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Assessment of changes in plasma hemoglobin and potassium levels in red cell units during processing and storage

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ABSTRACT

Red cell units undergo changes during storage and processing. The study was planned to assess plasma potassium, plasma hemoglobin, percentage hemolysis during storage and to determine the effects of outdoor blood collection and processing on those parameters. Blood collection in three types of blood storage bags was done – single CPDA bag (40 outdoor and 40 in-house collection), triple CPD + SAGM bag (40 in-house collection) and quadruple CPD + SAGM bag with integral leukoreduction filter (40 in-house collection). All bags were sampled on day 0 (day of collection), day 1 (after processing), day 7, day 14 and day 28 for measurement of percentage hemolysis and potassium levels in the plasma of bag contents. There was significant increase in percentage hemolysis, plasma hemoglobin and plasma potassium level in all the groups during storage ($p < 0.001$). No significant difference was found between any parameter analyzed for outdoor and in-house collected single CPDA red cell units. There was significant lower percentage hemolysis ($p < 0.001$) and potassium (day 7 to day 14 – $p < 0.05$ and day 14 to day 28 – $p < 0.001$) in red cell units from day 7 onward until day 28 of storage in the leukoreduced quadruple bag as compared to the triple bag. The in-house single CPDA red cell units showed significantly more hemolysis ($p < 0.001$) as compared to the triple bags with SAGM additive solution after 28 days of storage. There is gradual increase in plasma hemoglobin and plasma potassium levels during the storage of red blood cells. Blood collection can be safely undertaken in outdoor blood donation camps even in hot summer months in monitored blood transport boxes. SAGM additive solution decreases the red cell hemolysis and allows extended storage of red cells. Prestorage leukoreduction decreases the red cell hemolysis and improves the quality of blood.

1. Introduction

Changes which take place in red blood cell units during processing and storage over a period of time are referred to as storage lesions. These include changes in plasma hemoglobin and potassium levels which can have significant clinical implications for transfusion recipients. Circulating free hemoglobin can bind with nitric oxide (NO) at endothelium, depletion of which can lead to vasoconstriction, endothelial dysfunction and platelet activation, which increases the risk for thrombus formation [1,2]. Hyperkalemia can cause complications in neonates, massively transfused patients and those with renal failure. Cardiac arrest has been reported in patients following massive transfusion because of transient hyperkalemia [3–5]. The conditions and the ambient temperature, under which blood units are collected and transported in Indian settings, differ from that in the west. Also, in India there are no definite guidelines available for acceptable limits of hemolysis in red cell units. Therefore, it would be useful to assess the changes occurring in red cell units during storage and processing.

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Table 1
Percentage hemolysis, plasma hemoglobin and plasma potassium of outdoor single CPDA blood bags on day 1, day 7, day 14 and day 28.

	Day 1	Day 7	Day 14	Day 28
Percentage hemolysis ± SD	0.073 ± 0.021	0.172 ± 0.025	0.269 ± 0.036	0.422 ± 0.065
Plasma hemoglobin (mg dL^{-1}) ± SD	15.13 ± 4.53	36.57 ± 4.12	56.33 ± 5.28	87.05 ± 9.30
Potassium levels (mEq L^{-1}) ± SD	3.37 ± 0.31	10.89 ± 1.09	16.10 ± 1.80	22.76 ± 2.26

2. Material and methods

In this prospective study, a total of 160 blood units were collected. Of these 160 units, 120 blood units were collected in blood bank (in-house collection) and 40 blood units were collected in the blood donation camps (outdoor collection) as follows:

(i) single CPDA bag – 40 outdoor collection
(ii) single CPDA bag – 40 in-house collection
(iii) triple CPD + SAGM bag – 40 in-house collection
(iv) quadruple CPD + SAGM bag with integral leukoreduction filter (3–4 log leukoreduction) – 40 in-house collection

Blood collection was done strictly as per the guidelines prescribed by DGHS, MOH & FW, New Delhi [6]. The blood collected in single blood bags at camp were stored and transported in battery operated blood transport boxes (Insignia) equipped with digital temperature display. These transport boxes are capable of maintaining temperature around 4 °C. It was ascertained that the temperature during storage at blood donation camp and transportation did not exceed 10 °C.

Samples were collected from single bag on the day of collection (day 1) and then on day 7, day 14 and day 28. Samples from the triple bag and quadruple bag were collected before processing (day 0) and after processing (day 1) and subsequently on day 7, day 14 and day 28. After sampling, hematocrit and hemoglobin of the blood sample were measured using fully automated cell counter (Cell Tech Hematology analyzer). The blood sample was then centrifuged at 1000 rpm for 10 minutes and the supernatant plasma was then removed into cryovials and stored below minus 20 °C in deep freezer. The plasma samples were processed within 1 month of storage after appropriately thawing them.

2.1. Measurement of parameters

2.1.1. Plasma hemoglobin

3, 3′, 5, 5′ Tetramethyl benzidine (TMB) method was used to measure the plasma hemoglobin. The absorbance of colorimetric product was compared with standard hemoglobin solution (60 mg/dL) read at 620 nm by spectrophotometer (Lab Systems – Multiskan ELISA plate reader, Thermo Fisher Scientific, USA) [7,8] Plasma hemoglobin in a red cell unit was calculated using the formula [9]:

$$\frac{=(\text{absorbance of test}-\text{absorbance of blank})\times 60\,\text{mg/dL}}{(\text{absorbance of standard}-\text{absorbance of blank})}$$

2.2. Percentage hemolysis

In a red cell unit was calculated as [9]:

$$\frac{=(100-\text{hematocrit})\times \text{plasma hemoglobin (gm/dL)}}{\text{total hemoglobin (gm/dL)}}$$

2.2.1. Plasma potassium

Plasma potassium was evaluated using instrument ABL800 FLEX Analyzer, Radiometer, Denmark [10].

3. Statistical analysis

Discrete categorical data were presented as *n* (%); continuous data were given as mean ± SD and range. Normality of quantitative data was checked by measures Kolmogorov–Smirnov tests of normality. For normally distributed data, t-test was applied for comparison between two groups. For multiple comparisons (between different days), one way ANOVA followed by post hoc multiple comparisons was applied. All statistical tests were two-sided and performed at a significance level of $\alpha = 0.05$. All analyses were conducted using SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Effect of storage on percentage hemolysis, plasma hemoglobin and plasma potassium level

There was gradual and significant increase in hemolysis, plasma hemoglobin and plasma potassium levels in all the four groups, with increase in the storage period ($p < 0.001$) (Tables 1, 2, 3 and 4 and Figures 1, 2 and 3).

Table 2
Percentage hemolysis, plasma hemoglobin and plasma potassium of in-house single CPDA blood bags on day 1, day 7, day 14 and day 28.

	Day 1	Day 7	Day 14	Day 28
Percentage hemolysis ± SD	0.073 ± 0.029	0.171 ± 0.031	0.263 ± 0.043	0.402 ± 0.070
Plasma hemoglobin (mg dL^{-1}) ± SD	15.71 ± 6.10	37.84 ± 4.86	57.88 ± 4.38	86.40 ± 6.64
Potassium levels (mEq L^{-1}) ± SD	3.37 ± 0.30	11.26 ± 1.09	16.73 ± 1.69	23.06 ± 2.07