ME, ed. Preparation of red blood cell, Method 6.4. AABB, Technical manual. Bethesda, Maryland. 15th ed. USA: American Association of Blood Bank; 2005. p. 804-6.

6c. Saran RK.Transfusionmedicine, Technical Manual. 2nd ed. New Delhi, India: Directorate General of Health Services (DGHS), Ministry of Health and Family Welfare, Government of India; 2003. p. 353-4.

7. Kreuger A, Akerblom O, Hogman CF. A clinical evaluation of citrate-phosphate-dextrose-adenine blood.Vox Sang. 1975; 29:81-9.
8. Beutler E, Wood C. The storage of hard-packed red blood cells in citrate-phosphate-dextrose (CPD) and CPD-adenine (CPDAI).Blood.1979; 54:280-84.
9. Akerblom O, Kreuger A. Studies on citrate-phosphate-dextrose (CPD) blood supplemented with adenine. Vox Sang. 1975; 29:90- 100.
10. Simon ER, Chapman RG, Finch CA. Adenine in red cell preservation. J Clin Invest. 1962; 41:351-59.
11. Wood L, Beutler E. The viability of human blood stored in phosphate adenine media. TRANSFUSION.1967; 7:401-08.
12. Haradin AR, Weed RI, Reed CF. Changes in physical properties of stored erythrocytes: Relationship to survival in vivo. TRANSFUSION.1969; 9:229-37.
13. Hogman CF, de Verdier CH, Ericson A. Studies on the mechanism of human red cell loss of viability during storage at +4"C in vitro. Vox Sang 1985; 48:257-68.
14. La Celle PL. Alteration of deformability of the erythrocyte membrane in stored blood. TRANSFUSION.1969; 9:238-45.
15. Beutler E, Kuhl W, West C. The osmotic fragility of erythrocytes after prolonged liquid storage and after reinfusion.Blood.1982; 59:1141-47.
16. Lutz HU, Liu SC, Palek J. Release of spectrin-free vesicles from human erythrocytes during ATP depletion: I.Characterization of spectrin-free vesicles. J Cell Biol. 1977; 73:548-60.
17. Rumsby MG, Trotter J, Allan D. Recovery of membrane micro-vesicles from human erythrocytes stored for transfusion:A mechanism for the erythrocyte discocyte-to-spherocyte shape transformation. Biochem SOC Trans. 1977; 5:126-28.
18. Moore GL, Peck CC, Sohmer PR. Some properties of blood stored in anticoagulant CPDA-I solution: A brief summary. TRANSFUSION.1981; 21:135-37.
19. Latham JT Jr, Bove JR, Weirich FL. Chemical and hematologic changes in stored CPDA- 1 blood. TRANSFUSION.1982; 22:158-59.
20. Valeri CR, Valeri DA, Gray A. Viability and function of red blood cell concentrates stored at 4°C for 35 days in CPDA-I, CPDA-2, or CPDA-3. TRANSFUSION.1982; 22:210-216.
21. Moroff G, Dende D. Characterization of biochemical changes occurring during storage of red cells:Comparative studies with

CPD and CPDA- 1 anticoagulant-preservative solutions. TRANSFUSION.1983; 23:484-89.
22. Sohmer PR, Scott RL. Massive transfusion. Clin Lab Med. 1982; 2:21-34.
23. Shields CE. Effect of adenine on stored erythrocytes evaluated by autologous and homologous transfusions. TRANSFUSION. 1969; 9:115-19.
24. AuBuchon JP, Birkmeyer JD, Busch MP. Safety of the blood supply in the United States: opportunities and controversies. Ann Intern Med. 1997; 127:904-09.
25. Monica C. District Laboratory practice in Tropical countries, part 2. Cambridge University Press, Great Britain. 2003:348-361.
26. Nakao K, Wada T, Kamiyama T, Nakao M, Nagano K. A direct relationship between adenosine triphosphate-level and in vivo viability of erythrocytes.Nature.1962; 194:877-8.
27. Valeri CR, Hirsch NM. Restoration in vivo of erythrocyte adenosine triphosphate, 2,3-diphosphoglycerate, potassium ion, and sodium ion concentrations following the transfusion of acid-citrate-dextrose-stored human red blood cells. J Lab Clin Med. 1969; 73:722-33.
28. Sawant RB, Jathar SK, Rajadhyaksha SB, Kadam PT. Red cell hemolysis during processing and storage. Asian J Transfu Sci. 2007; 1:47-51.
29. Seghatchian MJ, Vickers M, Ip AH, Stivala JF, de Silva PM. The potassium haemoglobin and acid content of CPDA-1 whole blood, plasma reduced red cells and red cells suspended in SAG-M over 35 days. Transfus Med. 1993; 3(Suppl 2): 58-9.
30. Adias TC, Moore-Igwe B, Jeremiah ZA. Storage Related Haematological and Biochemical Changes of CPDA-1 Whole Blood in a Resource Limited Setting. J Blood Disorders.TRANSFUSION.2012; 3:124.
31. Uvizl R, Klementa B, Adamus M, Neiser J. Biochemical changes in the patient's plasma after red blood cell transfusion. Signa Vitae.2011; 6:64-71.
32. Hess JR, Sparrow RL, Van der Meer PF, Acker JP, Cardigan RA. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. TRANSFUSION.2009; 49:2599-03.
33. Strauss RG. Data-driven blood banking practices for neonatal RBC transfusions. TRANSFUSION.2000; 40:1528-40.
34. Ono T, Kitaguchi K, Takehara M, Shiliba M, Hayami K. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. Clinical chemistry.1981; 27:35-8.
35. Strauss RG, Sacher RA, Blazina JF, Blanchette VS, Schloz LM, Butch SH. Commentory on small-volume red cell transfusions for neonatal patients. TRANSFUSION.1990; 30:565-70.
36. Strauss RG. Transfusion therapy in neonates. Am J Dis Child.1991; 145:904-11.
37. Hall TL, Barnes A, Miller JR, Bethencourt DM, Nestor L. Neonatal mortality following transfusion of red cells with high plasma potassium levels. TRANSFUSION.1993; 33:606-9.
38. Latham JT Jr, Bove JR, Weirich FL. Chemical and hematologic changes in stored CPDA-1 blood. TRANSFUSION.1982; 22:158-9.
39. Bailey DN, Bove JR. Chemical and hematological changes in stored CPD blood. Transf. 1975; 15:244-49.
40. Frank JJ, Bermes EW, Bickel MJ, Watkins BF. Effect of in vitro hemolysis on chemical values for serum. Clin Chem. 1978; 24:1966-70.
41. Simon ER. Red cell preservation: further studies with adenine. Blood.1962; 20:485-91.
42. Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. Blood.2003; 101:1257-61.

Table I: Results of quality control parameters in whole blood with CPDA-1

Source	DGHS criteria*		Present study	
	Volume (ml)	Haematocrit (%)	Volume (ml)	Haematocrit (%)
Whole blood	350 ± 10	40 ± 5	347.9 ± 11.1	44.85 ± 8.7

*Directorate General Health Services, Transfusion Medicine Technical Manual 2003[6c]

Table 2: Tests of within-subjects effects for the parameters: pH, sodium, potassium and glucose concentrations in CPDA-1 stored whole blood.

	Descriptive Statistics			Parameter estimates				
Dependent variable	Time points	Mean	Std. Deviation	Std. Error	T	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Sodium (meq/L)	Day 1	147.70	1.625	.363	406.369	.000	146.939	148.461
	Day 2	144.10	2.100	.470	306.855	.000	143.117	145.083
	Day 3	140.75	2.173	.486	289.616	.000	139.733	141.767
	Day 4	140.55	1.932	.432	325.272	.000	139.646	141.454
	Day 5	140.40	2.088	.467	300.776	.000	139.423	141.377
	Day 6	139.00	2.340	.523	265.699	.000	137.905	140.095
	Day 7	137.50	2.236	.500	275.000	.000	136.453	138.547
Potassium (meq/L)	Day 1	3.23	.558	.125	25.874	.000	2.969	3.491
	Day 2	4.15	.298	.067	62.230	.000	4.010	4.290
	Day 3	5.11	.619	.138	36.909	.000	4.816	5.394
	Day 4	7.15	.796	.178	40.158	.000	6.773	7.517
	Day 5	7.82	.805	.180	43.428	.000	7.438	8.192
	Day 6	9.80	.680	.152	64.472	.000	9.482	10.118
	Day 7	11.70	1.766	.395	29.633	.000	10.874	12.526
Glucose (mg/dL)	Day 1	464.40	40.840	9.132	50.853	.000	445.286	483.514
	Day 2	418.10	42.232	9.443	44.274	.000	398.335	437.865
	Day 3	374.45	44.916	10.043	37.283	.000	353.429	395.471
	Day 4	345.85	32.285	7.219	47.907	.000	330.740	360.960
	Day 5	306.15	31.064	6.946	44.075	.000	291.612	320.688
	Day 6	265.00	39.741	8.886	29.821	.000	246.401	283.599
	Day 7	233.10	49.792	11.134	20.936	.000	209.797	256.403
pH	Day 1	7.1643	1.41285	0.308	23.237	0.000	6.521	7.807
	Day 2	7.1262	1.17501	0.256	27.792	0.000	6.591	7.661
	Day 3	7.1038	0.94137	0.205	34.581	0.000	6.675	7.532
	Day 4	7.0757	0.70730	0.154	45.843	0.000	6.754	7.398
	Day 5	6.9705	0.46141	0.101	69.229	0.000	6.760	7.181
	Day 6	6.8710	0.23879	0.052	131.861	0.000	6.762	6.980
	Day 7	6.767	0.1742	0.038	178.043	0.000	6.687	6.846

Table 3: Mauchly's Test of Sphericity[b] and its correction.

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Sodium(meq/L)	0.010	77.942	20	0.000	0.359	0.406	0.167
Potassium(meq/L)	0.000	134.675	20	0.000	0.326	0.363	0.167
Glucose(mg/dL)	0.000	195.116	20	0.000	0.209	0.217	0.167
pH	0.000	819.793	20	0.000	0.167	0.167	0.167

a. May be used to adjust the degrees of freedom for the averaged tests of significance.
b. To test the extra assumptions of repeated measures ANOVA.

Figure 1: Serial time points of Red blood cell count in CPDA-1 stored whole blood

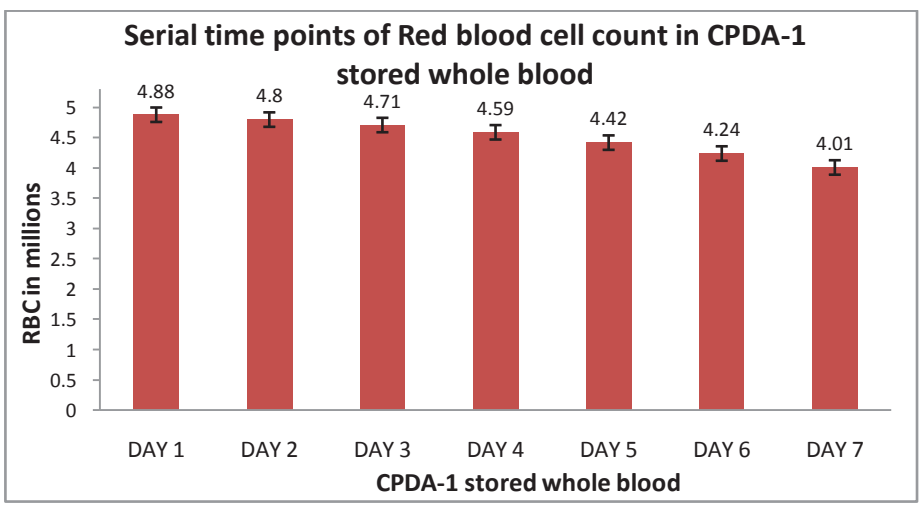

Figure 2: Serial time points of White blood cell count in CPDA-1 stored whole blood