Original Article

Storage Lesions in Red Blood Cell-Saline Adenine Glucose Mannitol: *In-vitro* and *In-vivo* Analysis over 42 Days and its Implications in Routine Transfusion Practice

Geet Aggarwal, Aseem Kumar Tiwari, Swati Pabbi, Gunajn Bhardwaj, Ganesh Rawat, Nandram, Arun Kumar Harith[1], Kamini Vinayak[1]

Departments of Transfusion Medicine and [1]Biochemistry, Medanta-The Medicity, Gurgaon, Haryana, India

Abstract

Background and Objectives: Indian studies on evaluation of storage lesions in red blood cells (RBCs) are either limited to 21 or 28 days or have evaluated limited parameters for 42 days. Moreover, issue of transfusion of "fresh" versus "old" RBC is far from settled. The study serially assesses, up to 42 days, *in vitro* and *in vivo* RBC storage lesion parameters, including di-(2-ethylhexyl) phthalate (DEHP) leaching and its comparison with published literature. **Methods:** The study serially assessed *in vitro* RBC storage lesion parameters including potassium, lactate, glucose, pH, supernatant hemoglobin, percentage-hemolysis, and DEHP leaching of RBC unit during storage till 42 days. The study also evaluated *in vivo* recovery of potassium after transfusion of "older" RBC. **Results:** Serial monitoring of *in vitro* biochemical parameters showed increase in potassium, lactate, supernatant Hb, and hemolysis% and reduction in glucose and pH. DEHP content of the RBC bag was within no-observed adverse effect limit on days 42. Measurement of serum potassium after transfusion of "older" RBC unit revealed that levels of potassium were within normal limit in all four patients. Sterility testing done on days 42 was negative for all 24 bags. **Conclusion:** Development of storage lesions is inevitable. Appropriate storage limits the RBC lesions to within normal limits. The increase in potassium, lactate, or hemolysis consequent to aging of blood has little clinical significance in routine transfusion practice.

Keywords: *42 days, in vivo, leaching, red blood cell, storage lesions*

Submitted: 05-Dec-2020.
Revised: 09-Feb-2022.
Accepted: 17-Mar-2022.
Published: 29-Apr-2022.

Introduction

Stored red blood cells (RBCs) undergo certain time-dependent adverse changes.[1] The term "storage lesion" is used to encompass "deterioration" that happens to RBCs during their storage period.[2,3] Stored RBCs are damaged by accumulation of their own waste products, by enzymatic and oxidative injury. These lesions are defined by changes in parameters including pH, lactate, potassium, glucose, etc.[4] The outcomes of these storage lesions are reduction in oxygen-carrying capacity of RBC, reduced posttransfusion RBC survival and can potentially be responsible for adverse transfusion reactions. However, at the same time, RBC has an innate capability of recovery when transfused.[5]

Numerous studies have been carried out throughout the world to identify possible relationship between the duration of storage of RBCs and side effects in transfused patients.[6-8] Indian studies on evaluation of RBC product are either limited to 21–28 days storage[9-11] or have evaluated limited parameters, e.g., only hemolysis.[12,13] Moreover, issue of "fresh" versus "old" is far from settled.

Aims and objectives

The study was planned to serially assess *in vitro* RBC storage lesion parameters including serum potassium, serum lactate,

Address for correspondence: Dr. Aseem Kumar Tiwari, E-mail: draseemtiwari@gmail.com

How to cite this article: Aggarwal G, Tiwari AK, Pabbi S, Bhardwaj G, Rawat G, Nandram, *et al.* Storage lesions in red blood cell-saline adenine glucose mannitol: *In-vitro* and *in-vivo* analysis over 42 days and its implications in routine transfusion practice. Glob J Transfus Med 2022;7:12-7.

Access this article online

Quick Response Code:

Website: www.gjtmonline.com

DOI: 10.4103/gjtm.gjtm_113_20

serum glucose, pH, supernatant hemoglobin with hemolysis; di-(2-ethylhexyl) phthalate (DEHP) leaching during RBC storage upto 42 days and its comparison with published literature. The study also evaluated *in vivo* recovery of potassium after transfusion of "older" RBC.

Materials and Methods

Study design

This was prospective analytical study conducted at the department of Transfusion Medicine of tertiary care hospital in north India from January 2018 to June 2018. Departmental standard operating procedures were followed for all processes involved in study.

Donor selection and exclusion

Blood donors were selected based on routine donor screening criteria laid down in the Drugs and Cosmetics Act and Rules, Ministry of Health and Family Welfare, Government of India.[14] The donors were administered medical history questionnaire and underwent a physical examination. The selection criteria included age >18 years, weight >55 kg, hemoglobin ≥12.5 g %, normal vitals including temperature <98.4°F, pulse 60–100/min, systolic blood pressure 100–140 mm Hg, diastolic blood pressure 60–90 mmHg, and overall sense of well-being. Donors who did not fulfill screening criteria or did not provide study-specific consent were excluded from study.

Donation process

Whole blood (450 ml) was collected from 28 consecutive voluntary donors using triple blood bag with citrate-phosphate-dextrose-Saline Adenine Glucose Mannitol (SAGM) (SangX, BioLife Medical, Delhi, India).

Component preparation

Day 0, RBC units were prepared by platelet-rich plasma (PRP) method. First centrifugation was done at 2350 rpm (rcf-1834 g) at 22°C for 7 min (Heraeus Cryofuge 6000i, Thermo, US). This allowed separation of RBC and PRP. Later, PRP was subjected to 3450 rpm (rcf-3952 g) at 22°C for 8 min and separated into platelet-poor plasma and random donor platelet concentrate. SAGM (RBC additive solution) was then added to prepare RBC before storing them at 4°C. Next day, stored RBC units were leukoreduced by filtration (BioR, Fresinius Kabi AG, Homburg, Germany). Of the 28 units prepared, 20 RBC units were used to study the *in vitro* parameters and eight RBC units were evaluated for *in vivo* parameter. All samples were drawn after leukoreduction.

Study protocol

In vitro

Parameters including potassium, lactate, glucose, pH, supernatant hemoglobin, percentage-hemolysis, and DEHP leaching in RBC units were serially assessed during storage. Samples were collected on day 1, postleukoreduction, and weekly thereafter for 6 weeks. Ten-milliliter aliquots were drawn aseptically on storage days 1, 7, 14, 21, 28, 35, and 42 by using a sterile connecting device (CompoDock, Fresenius Kabi AG, Homburg, Germany). Parameters including plasma potassium, plasma glucose, and serum lactate were measured weekly till 42nd day (days 1, 7, 14, 21, 28, 35, 42). Parameters such as pH, plasma hemoglobin, and hemolysis% were measured at alternate weeks (days 1, 14, 28, 42). These parameters were measured in all 20 RBC units. Leaching for DEHP content was measured for four RBC bags only on days 1, 35, and 42.

In vivo

Eight RBC units were transfused to eight different patients in the last week of their storage period, i.e., 36th–42nd day. Serum potassium was measured of RBC unit and patient before transfusion. Serum potassium was measured at 1–2 h and between 18 and 24 h, posttransfusion. Patient selected for transfusion did not have any renal impairment, were not on any potassium-metabolism altering medication, and were not being given any potassium supplements.

Sterility test

Sterility testing was performed on all 28 bags. Twenty bags, reserved for *in vitro* testing, were sent for sterility testing on 42nd day after last sample collection. For the remaining 8 bags, a segment was removed at the time of issue and sent for sterility testing.

Equipment used for measurement of parameters

All parameters were measured simultaneously in-house at the department, except DEHP, which was carried out at an out-sourced (National Accreditation Board for Laboratories) accredited laboratory. Complete blood counts were performed on Sysmex XE 2100 analyzer (Sysmex Corporation, Japan). Serum potassium, glucose, and lactate were measured using Vitros immunoassay analyzer (Ortho-Clinical Diagnostics, United States). pH 700-Oakton (EUTECH Instruments, Singapore) and HEMOCUE Plasma/Low Hb (Hemocue AB, Ängelholm, Sweden) were used to measure pH and plasma Hb, respectively. Leaching for DEHP was performed at outsourced laboratory using mass spectrometry with appropriate controls. Hemolysis% was calculated using the formula= ({Plasma Hb× [1 − Hct]}/Hb-bag) ×100

Sample size calculation and statistical analysis

Sample size calculation is based on parameter of interest-change in hemolysis% at days 42 from day 1.

The minimum sample size worked out as 20, with confidence level at 95% and with 80% power.

Independent *t*-test was applied to detect statistical significant. Pearson's correlations coefficient (r) was applied for biochemical parameters. $P < 0.05$ was considered statistically significant.

Ethical clearance

Institutional review board and an independent ethics committee approved the study.

Results

All 28 voluntary blood donors were men (age 20–30 years). All 28 RBC units prepared fulfilled quality control standards prescribed by Director General of Health Services, MoHFW, Government of India.[14] Mean volume of RBC unit was 318.9 ± 20.6 mL.

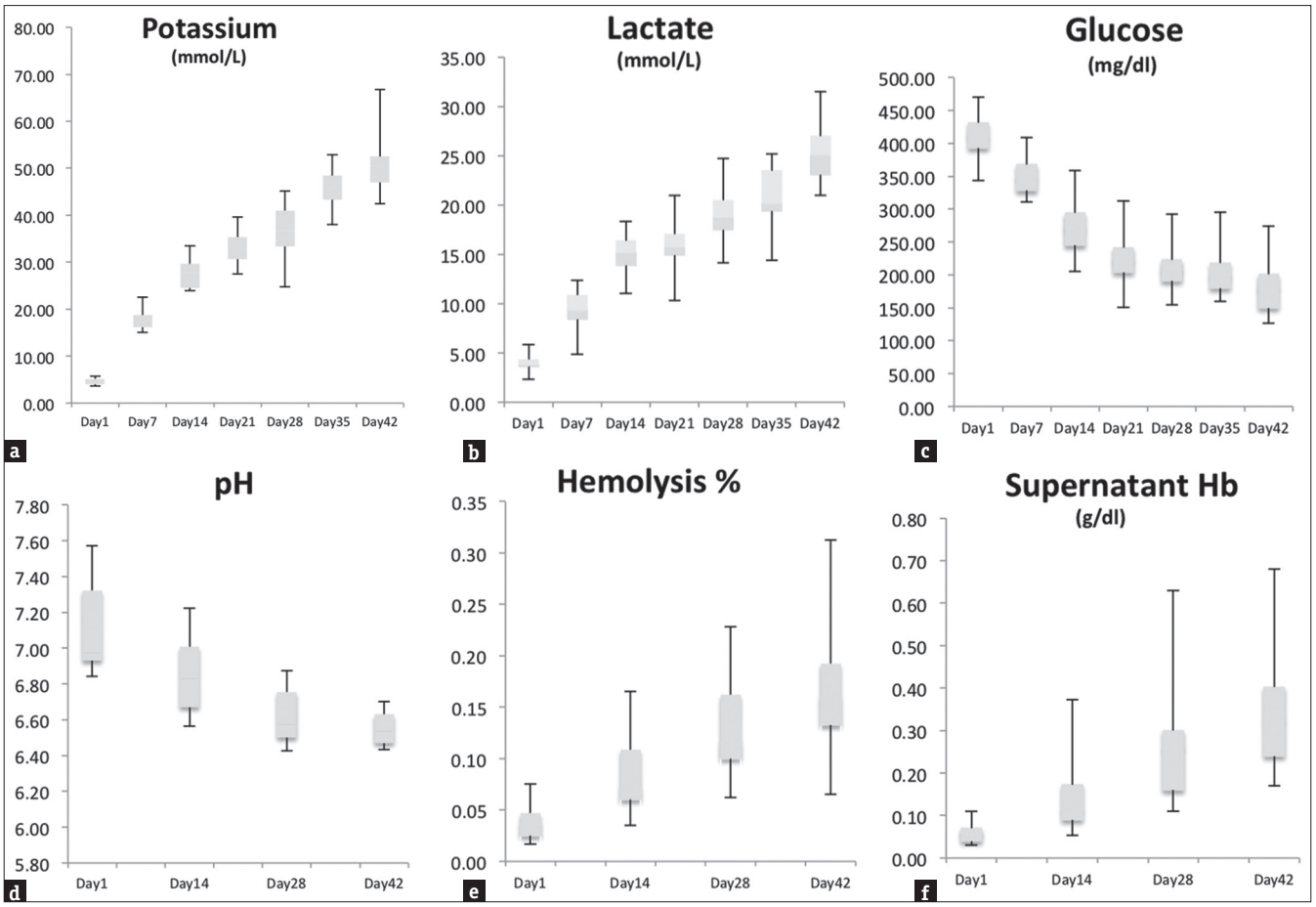

Figure 1: (a-f): Graphical representation of serial assessment of *in vitro* parameters using box and whisker graphs

Table 1 and Figure 1a-f represent results of *in vitro* serial monitoring of biochemical parameters. As expected, there was increase in potassium, lactate, supernatant Hb, and hemolysis% and reduction in glucose and pH. The increase in concentration of potassium and lactate and decrease in glucose concentration over the storage period was statistically significant ($P < 0.0001$). Hemolysis% and supernatant Hb of RBC units increased significantly during storage period ($P < 0.0001$). Mean hemolysis rate (0.23 ± 0.06; clinically insignificant) for all units ($n = 20$) remained below maximum acceptance limit of 0.8% as stated by the Council of Europe guideline.[15] Percentage-hemolysis was lower than laid down standards.

Mean DEHP content, as result of leaching, for four RBC bags on day 1st, 35th, and 42nd was 0.56 mg/kg, 2.43 mg/kg, and 3.39 mg/kg, respectively, and was within prescribed limit.

Measurement of serum potassium after transfusion of "older" RBC unit revealed that levels of potassium were within normal limit in all eight patients on both occasions [1–2 h and 18-24 h posttransfusion; Table 2]. All eight patients were admitted in internal medicine ward and transfused RBC for correction of anemia.

Discussion

From biomolecular point of view, accumulation of changes in RBC is numerous and in proportion to their storage period.[1] Few published studies either analyze storage lesions up till 21 days (Mukherjee *et al*.[9]) or 28 days (Sawant *et al*.[10]) or analyzed only limited number of storage lesions up till 42 days (Arif *et al*.[13]). The present study, probably the first of its kind, from Indian subcontinent serially measured and analyzed biochemical RBC storage lesions including DEHP leaching till 42 days, i.e., maximum allowable storage period for RBC unit in India and recovery of potassium levels in patients after transfusion of "older" RBC units.

Biochemical parameters

Reduction in rate of cellular metabolism and energy demands, when RBC units are stored at 2–6°C, allows storage for 42 days. This temperature makes sodium-potassium pumps in cell membrane inoperable and allows extra-cellular leakage of potassium. The extracellular potassium levels of stored blood increase daily at approximately 1 mmol/liter with the higher concentrations observed during the early days of storage.[1] Potassium levels in the present study were either comparable[9,10] or slightly higher than levels reported.[13] In study by Strauss[16] and Zubair,[17] supernatant plasma level after 42 day of RBC storage in additive solution rose to 50 mmol/L and 63 meq/L, respectively.

Glucose is primary source of adenosine triphosphate (ATP) production, through glycolytic pathway, for RBC. Since

Table 1: Paired comparison of *in vitro* parameters over 42 days from day 1

Parameters	Statistic	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Potassium	Mean±SD	4.83±0.7	18.9±2.14	28.12±2.98	36.45±3.17	45.12±5.03	49.72±5.95	55.75±5.86
	Δ	-	−14.07±2.01	−23.29±2.79	−31.62±2.89	−40.29±4.92	−44.88±5.84	−50.92±6.19
	95% CI of Δ	-	−15.01-−13.13	−24.59-−21.98	−32.97-−30.26	−42.59-−37.98	−47.61-−42.15	−53.82-−48.02
	P	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
pH	Mean±SD	7.2±0.24	-	6.95±0.2	-	6.69±0.14	-	6.59±0.09
	Δ	-	-	0.26±0.15	-	0.51±0.19	-	0.61±0.19
	95% CI of Δ	-	-	0.18-0.33	-	0.42-0.6	-	0.52-0.7
	P	-	-	<0.0001*	-	<0.0001*	-	<0.0001*
Lactate	Mean±SD	5.24±0.85	12.96±1.91	17.91±1.89	20.78±2.53	22.72±2.65	25.52±3.12	27.55±2.98
	Δ	-	−7.72±1.92	−12.67±1.47	−15.54±2.22	−17.48±2.43	−20.28±2.97	−22.31±2.91
	95% CI of Δ	-	−8.62-−6.82	−13.36-−11.98	−16.57-−14.5	−18.61-−16.34	−21.67-−18.89	−23.67-−20.95
	P	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Hb	Mean±SD	0.07±0.02	-	0.18±0.09	-	0.32±0.13	-	0.42±0.14
	Δ	-	-	−0.12±0.08	-	−0.25±0.13	-	−0.35±0.13
	95% CI of Δ	-	-	−0.16-−0.08	-	−0.31-−0.19	-	−0.41-−0.29
	P	-	-	<0.0001*	-	<0.0001*	-	<0.0001*
Glucose	Mean±SD	460.3±30.35	369.65±31.18	314.3±38.45	280.25±37.97	248.05±34.4	217.6±45.81	195.25±55.45
	Δ	-	90.65±19.54	146±30.78	180.05±32.66	212.25±31.5	242.7±44.53	265.05±55.88
	95% CI of Δ	-	81.51-99.79	131.6-160.4	164.76-195.34	197.51-226.99	221.86-263.54	238.9-291.2
	P	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Hemolysis	Mean±SD	0.05±0.02	-	0.11±0.03	-	0.17±0.04	-	0.23±0.06
	Δ	-	-	−0.06±0.03	-	−0.12±0.04	-	−0.18±0.06
	95% CI of Δ	-	-	−0.08-−0.05	-	−0.14-−0.1	-	−0.22-−0.16
	P	-	-	<0.0001*	-	<0.0001*	-	<0.0001*
HCT	Mean±SD	58.85±1.74	64.07±4.69	65.15±8.87	64.24±4.6	65.71±4.08	65.86±3.06	65.69±5.12
	Δ	-	−5.22±3.9	−6.28±7.74	−5.39±4.08	−6.86±3.96	−6.57±3.27	−6.84±5.13
	95% CI of Δ	-	−7.05-−3.39	−10.01-−2.55	−7.29-−3.48	−8.71-−5	−8.91-−4.23	−9.24-−4.44
	P	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Δ: (Mean±SD) of difference; **P*<0.05, statistically significant. SD: Standard deviation, CI: Confidence interval, Hb: Hemoglobin, HCT: Hematocrit

Table 2: *In vivo* analysis of s. potassium levels pre and posttransfusion of "older" red blood cell (normal adult range: 3.8-5.1 mEq/L)

Blood bag number	Day of RBC storage	Serum potassium (blood bag) (mmol/L) on day of transfusion	Serum potassium (mmol/L) of patient		
			Preransfusion	1-2 h posttransfusion	18-24 h posttransfusion
13,460	40th	49.9	3.4	3.8	3.4
17,645	37th	44.6	4.3	4.5	4.4
17,646	38th	52.0	4.3	4.4	3.9
17,647	40th	44.4	5.0	5.1	4.3
2341	38th	45.2	3.5	3.9	3.5
2357	38th	51.4	4.2	4.4	4.2
2381	40th	48.3	4.4	4.5	3.5
2403	39th	47.2	4.9	5.0	4.7
Mean±SD			4.25±0.65	4.45±0.53	4.00±0.45
Δ			-	−0.20±0.14	0.25±0.37
95% CI of Δ			-	−0.42-0.02	−0.34-0.84
*P**			-	0.066	0.269

Δ: (Mean±SD) of difference. **P*<0.05, statistically significant. SD: Standard deviation, CI: Confidence interval

during storage availability of glucose is limited, there is concomitant reduction in ATP production. Additive solutions like SAGM provide sufficient glucose (additional 900 mg dextrose) to allow RBC viability till 42 days. In the present study, glucose values after 42-day period of storage were intermediate between values reported by Mukherjee *et al*.[9] and Picker *et al*.[18]

Glucose consumption and lactate production are negatively correlated. During course of storage, lactate accumulates as

by-product of glycolytic pathway. Lactate has been defined useless end product, poisonous at times, of which cells must discard off quickly. High lactate concentration can adversely affect pH of storage medium. Mukherjee *et al*. reported 2.7 times to increase in lactate concentration by days 21 as compared to 3.9 times in the present study. Lactate concentration on days 42 in the present study was lower in comparison to reports by Picker *et al*.[18] and Zimmermann *et al*.[19]

Hematological parameters

RBC metabolism slows down during course of storage period as pH falls. At pH below 6.2, RBC had decreased ATP production as mathematically deduced by pH curve of many samples of stored blood in various storage solutions.[20] Although ATP measurement was not done in the present study, mean pH was above the lower limit of pH threshold of 6.2 throughout storage period. Similar pH trends have been shown by other reports.[9,18,19]

Any breakdown in RBC membrane and subsequent release of hemoglobin is termed as hemolysis. This may occur during processing, storage, and transportation. Additive solutions and leukoreduction reduce hemolysis; however, some amount of hemolysis is inevitable. The presence of free hemoglobin in supernatant plasma or additive solution is representation of hemolysis. In the present study, both hemolysis% and free supernatant Hb were with prescribed limits. Similar results have been reported by earlier studies.[9,10,12,13,18,19]

Leaching

DEHP, a plasticizer, is added to plastics to increase its flexibility either by weakening intermolecular forces or by increasing free volume. During storage, DEHP can migrate from PVC storage bags into blood and blood products (platelets, plasma, packed RBCs) because of the lipophilic nature of these biological fluids and cells. Although the DEHP concentration increased from days 1–42 by more than six times, it was within the prescribed no observed adverse level of 4·8 mg/kg/day by European Council.[14]

In *vivo* recovery of potassium

"Older" RBC units have increased concentration of extracellular potassium; however, as the present study depicts, posttransfusion ionic potassium levels returned to normal levels with 24 h. There was no adverse transfusion event reported in any patient. Strauss[21] also showed that no significant differences in comparative changes between pretransfusion and posttransfusion blood chemistries including potassium, whether fresh Citrate-phosphate-dextrose-adenine RBCs or stored AS-1 or AS-3 RBCs were transfused. Moreover, daily adequate intake for adult males and females is 2600–3400 mg/day (34.7–45.3 meq/day).[22] Adult patients requiring single unit transfusion are definitely not at risk of hyperkalemia if transfused "older" unit.

Old versus. new: International randomized trials

The debate on transfusion of "old" versus "fresh" RBC has been addressed in several randomized trials. Debate began in 2008 when Koch *et al*.[8] concluded that there was significantly higher risk of postoperative complications as well as reduced short-term and long-term survival associated with transfusion of red cells that had been stored for more than 2 weeks in patients undergoing cardiac surgery. Retrospective study of Koch *et al*. was later challenged in several well-designed prospective trials, such as ABLE,[23] RECESS,[24] TOTAL,[25] ARIPI,[26] INFORM,[27] and TRIBE.[28] The ABLE study (Age of transfused blood in critically ill adults) concluded that transfusion of standard-issue (oldest available compatible blood) red cells did not decrease the 90-day mortality among critically ill adults in comparison to transfusion of fresh units (<8 days). The RECESS trial concluded that transfusion of "fresh" red cells (<10 days) was not superior to transfusion of "older" red cells (>21 days) among patients 12 years of age or older who underwent complex cardiac surgery. The TOTAL randomized control trial concluded that transfusion of longer-storage RBC (25–35 days) unit did not result in inferior reduction of elevated blood lactate levels in children with lactic acidosis due to severe anemia in comparison to shorter-storage RBC units (1–10 days). The ARIPI randomized trial concluded that the use of fresh RBCs (<8 days) did not improve outcomes in premature, very low-birth-weight infants requiring a transfusion in comparison to standard blood bank practice (2–42 days). The multicentric, randomized controlled INFORM trial concluded that transfusion of blood stored for longer than 35 days has no effect on in-hospital patient mortality. Secondary analyses of multicenter randomized TRIBE study that despite massive blood transfusion, including very old blood (>35 days), duration of red-cell storage did not influence outcome in burn patients. All these trials have more-or-less settled the debate that "fresher units have superior outcome in comparison to older units."

However, this debate is "less than settled" in India, where there is perennial shortage of blood and transfusion based on age of blood results in further shortage. Physicians and surgeons continue to demand "fresh" blood units for their patients. The present study adds evidence to safety data of transfusing "older" RBC units.

Limitations

The study did not include serial analysis of ATP and 2,3-DPG for want of reagents and funds.

Conclusion

This study reiterates the biochemical evidence associated with the development of storage lesion in "older" RBC units and provides clinical evidence to corroborate with other published literature that transfusion of "older" RBC in adult patient would be completely safe. It is desirable and safe to follow (first-in first-out) transfusion policy in a resource-constrained country like India. Although potassium, lactate and supernatant Hb, and hemolysis were higher in stored blood, all of them were within acceptable limits. Hence, authors conclude that age of blood has little clinical relevance in routine transfusion practice although there could be specific situations where the same needs consideration.

Acknowledgment

Authors acknowledge the blood bank technical staff for their help for smooth conduct of the study and the timely issue of appropriate blood components.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. Vox Sang 2009;96:93-103.
2. Kor DJ, Van Buskirk CM, Gajic O. Red blood cell storage lesion. Bosn J Basic Med Sci 2009;9 Suppl 1:21-7.
3. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: Role of red blood cell breakdown. Transfusion 2011;51:844-51.
4. D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. Red blood cell storage: The story so far. Blood Transfus 2010;8:82-8.
5. Yoshida T, Prudent M, D'alessandro A. Red blood cell storage lesion: Causes and potential clinical consequences. Blood Transfus 2019;17:27-52.
6. Murrell Z, Haukoos JS, Putnam B, Klein SR. The effect of older blood on mortality, need for ICU care, and the length of ICU stay after major trauma. Am Surg 2005;71:781-5.
7. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, *et al.* Age of transfused blood is an independent risk factor for postinjury multiple organ failure. Am J Surg 1999;178:570-2.
8. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, *et al.* Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.
9. Mukherjee S, Marwaha N, Prasad R, Sharma RR, Thakral B. Serial assessment of biochemical parameters of red cell preparations to evaluate safety for neonatal transfusions. Indian J Med Res 2010;132:715-20.
10. Sawant RB, Jathar SK, Rajadhyaksha SB, Kadam PT. Red cell hemolysis during processing and storage. Asian J Transfus Sci 2007;1:47-51.
11. Saini N, Basu S, Kaur R, Kaur J. Assessment of changes in plasma hemoglobin and potassium levels in red cell units during processing and storage. Transfus Apher Sci 2015;52:319-25.
12. Makroo RN, Raina V, Bhatia A, Gupta R, Majid A, Thakur UK, *et al.* Evaluation of the red cell hemolysis in packed red cells during processing and storage. Asian J Transfus Sci 2011;5:15-7.
13. Arif SH, Yadav N, Rehman S, Mehdi G. Study of hemolysis during storage of blood in the blood bank of a tertiary health care centre. Indian J Hematol Blood Transfus 2017;33:598-602.
14. Malik V. Drugs and Cosmetics Act, 1940. 16th ed. New Delhi: Eastern Book Company; 2003. p. 279-303.
15. European Directorate for the Quality of Medicines. Health Care. Guide to the Preparation, Use and Quality Assurance of Blood Components. Strasbourg, France: Council of Europe. Recommendation No.; 2013.
16. Strauss RG. Data-driven blood banking practices for neonatal RBC transfusions. Transfusion 2000;40:1528-40.
17. Zubair AC. Clinical impact of blood storage lesions. Am J Hematol 2010;85:117-22.
18. Picker SM, Radojska SM, Gathof BS. *In vitro* quality of red blood cells (RBCs) collected by multicomponent apheresis compared to manually collected RBCs during 49 days of storage. Transfusion 2007;47:687-96.
19. Zimmermann R, Wintzheimer S, Weisbach V, Strobel J, Zingsem J, Eckstein R. Influence of prestorage leukoreduction and subsequent irradiation on *in vitro* red blood cell (RBC) storage variables of RBCs in additive solution saline-adenine-glucose-mannitol. Transfusion 2009;49:75-80.
20. 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.
21. Strauss RG. Transfusing Neonates and Infants. Practical Transfusion Medicine 2017:371-82.
22. Oria M, Harrison M, Stallings VA. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Food and Nutrition Board; Committee to Review the Dietary Reference Intakes for Sodium and Potassium. Dietary Reference Intakes for Sodium and Potassium. 2019.
23. Lacroix J, Hébert PC, Fergusson DA, Tinmouth A, Cook DJ, Marshall JC, *et al.* Age of transfused blood in critically ill adults. N Engl J Med 2015;372:1410-8.
24. Steiner ME, Ness PM, Assmann SF, Triulzi DJ, Sloan SR, Delaney M, *et al.* Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med 2015;372:1419-29.
25. Dhabangi A, Ainomugisha B, Cserti-Gazdewich C, Ddungu H, Kyeyune D, Musisi E, *et al.* Effect of transfusion of red blood cells with longer vs. shorter storage duration on elevated blood lactate levels in children with severe anemia: The TOTAL randomized clinical trial. JAMA 2015;314:2514-23.
26. Fergusson DA, Hébert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, *et al.* Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: The ARIPI randomized trial. JAMA 2012;308:1443-51.
27. Cook RJ, Heddle NM, Lee KA, Arnold DM, Crowther MA, Devereaux PJ, *et al.* Red blood cell storage and in-hospital mortality: A secondary analysis of the INFORM randomised controlled trial. Lancet Haematol 2017;4:e544-52.
28. Cartotto R, Taylor SL, Holmes JH 4th, Peck M, Cochran A, King BT, *et al.* The effects of storage age of blood in massively transfused burn patients: A secondary analysis of the randomized transfusion requirement in burn care evaluation study. Crit Care Med 2018;46:e1097-104.