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A study to assess the relationship between donor uric acid levels and supernatant hemolysis in stored packed red blood cell units

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Abstract:

BACKGROUND: Most of the red blood cell (RBC) storage lesions can be attributed to oxidative stress encountered by the RBCs throughout the duration of their storage. Various donor variables at the time of donation may be responsible for the total antioxidant capacity of the supernatant and thus, the "storability" and the magnitude of development of these RBC storage lesions. It is known that uric acid (UA) is responsible for more than 60% of the TAC of the blood. This study aims to explore the relationship between donor UA levels and the difference in percentage hemolysis, an important RBC storage lesion, on day 1 and day 21, in stored packed RBCs (PRBCs) units.

MATERIALS AND METHODS: The serum UA of 100 healthy voluntary male blood donors was estimated at the time of blood donation. The percentage hemolysis in the supernatant of the leukoreduced citrate phosphate dextrose/saline–adenine–glucose–mannitol RBC units (n = 100) prepared from these donors was calculated on day 1 and day 21. The difference in percentage hemolysis between donors with high normal serum UA levels (>7 mg/dL) was compared to that of the donors with low normal serum UA levels (<5 mg/dL) to observe the effect of donor UA levels on the difference in percentage hemolysis.

RESULTS: The mean of the differences in percentage hemolysis in the supernatant in low UA group (<5 mg/dL) was higher than the mean of the differences in percentage hemolysis in the supernatant in high UA group (>7 mg/dL) and this was statistically significant (P < 0.001). The donor serum UA level and difference in percentage hemolysis on day 21 and day 1 were found to be negatively co-related.

CONCLUSION: Higher levels of serum UA of blood donors seem to have a protective effect on the stored PRBC units as shown in this study. Hence, the potential of UA as one of the constituents of RBC additive solutions might lead to the enhancement of the quality of stored PRBC units by decreasing the RBC storage lesions.

Keywords:

Donor variables, percentage hemolysis, serum uric acid, supernatant free hemoglobin

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Introduction

The continuous evolution of blood storage systems including the constant tinkering and fine tuning of the additive solutions has led to the present scenario where packed (red blood cells [PRBCs]) are routinely stored for up to 42 days using (citrate phosphate dextrose/saline–adenine–glucose–mannitol [CPD/SAGM]) bags.[1] However, the stored RBCs tend to undergo various metabolic and physical changes during their storage which are collectively known as "RBC storage lesions" and although we have succeeded in slowing down these "storage lesions"

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substantially over the last few decades, these changes are inevitable.

These storage lesions are both metabolic (decline in the concentrations of 2,3-diphosphoglycerate, reduction of adenosine 5'-triphosphate, declining pH, and increase in the extracellular potassium concentration) and physical (loss of RBC membrane, changes in RBC shape and rheology, and the consequent increase in the supernatant free hemoglobin [fHb] concentration).[2]

Out of the various putative mechanisms proposed for the development of these RBC storage lesions, the cumulative oxidative injury to the stored RBC units and the accompanying reduction in the antioxidant capacity (AOC) of both stored RBCs and the supernatant have gained the maximum acceptance.[3]

The "storability" of the PRBCs is determined by a number of factors including donor variables, the storage system used for their preservation, and the duration of their storage.[4,5] Donor characteristics differ widely and these factors may determine the AOC of the PRBCs as well as their supernatant. These factors will subsequently result in a variation in the rate and magnitude of the development of RBC storage lesions in these stored RBC units.[6]

Uric acid (UA) is an important antioxidant present in the plasma and has been found to be responsible for more than 60% of the total AOC of the plasma.[7] The serum UA levels of the donors may be influenced by a number of factors. Serum UA levels are found to be relatively higher in males, individuals consuming a purine-rich diet (meat and seafood), alcohol consumption (beer), moderate to heavy exercise, and with high muscle mass.[8] Serum UA levels may also be transiently increased in individuals with chronic inflammatory conditions. Age has not been found to have a significant effect on serum UA levels.[4,8] It acts as a scavenger for the reactive oxygen species (ROS) and mops up the free radicals, thereby reducing the oxidative RBC membrane damage. Donor variations in serum UA levels at the time of blood donation, in addition to various other factors, determine the capacity of the stored RBCs to resist the accumulation of storage lesions by reducing the oxidative damage sustained by them due to the ROS.[9]

This study aimed at finding whether the serum UA levels of blood donors have an effect on the increase in the supernatant fHb levels in leukoreduced CPD/SAGM stored PRBC units.

Materials and Methods

Study population

This single-center prospective observational study was carried out in the department of immunohematology and blood transfusion in a tertiary care hospital in western India for a period of 2 years (September 2018 to September 2020). The study population included healthy voluntary male blood donors who met the latest (2017) donor selection criteria laid down by National Blood Transfusion Council, India. A total of 100 cases were evaluated. Ethical approval for this study was obtained from the institutional ethics committee.

Sample size estimation

The sample size was estimated using the two-tailed test for hypothesis testing about the difference between means. The sample size was estimated to be a minimum of 24 in each group, with a sample error of 5% at a 95% confidence interval. The study was powered at 90%.

Inclusion criteria

All healthy voluntary male blood donors who met the latest donor selection criteria and consented to participate in the study were included. Only PRBC units collected in 450 ml CPD-SAGM bags with integral leukofilters were included in the study. Written informed consent was obtained from all the volunteers.

Exclusion criteria

Female donors and male smokers were excluded. Female donors were excluded from this study because the average serum UA level in females is lower than that in males[10,11] and also because the female hormone (estrogen) has been found to reduce the RBC storage lesions[12-14] and thus, would have been a confounding factor in this study.

Methodology

Blood samples for serum UA estimation of donors were collected from the diversion pouch attached to the blood bag. The serum UA levels were estimated on the same day using Siemens Dimension® EXL™ 200 Integrated Chemistry System using Kalckar modification of the uricase method.[15] Thereafter, the collected whole blood was separated into components – leukoreduced PRBC units, fresh frozen plasma, and RDP. These PRBC units were then stored at 2°C–6°C post processing.

The PRBC units from donors with serum UA >7 mg/dL constituted the high UA group. Those with serum UA <5 mg/dL constituted the low UA group. PRBC units from donors with serum UA levels between 5 and 7 mg/dL were not included in the study. Representative samples (10 ml) were taken from the PRBC units through the pilot tube of the blood bag while observing strict aseptic precautions. The samples were collected in Falcon tubes (graduated conical bottom centrifuge tube) of 14 ml capacity which was centrifuged at 1500 g for 5 min to separate the supernatant.

Thereafter, the supernatant FHb levels were estimated on day 1. The blood bags were then stored back at 2°C–6°C for another 20 days and assessed again on the 21st day of collection for supernatant fHb levels as stated above.

The hematocrit of the PRBC in the blood bags was determined using five-part fully automated cell counter (COULTER® LH 750 Hematology Analyzer) on both day 1 and day 21. These hematocrit values were later used for the calculation of percentage hemolysis. fHb was estimated using plasma low hemoglobin system with a microcuvette (HemoCue®, Angelholm, Sweden).

In a unit of stored PRBCs, the concentration of fHb depends on the number of disintegrated RBCs and the volume of plasma.[16] The same percentage hemolysis may thus give a much higher concentration of free plasma hemoglobin in a red cell concentrate with higher hematocrit.[17-19] Therefore, the degree of hemolysis is often described as the percent of fHb in relation to the total. It is essential to correct for the hematocrit to avoid overestimation of the percent hemolysis in a product. The formula for calculating the percent hemolysis is given below:

Percent hemolysis (%) = (100 − hematocrit) × fHb in plasma or suspending medium (g/dL)/total Hb (g/dL)

Statistical analysis

Data were captured in MS Excel spreadsheet. For data analysis, computer software (Statistical Package for Social Sciences, IBM SPSS, Version 23.0 for Windows, IBM Corp., Armonk, NY, USA) was used. Descriptive statistics were elaborated in the form of means and standard deviations for continuous variables, and frequencies and percentages for categorical variables. The difference in the percentage hemolysis was calculated by subtracting percentage hemolysis in the supernatant on day 1 from that on day 21 for each blood bag. Independent samples *t*-test assuming unequal variances was used for comparison of the difference in percentage hemolysis on day 1 and day 21 in the two groups of blood donors, i.e., those with serum UA >7 mg/dL versus those with serum UA <5 mg/dL at the time of donation. Pearson's correlation coefficient was calculated to explore the linear correlation between two continuous variables. The level of significance was taken as $P < 0.05$.

Results

Three hundred and fifty seven blood donors who fulfilled the inclusion criteria were selected for the estimation of serum UA levels. The age of the donors ranged from 19 to 52 years and it showed a normal distribution [Figure 1]. The mean age of the donors was 35.07 ± 5.02 years [Table 1]. The serum UA levels showed normal distribution [Figure 2]. The mean donor serum UA level was 5.75 ± 1.15 mg/dL [Table 1]. Out of these 357 donors, PRBC units collected from 100 donors (50 donors with serum UA >7 mg/dL who constituted the high UA group and 50 donors with serum UA <5 mg/dL who constituted the low UA group) were selected for the final study.

Only 14% of the donors had serum UA >7 mg/dL and 22% had serum UA <5 mg/dL [Figure 3].

In the low UA group (serum UA <5 mg/dL), the mean percentage hemolysis in supernatant on day 1 was 0.105 ± 0.022 and on day 21, it was 0.246 ± 0.048. The mean of difference in percentage hemolysis in supernatant on day 21 and day 1 was 0.140 ± 0.031 [Table 2 and Figure 4]. Whereas, in the high UA group (serum UA >7 mg/dL), the mean percentage hemolysis in supernatant on day 1 was 0.104 ± 0.022 and on day 21 it was 0.198 ± 0.036. The mean of difference in percentage hemolysis in supernatant on day 21 and day 1 was 0.092 ± 0.019 [Table 2 and Figure 4].

The mean of the differences in percentage hemolysis in the supernatant in low UA group (<5 mg/dL) was higher than the mean of the differences in percentage hemolysis in the supernatant in high UA group (>7 mg/dL) and this was statistically significant ($P < 0.001$) using the two samples *t*-test assuming unequal variances. The percentage hemolysis on day 21 was within regulatory limits (<1%) in all of the bags included in this study. The donor serum UA level and difference in percentage hemolysis on day 21 and day 1 were found

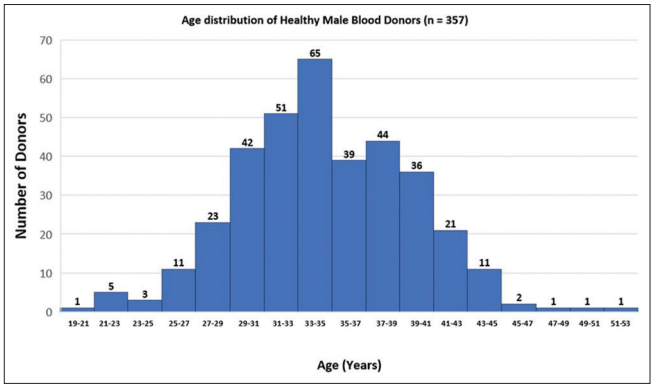

Figure 1: Age distribution of blood donors (*n* = 357). Age of blood donors is represented on X-axis and total no of blood donors on Y-axis

Table 1: The age distribution and serum uric acid levels of blood donors

	Mean	SD	Minimum	Maximum
Age (Years)	35.07	5.02	19	52
Serum Uric Acid level (mg/dL)	5.75	1.15	2	9

Table 2: Mean supernatant percentage hemolysis in low uric acid and high uric acid groups

	Day 1 (mean Supernatant percentage hemolysis)	Day 21 (mean Supernatant percentage hemolysis)	Mean of Difference in Supernatant percentage hemolysis	*P* (Mean of Difference in Supernatant percentage hemolysis)
UA <5 mg/dL	0.105±0.022	0.246±0.048	0.140±0.031	< 0.05
UA >7 mg/dL	0.104±0.022	0.198±0.036	0.092±0.019	< 0.05

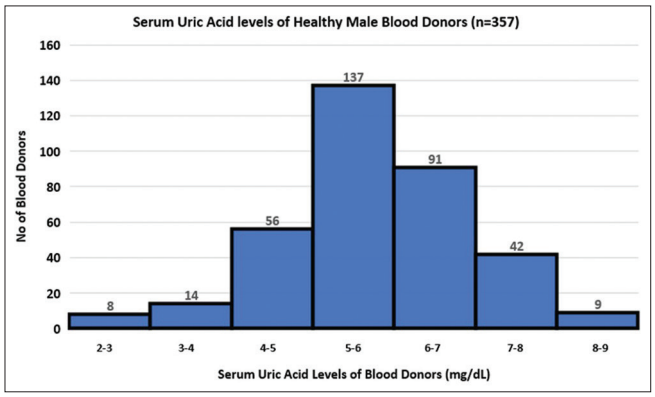

Figure 2: Showing serum uric acid levels of blood donors (*n* = 357). Serum uric acid levels of blood donors are represented on X-axis and total no of blood donors on Y-axis

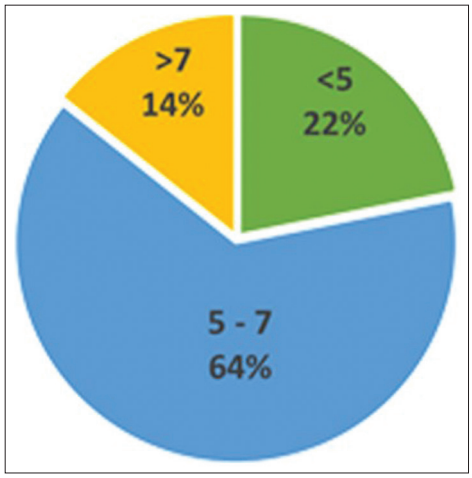

Figure 3: Pie chart showing the distribution of serum uric acid levels in blood donors (*n* = 357)

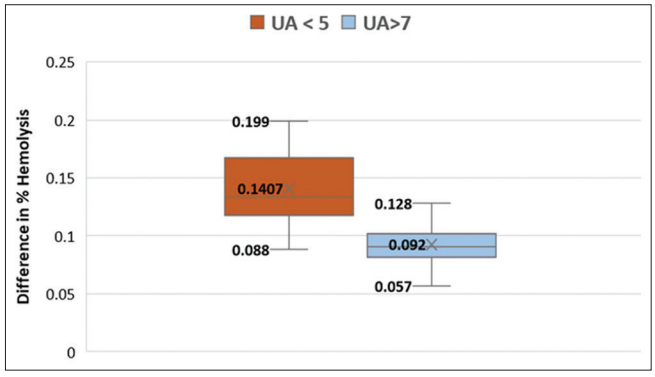

Figure 4: Box and whisker plot showing the mean of difference in percentage hemolysis in supernatant on day 21 and day 1 in low uric acid and high uric acid groups

to be negatively co-related with Pearson's correlation coefficient $r = -0.62$ [Figure 5].

Discussion

This study evaluated whether the donor-related variation in serum UA levels is associated with the storability of donated RBCs under storage in CPD/SAGM.

The "storability" of the blood collected from healthy regular blood donors depends on a number of factors including the blood bag/anticoagulant – preservative system used, processing techniques, storage conditions, and donor characteristics.[5] Donor characteristics will decide various donor variable associated factors in the stored RBCs and the supernatant, and therefore, the resistance to the development of storage lesions as well as the posttransfusion recovery of the red cells.[20] The rate and magnitude of development of RBC storage lesions depend on the ability of stored units to cope with a range of oxidative stimuli and defects.[14,17] Intrinsic variability in donor UA levels seems to influence RBC storage lesions. Although it is not known at present whether this protective effect conferred by UA is by a direct antioxidant function of UA inside the RBCs unit or due to its capability to reveal the redox homeostasis of the individual donor, normal-range variation in UA might be used to evaluate the susceptibility of stored PRBC units to storage lesions.[12,18]

The biochemical and biophysical profile of different donors differs widely because of the inherent heterogeneity of the population. Hence, the capacity to resist oxidative stress during storage will also differ even if all other factors are constant.[20] It has been shown in various studies that various characteristics reflecting the storage lesions in stored PBC units such as RBC shape, fragility, hemolysis, metabolism, redox homeostasis, and other aging-related variables are a function of donor factors as well as the storage system used.[20,21]

Our study, by assessing 100 voluntary, regular donors, showed that serum UA might be responsible for a significant part of the donor-related AOC of the supernatant. In this study, the difference in percentage hemolysis in the supernatant decreased as the baseline UA of the donor increased. This might be due to the antioxidant effect provided by the UA present in the supernatant and/or its influx into the stored RBCs.

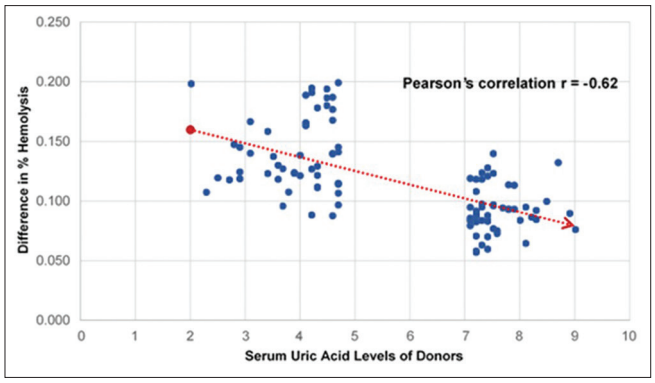

Figure 5: Scatter plot showing the difference in the percentage hemolysis on day 21 and day 1 in individual packed red blood cell units on Y-axis versus serum uric acid levels of the donors on X-axis

The serum UA levels among the donors showed a normal distribution profile, and hence, we selected the "tails" of the Gaussian curve to study the potential impact of widely different baseline levels of UA on hemolysis in the supernatant. PRBC units from the donors located at the higher levels of the curve (higher-UA donors) exhibited lower difference in percentage hemolysis in the supernatant at day 21 of the storage period compared to the units of low-UA donors.

A similar study by Tzounakas *et al*.[4] did not find any statistical difference between supernatant fHb in the low and high UA groups. However, their sample size was very small (n = 8) and they used supernatant fHb for analyzing their data, whereas we used percentage hemolysis. Furthermore, the PRBC units used in their study were nonleukoreduced. Another study by Bardyn *et al*.[22] concluded that the antioxidant power (AOC) in RBC concentrates and the extracellular UA concentration were positively correlated.

Donor UA presents in the residual plasma of the RBCs unit might reduce the ROS and reactive nitrogen species accumulation, leading to fewer oxidative hits to protein and lipid targets in the soluble component and the RBC surface.[23-25] Hence, the choice of a storage system with UA as one of the constituents of the red cell additive solution can lead to a further reduction in storage lesions and potentially enhance the quality of the RBC units.[7,8,22]

Conclusion

Accurate evaluation of the RBC storage lesions and their correlation with the donor characteristics will help us in determining which donor factors are important and which are not. This will help the transfusion services concentrate on the factors which they should study in detail. Moreover, this might lead to small changes in the RBC storage strategies (storage conditions and composition of the RBC additive solutions) which will accrue over time and lead to improvement in the quality of stored packed red cells.

Although this study had a few limitations namely, only serum UA levels of the donors were taken into consideration, whereas other factors contributing to inter-donor variability (age, sex, dietary habits, exercise, alcohol consumption, smoking, obesity, etc.,) were left unexplored and supernatant hemolysis was the only red cell storage lesion that was analyzed in this study, there is ample evidence that higher donor UA levels improve the storability of PRBC units. Hence, we strongly recommend UA for consideration and evaluation in future large-scale studies on donor variables, to explore the potential usefulness of UA as a constituent of RBC additive solutions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Hess JR. Red blood cell storage. In: McManus LM, Mitchell RN, editors. Pathobiology of Human Disease. San Diego: Elsevier; 2014. p. 3081-7.
2. Chin-Yee I, Arya N, d'Almeida MS. The red cell storage lesion and its implication for transfusion. Transfus Sci 1997;18:447-58.
3. Racek J, Herynková R, Holecek V, Jerábek Z, Sláma V. Influence of antioxidants on the quality of stored blood. Vox Sang 1997;72:16-9.
4. Tzounakas VL, Georgatzakou HT, Kriebardis AG, Papageorgiou EG, Stamoulis KE, Foudoulaki-Paparizos LE, *et al*. Uric acid variation among regular blood donors is indicative of red blood cell susceptibility to storage lesion markers: A new hypothesis tested. Transfusion 2015;55:2659-71.
5. Roubinian NH, Plimier C, Woo JP, Lee C, Bruhn R, Liu VX, *et al*. Effect of donor, component, and recipient characteristics on hemoglobin increments following red blood cell transfusion. Blood 2019;134:1003-13.
6. Tzounakas VL, Georgatzakou HT, Kriebardis AG, Voulgaridou AI, Stamoulis KE, Foudoulaki-Paparizos LE, *et al*. Donor variation effect on red blood cell storage lesion: A multivariable, yet consistent, story. Transfusion 2016;56:1274-86.
7. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc Natl Acad Sci U S A 1981;78:6858-62.
8. De Oliveira EP, Burini RC. High plasma uric acid concentration: Causes and consequences. Diabetol Metab Syndr 2012;4:12.
9. Bartosz G. Total antioxidant capacity. In: Spiegel HE, Nowacki G; Hsiao K-JBT-A in CC, editors. Advances in Clinical Chemistry. NewYork: Elsevier; 2003. p. 219-92.
10. Chiou WK, Wang MH, Huang DH, Chiu HT, Lee YJ, Lin JD. The relationship between serum uric acid level and metabolic syndrome: Differences by sex and age in Taiwanese. J Epidemiol 2010;20:219-24.
11. Tani S, Matsuo R, Imatake K, Suzuki Y, Takahashi A, Yagi T, *et al*. The serum uric acid level in females may be a better indicator of metabolic syndrome and its components than in males in a Japanese population. J Cardiol 2020;76:100-8.

12. Raval JS, Waters JH, Seltsam A, Scharberg EA, Richter E, Kameneva MV, *et al*. Menopausal status affects the susceptibility of stored RBCs to mechanical stress. Vox Sang 2011;100:418-21.
13. Massafra C, Gioia D, De Felice C, Picciolini E, De Leo V, Bonifazi M, *et al*. Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalase and glutathione peroxidase activities during the menstrual cycle. J Endocrinol 2000;167:447-52.
14. Tzounakas VL, Anastasiadi AT, Drossos PV, Karadimas DG, Valsami SÉ, Stamoulis KE, *et al*. Sex-related aspects of the red blood cell storage lesion. Blood Transfus 2021;19:224-36.
15. Galbán J, Andreu Y, Almenara MJ, de Marcos S, Castillo JR. Direct determination of uric acid in serum by a fluorometric-enzymatic method based on uricase. Talanta 2001;54:847-54.
16. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: Role of red blood cell breakdown. Transfusion 2011;51:844-51.
17. Sawant RB, Jathar SK, Rajadhyaksha SB, Kadam PT. Red cell hemolysis during processing and storage. Asian J Transfus Sci 2007;1:47-51.
18. Sowemimo-Coker SO. Red blood cell hemolysis during processing. Transfus Med Rev 2002;16:46-60.
19. 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.
20. Tzounakas VL, Kriebardis AG, Papassideri IS, Antonelou MH. Donor-variation effect on red blood cell storage lesion: A close relationship emerges. Proteomics Clin Appl 2016;10:791-804.
21. Tarasev M, Alfano K, Chakraborty S, Light L, Doeden K, Gorlin JB. Similar donors-similar blood? Transfusion 2014;54:933-41.
22. Bardyn M, Maye S, Lesch A, Delobel J, Tissot JD, Cortés-Salazar F, *et al*. The antioxidant capacity of erythrocyte concentrates is increased during the first week of storage and correlated with the uric acid level. Vox Sang 2017;112:638-47.
23. Costantini D. On the measurement of circulating antioxidant capacity and the nightmare of uric acid. Methods Ecol Evol 2011;2:321-5.
24. Kanias T, Acker JP. Biopreservation of red blood cells – The struggle with hemoglobin oxidation. FEBS J 2010;277:343-56.
25. D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. Red blood cell storage: The story so far. Blood Transfus 2010;8:82-8.