

Biochemical changes in stored donor units: implications on the efficacy of blood transfusion

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Background: Blood transfusion with allogeneic blood products is a common medical intervention to treat anemia or prepare patients for surgical procedures. Generally, the blood units are secured and stored prior to expected transfusion. During storage, a number of biochemical changes occur (generally known as storage lesion), which can affect the efficacy of blood transfusion. The aim of the study was to evaluate the biochemical changes that occur in blood units during storage and to project the impact of these changes on transfusion.

Methods: The study protocol was approved by the Faculty of Medicine Research Ethics committee of Mbarara University of Science and Technology. A total of 200 blood recipients were categorized into two study arms: group I received fresh blood (n=100) and group II received old blood (n=100), who were formally consented and recruited consecutively. A total of 2 mL of venous blood was collected from each participant in EDTA tubes before transfusion (for pre-transfusion hemoglobin [Hb] estimation) and after transfusion (for post-transfusion Hb estimation). Each crossmatched unit was sampled to collect plasma for pH, lactate and potassium assays. Data were analyzed with STATA version 12.0.

Results: A total of 200 blood transfusion recipients aged 1–60 years were enrolled in the study. Up to 60% of the participants were females. The pH of the stored blood dropped from 7.4 to 7.2 in the first 3 days to ~7.0 by day 11 and to <7.0 by day 35 ($p=0.03$). The average rise in lactate level was 25 g/dL in blood stored for 0 to 11 days and 32.4 g/dL in blood stored for 21–35 days. The highest increase was encountered in blood stored beyond 28 days: 40–57 g/dL by 35 days ($p=0.001$). Potassium levels equally increased from ~4.6 mmol/L in the first 5 days of storage to ~14.3 mmol/L by 11 days. From the third week of blood storage and beyond, there was exponential increase in potassium levels, with the highest record in blood units stored from 30 to 35 days ($p=0.068$).

Conclusion: Whole blood stored for >14 days has reduced efficacy with increased markers of red cell storage lesion such as increased potassium level, lactate and fall in pH. These lesions increase the length of hospital stay.

Keywords: blood transfusion, storage lesion, biochemical changes, anemia, Uganda

Background

Blood transfusion with allogeneic blood products is a common medical intervention to treat several medical disorders,^{1,2} such as anemia resulting from infection, blood synthesis disorders, drug-related cytotoxicity and blood loss in accidents, during childbirth or during operation.^{2,3} Therefore, transfusion is intended to improve oxygen delivery to tissues and reduce the complications of anemia or related medical procedures.⁴ In most cases, patients are transfused in emergency situations to save lives and presence

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of ready stored blood units is important. For this reason, blood transfusion bodies enacted guidelines which ensures the preparation and storage of blood products ahead of any medical emergency requiring blood transfusion. Allogeneic blood products are always stored *in vitro* for a number of days (usually up to 35 days) in additives such as citrate phosphate dextrose adenine (CPDA)^{1,4-7} at 2–6°C. During storage, several physical and biochemical changes take place in blood products that await transfusion, and the most affected product is whole blood.⁸ These changes known collectively as red cell storage lesion are progressive events that affect blood products stored for longer period than products stored for a short period.⁹ These changes include increased lactate level with resulting lower pH, increased lysis, increased potassium level and reduced 2,3-biphosphoglycerate and ATP level.^{4,9,10} Red cell storage lesion therefore reduces efficacy of the transfused blood products by increasing the rate of removal of the transfused red blood cells (RBCs) by macrophages and immunomodulation, which increases the chances of transfusion-related morbidities such as acute lung injuries, increased hospital stay and increased mortality.⁴ Several studies have demonstrated a reduced efficacy of stored whole blood units as it is a product mostly affected by storage lesion.^{4,10,11} This study aimed to establish the efficacy of fresh vs old units of blood.

Methods

This study protocol was approved by the Faculty of Medicine Research Ethics committee of Mbarara University of Science and Technology.

This was a prospective randomized study where blood recipients were consecutively enrolled and randomized into one of the two groups of the study arms, each with 100 participants. Written informed consent was obtained from the participants aged ≥ 18 years and from the parents or guardians on behalf of recipients aged < 18 years. Group I participants were recipients who received whole blood that had been stored for < 14 days, whereas group II participants were recipients who received whole blood that had been stored for > 21 days. The blood units stored for < 14 days were considered fresh and those > 21 days were considered old.⁴ All the blood units were stored at 1–6°C until transfused or expired, according to the local blood transfusion guideline.

Recipients were assessed at 24 hours, 48 hours or at the end of the transfusion episode. Two milliliters of blood specimen was collected in EDTA vacutainer tubes from every recipient before blood transfusion to estimate pre-transfusion hemoglobin (Hb). Recipients were monitored closely every

day for any transfusion reaction, whether they were discharged normally, died or got any transfusion complications.

After a full transfusion episode, another 2 mL of blood was collected in EDTA vacutainer tubes from every recipient and was used for the estimation of post-transfusion Hb.

Four milliliters of the corresponding donor units for respective recipients was aseptically collected in a plain container for biochemical tests (pH, potassium and lactate levels) and for blood culture.

The Hb level was estimated using a Beckman Coulter full hemogram machine (Brea, CA, USA); donor lactate level was determined using Humastar 80 (Human Diagnostics, Wiesbaden, Germany); and the potassium and pH in the donor units were determined by Humalites (Human Diagnostics). Donor units were cultured in blood agar medium (Oxoid, Hampshire, UK) and incubated for 24 hours at 37°C.

Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA), and differences in Hb rise, lactate, pH and potassium level and the length of hospital stay were assessed using Student's *t*-test. Associations between predictor variables and outcome variables were assessed using multivariate analyses. A 95% confidence level was used with an alpha of 0.05, and a *p* value of < 0.05 was considered small enough for the association or differences to have occurred by chance.

Results

Demographic characteristics of participants

A total of 200 blood transfusion recipients aged 1–60 years were enrolled in the study. Up to 60% of the participants were females (Table 1). The recipients were divided into two study groups: group I comprised 100 recipients who received blood products stored for 0–11 days with an average storage age of 5 days, and group II comprised 100 recipients who received blood products stored for 21–35 days with an average storage age of 28 days. The recipients were from the following four wards: 29.0% from medical, 32.0% from pediatric, 25.0% from maternity and 14.0% from emergency.

Indication(s) for blood transfusion

The clinical indications for blood transfusion were malaria (38.0%), surgery (11.0%), bleeding (26.0%) and other infections (25.0%).

The average number of blood units received was 2 and 4 for group I and group II, respectively.

All recipient groups had mean pre-transfusion Hb levels of 10.0 g/dL (the pre-transfusion Hb ranged from 8 to

Table 1 Demographic characteristics of the participants (n=200)

	Group I	Group II
Males, frequency (%)	42 (52.5)	38 (47.5)
Females, frequency (%)	58 (48.3)	62 (51.7)
Age (years), median (IQR)	37 (33, 41)	35 (31, 39)
Age group (years), frequency (%)		
1–10	7 (63.6)	4 (36.4)
11–20	24 (57.1)	18 (42.9)
21–30	30 (44.8)	37 (55.2)
31–40	21 (53.8)	18 (46.2)
>40	18 (43.9)	23 (56.1)

Note: Group I comprised 100 recipients who received blood products stored for 0–11 days with an average storage age of 5 days, and group II comprised 100 recipients who received blood products stored for 21–35 days with an average storage age of 28 days.

Abbreviation: IQR, interquartile range.

12 g/dL). The post-transfusion Hb increments were 1–2 g/dL and 0.3–0.7 g/dL for group I and group II, respectively ($p=0.04$).

The blood pH of donor units decreased significantly with increase in storage age. The pH dropped from 7.4 to 7.2 in the first 3 days to ~7.0 by day 11 and to <7.0 by day 35 ($p=0.03$).

Lactate levels in the donor units increased significantly with increase in storage age and reduction in pH with an average increase of 25 g/dL for blood stored for not >11 days and 32.4 g/dL for blood stored from 21 to 35 days ($p=0.001$) (Table 2).

Potassium levels equally increased from an average of 4.6 mmol/L in the first 5 days of storage to ~14.3 mmol/L by 11 days. From the third week of blood storage and beyond, there was exponential increase in potassium levels with the highest record in blood units stored beyond 21 days (24.6 mmol/L) ($p=0.068$). The length of hospital stay was variable according to the study group, the highest was 14 days in group II recipients and the lowest was 2 days in group I ($p=0.056$).

Correlation and multiple regression analyses were conducted to examine the relationship between storage age, Hb increment and storage lesion. The descriptive statistics and analysis results are summarized in Table 3.

Discussion

Despite the benefits of blood transfusion (an effective measure to avert anemia, preparation of individuals for surgical procedures, reduce symptoms of blood loss), it may prove dangerous to recipients.^{12,13} There are several undesired aftermaths of blood transfusion even after careful laboratory techniques in processing and crossmatching the donors and the recipients. These undesired effects may be due to changes in the usual microenvironment of the red cells during storage.

The current study has demonstrated that pH of stored blood decreases during storage, and the fall in pH increases with

Table 2 Biochemical variations and Hb increment

	Group I (mean, CI)	Group II (mean, CI)	p value
Biochemical variation (mean, SD)			
Lactate (g/dL)	25 (1.3)	32.4 (2.2)	0.001
Potassium (mmol/L)	14.3 (1.9)	24.6 (1.7)	0.068
Hb increment, g/dL (mean, SD)			
	1.4 (0.08)	0.5 (0.06)	0.04

Note: Group I comprised 100 recipients who received blood products stored for 0–11 days with an average storage age of 5 days, and group II comprised 100 recipients who received blood products stored for 21–35 days with an average storage age of 28 days.

Abbreviations: Hb, hemoglobin; CI, confidence interval.

Table 3 Summary statistics and results from multivariate modeling

Storage age	R ²	SE	95% CI	p value
Hb increment	-2.6577	0.8205	-4.8673, -1.4512	0.002
Potassium	79.8438	78.1751	11.7172, 544.077	<0.0001
Lactate	9.689	7.9410	1.9439, 48.2950	<0.0001
pH	61.840	73.2718	6.0642, 54.1136	0.006

Abbreviations: Hb, hemoglobin; SE, standard error; CI, confidence interval.

increase in storage time ($p=0.03$). The fall in pH would be a result of the rise in lactate level from anaerobic metabolism of glucose^{6,14} and that the fall in pH was directly proportional to rise in lactate level.^{15,16} An experimental study by Wilson et al,¹⁷ in canine red cells, demonstrated that the fall in pH was higher from day 14 and correlates with increase in production of lactate. The biochemical rationale here is that, though at reduced rate, the stored cells have to be maintained alive through anaerobic respiration. In a normal biological system, the generated lactate is buffered off by kidneys and would not have much effect on the system's pH. However, the lack of similar buffering potential of the stored units leaves open the units to the lowering of blood pH without resistance. The raised lactate concentration with the reciprocal fall in pH would have devastating effect on blood recipients especially those who may receive numerous units of blood within a short time. This reduces blood efficacy and predisposes blood recipients to unwanted transfusion-related morbidity and mortality.^{18–21}

Normally, potassium is ~500 times higher in the cell than in plasma. Lysis of cells releases this cytosolic content into the plasma. There is increased cell lysis that occurs as blood is being stored awaiting transfusion. In the current study, storage age of blood units seems to directly correlate quantitatively with the rise in free potassium in the blood units, though it was statistically insignificant ($p=0.068$). Several observational studies have demonstrated that the rise in the potassium level in stored units has devastating effects on blood recipients.^{22,23} Some blood recipients do not benefit from blood transfusion since their bodies may need to resort

to the more extreme effects of hyperkalemia^{23–25} and others could lose the battle.^{23,26} It is not clear why there is increased lysis in such units; however, studies suggest a reduction in ATP, 2,3-diphosphoglycerate and increase in lactate with subsequent fall in pH with the resulting cell membrane rigidity as the culprits.^{17,27–29} These biochemical changes render the cell membrane too rigid and predispose the cells to lysis. These changes also explain why recipients of older blood units did not obtain the post-transfusion Hb increment as expected (0.5 vs 1.0, $p=0.04$). This reduced efficacy therefore increases the length of hospital stay for recipients of older blood units compared to recipients of newer blood units, though our study did not obtain significant difference ($p=0.056$). The lack of association in this study would have been as a result of using participants with varied disease conditions unified by close range of pre-transfusion Hb.

Multivariate logistic regression analysis related storage lesions with storage age. The rise in lactate level ($p<0.0001$) and potassium ($p<0.0001$) positively correlated with increase in storage age of the blood units. However, Hb increment after blood transfusion is lower with increase in storage age ($R^2=-2.6577$, $p=0.002$). There is increase in anaerobic metabolism of the red cells during storage and results in accumulation of lactate with the resulting drop in pH. These biochemical changes coupled with the decreased 2,3-DPG result in increased cell lysis. The lysis ultimately leads to the increase in potassium level with the resulting burden on the recipients.

Conclusion

Whole blood stored for >14 days has reduced efficacy with increased markers of red cell storage lesion such as increased potassium level, lactate and fall in pH. These lesions increase the length of hospital stay.

Recommendation

Prescription of older units should be effected with caution. An elaborate research to study effect and causality can be conducted to further understand how storage affects performance of older blood units.

Acknowledgments

The authors wish to thank all the participants whose contribution rendered this study a success. We also want to thank the staff of all the wards and Mbarara Regional referral Hospital laboratory for their support during recruitment for and analysis of the specimens.

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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