

Original Research Article

## **Behaviour of Uric Acid, the Final Metabolic Product of Purines, in Stored CPDA (Citrate-Phosphate-Dextrose-Adenine) Whole Blood Units**

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**Abstract:** Some nutrient media like CPDA (Citrate -phosphate-dextrose-adenine), for blood storage, contain adenine for improved storage of erythrocytes, but the dynamics of its final catabolic product i.e. uric acid (UA) has not been studied in detail. Sixteen CPDA-1 whole blood bags were used for baseline and subsequent weekly determination of UA, Potassium (K), Sodium (Na) and Albumin (Alb) in the extracellular fluid and Hemoglobin (WB Hb) in whole blood during the initial four weeks of storage using automated machines. Unidirectional changes were seen in concentrations of K (increase; ANOVA  $p < 0.0001$ ), Na (decrease; ANOVA  $p < 0.0001$ ) whereas no change was seen in WB Hb (ANOVA  $p = 0.99$ ) and a tri-modal pattern (increase-decrease-increase) was observed for UA (ANOVA  $p < 0.0001$ ) and Alb (ANOVA  $p = 0.01$ ) across the storage period. The changes in K and UA might prove useful in confirming the age of blood bags.

**Keywords:** CPDA, Uric acid, Potassium, Sodium, Albumin, Hemoglobin

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### **INTRODUCTION**

Apart from erythrocytes, the major constituent, whole blood consists mainly of leucocytes and thrombocytes [1]. This tissue which circulates gases and nutrients in the body is transfusable and consequently of great therapeutic importance. Prolongation of storage period of blood along with retention of its clinical effectiveness has helped in greater utilization of this scarce resource. One widely accepted anticoagulant-nutrient medium is 'citrate-phosphate-dextrose-adenine' (CPDA). It has shown that adenine along with citrate, phosphate and dextrose in solution can be used to store blood cells at 2-4 °C for 35 days because it resulted in higher adenosine triphosphate levels inside red blood cells (RBCs) and subsequently better preservation of RBC glycolytic ability [2].

As RBCs are devoid of nucleus and WBCs although nucleated are minimal in number, it can be hypothesised that uric acid (UA), the final metabolic product of purines, formed in CPDA blood bags is sourced at least partially from adenine provided in the nutrient medium. RBCs, in blood bags, have been

shown to take up extracellular adenine [3]. The adenine entering RBCs can be metabolised to hypoxanthine, which may move out into the plasma [4]. Not surprisingly RBCs and platelets have been reported to be the major source of hypoxanthine and xanthine in the serum [5] with hypoxanthine levels showing a 1000 fold increase when stored with blood cells for 24 hours [6]. Hypoxanthine and xanthine are further metabolized to UA by the enzyme *xanthine oxidase*. This enzyme is present in highest concentrations in hepatic tissue but also has been demonstrated in plasma [7-10].

Plausibly, the level of UA in blood storage bags might depend on (a) the un-physiological concentrations of adenine in nutrient mediums and (b) the closed environment with no escape route for UA, an excretory product. UA levels in turn can have a bearing on blood cell stability/viability both before and after transfusion as (a) UA has been reported to provide antioxidant protection to erythrocytes both in animals [11] as well as humans [12] and (b) UA also has also been identified as a pro-inflammatory and pro-thrombotic molecule *in vivo* [13]. These facts and

assumptions coupled with the dearth of reports on UA levels in CPDA blood storage bags prompted us to serially assess extracellular UA along with some other common parameters, including sodium (Na) and potassium (K), in CPDA-1 blood bags.

**MATERIALS AND METHODS**

This study was performed in a tertiary care hospital in India where serial samples from sixteen polyvinyl-chloride CPDA-1 blood bags (Mitra Pvt. Ltd., India) that were set aside for regular “quality control and stability tests” were used for evaluation of some additional biochemical parameters over a period of Jan 2016 to Feb 2017.

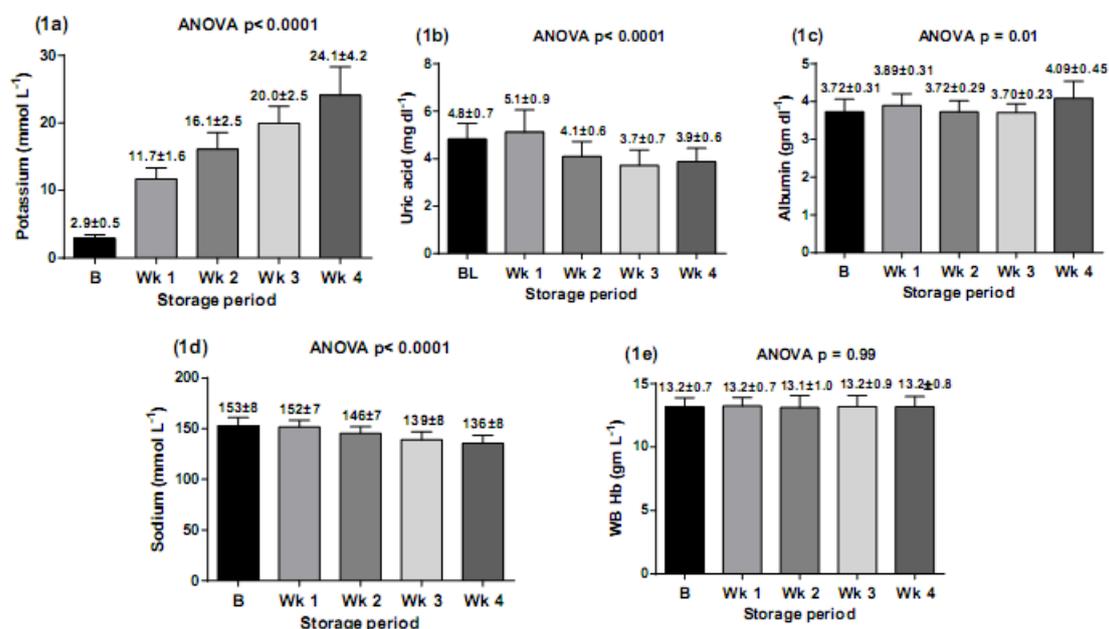
Standard guidelines were followed while collecting blood [14]. The bags were placed in blood storage cabinets (Terumo Penpol, India) which maintained a temperature of 2 to 6°C after 350 ml of blood was collected in 49 ml of CPDA-1. Before each sample collection (of around 10 ml) from the bag under aseptic conditions by sterile connecting device (Terumo Penpol, India), the contents of the bag were properly mixed. Immediately after sample collection the bags were placed back in blood storage cabinets. Baseline and subsequent weekly samples were collected on day one (the day of donation/ baseline), seven, 14, 21 and 28. The sample was divided into two portions. One part

was immediately centrifuged and the supernatant processed in fully automated XL series analyser (Vital Scientific, Netherlands) for spectrophotometric determination of UA (uricase method), K and Na (both by ion selective electrode ). The same analyser was also used for measuring concentration of albumin (Alb) by BCG method. The other portion of the sample was treated with EDTA and processed in LabLife H3D Premier automated hematology analyser (DIAGNOVA, India) to estimate whole blood hemoglobin (WB Hb).

Results are presented as mean ± SD. One way ANOVA (analysis of variance) was used to examine the (i) weekly levels and (ii) weekly percentage changes of various parameters over four weeks. Weekly percentage changes of parameters were calculated to measure degree of alteration of the same on subsequent weeks. A *p* value < 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism version 6.00 (GraphPad software, La Jolla California USA, www.graphpad.com). Ethical committee permission from the institute was taken.

**RESULTS**

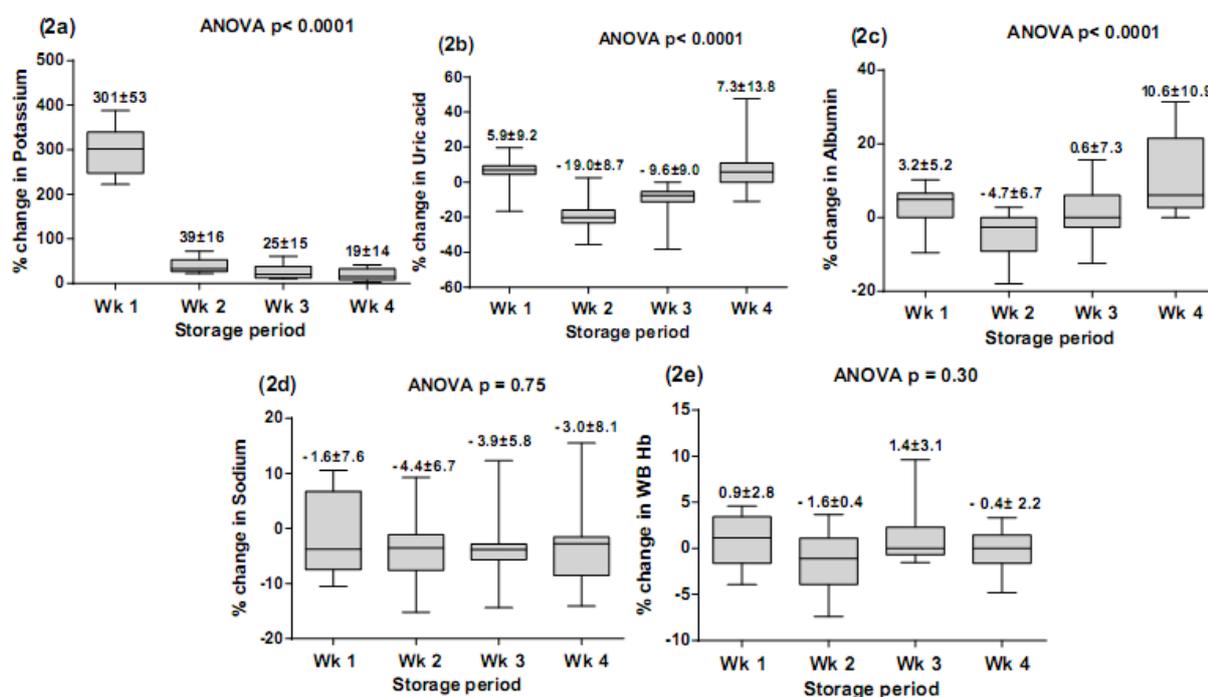
K levels showed an impressive rise throughout the storage period which was statistically very significant (Fig. 1a; ANOVA *p*< 0.0001).



**Fig-1: Extracellular levels of various parameters in CPDA-1 whole blood bags at the end of each of the first four weeks of storage. WB Hb: Whole blood hemoglobin. Values are expressed as Mean± SD, n=16**

As shown in Fig. 2a, the weekly percentage rise in K level was greatest at the end of first week (300%) whereas further weekly increases in K level show a constant falling trend with no increase greater than 75% (ANOVA  $p < 0.0001$ ). UA, levels, in contrast, increased till the end of first week and then decreased till the end of third (Fig. 1b). The changes in UA levels can be seen from a different perspective as weekly percentage change in its levels (Fig. 2b), which shows that there was a weekly increase at the end of first week followed by the greatest weekly decrease of 20% at the

end of second weekly and after that the magnitude of weekly decrease steadily fell to actually show a weekly increase at the end of fourth week (ANOVA  $p < 0.0001$ ). Alb mimics these patterns shown by UA (Fig. 1c; ANOVA  $p = 0.01$  and Fig. 2c; ANOVA  $p < 0.0001$ ), although to a lesser magnitude. Unlike UA and Alb, Na showed a continuous decrease over the storage period (Fig 1d; ANOVA  $p < 0.0001$ ). WB Hb (Fig.1d and 2d) showed an almost constant level throughout the storage period (ANOVA  $p = 0.99$ ) and likewise the weekly percentage change was almost zero (ANOVA  $p = 0.30$ ).



**Fig-2: Weekly percentage change in various parameters in CPDA-1 whole blood bags at the end of first four weeks of storage. WB Hb: Whole blood hemoglobin. Calculated values are expressed as Mean ± SD, n= 16**

**DISCUSSION**

K being the major intracellular cation, its levels in stored blood bags is positively associated with haemolysis. Ours and also other studies [15, 16] show a spectacular rise of K levels across the storage period of blood bags. A previous study by Downman *et al.* [17] supports our finding that the increase in K is many times higher in the first week when compared to subsequent weeks of storage. Continued haemolysis will cause dilution of extracellular fluid which corresponds well with the decreasing levels of extracellular Na (which has low intracellular levels) with the passage of time. However concentrations of UA and Alb show a raised level by the end of first week

followed by progressive decrease. It is also notable that the percentage decrease in UA and albumin was the greatest between the first and second week. These striking similarities shown by UA and Alb over storage time point towards either a possible decrease in extracellular fluid volume in the first week and beyond two weeks of storage or an increase in formation of extracellular UA and Alb in the same period. It has been reported that when stored in different mediums like Citrate, Citrate-glucose and Acid-citrate-glucose the mean corpuscular volume (MCV) of erythrocytes increases to various extents [18]. Sheep whole blood stored in CPDA-1 has also shown increasing MCV on day seven and 21[19]. It is plausible that during the

initial days of storage in CPDA-1 there is enlargement in RBC size which is accompanied with a relatively intact, selectively permeable, RBC membrane so that while water can freely enter RBCs, other constituents cannot, thereby increasing their concentration in plasma. Since haemoglobin was that of 'whole blood', as expected, it neither showed any change in levels nor followed the pattern of weekly percentage changes that was seen for UA or Alb. The deteriorating structure of plasma membrane with increase in storage time might be an explanation for the decreasing level of some parameters (UA and Alb) beyond the first week. Formed elements of blood are not known to produce Alb and we are unable to explain rise in Alb levels beyond the third week whereas a partial explanation for the observed levels of UA across the studied storage period has been provided below.

In our study, among the similar undulating patterns observed for a few parameters, the path followed by UA was the most striking and of the greatest amplitude. We suggest that these accentuations for UA might be contributed to by the production of UA from adenine in CPDA-1 blood bags coupled with its low solubility. Although RBCs have lost the capacity to carry out a number of biochemical reactions, they have retained the necessary pathways of nucleotide metabolism and some carbohydrate linked metabolisms [4]. The major pathway of glucose metabolism in RBCs is anaerobic glycolysis which generates ATP and lactic acid (which decreases the pH of blood). In stored whole blood, by 42 days, 95% of the adenine is cleared from the extracellular fluid while the cellular ATP concentrations stay over 60 % of the initial level [3]. While RBCs/ platelets utilize adenine (of the blood as well as the nutrient medium in CPDA bags) and contribute to the hypoxanthine-xanthine-UA pool, the inability of WBCs to do the same can be explained by the absence of adenase and guanase in these cells [20]. The final catabolic product of adenine i.e. UA followed a rising-falling-rising pattern which was more prominent than that for Alb. Two possible causative factors for the undulating pattern followed by UA and their explanations are attempted: (i) UA is soluble in alkaline medium and precipitates down as the medium becomes more acidic [21]. It is obvious that precipitation of UA would decrease its concentration in the solvent (extracellular fluid). Thus increased UA level at the end of the first week of storage can be hypothesized to be due to the initial high glycolytic activity (by relatively undamaged RBCs metabolizing plentiful of glucose and adenine) forming abundant ATP (followed by its consequent degradation into UA)

coupled with the not yet greatly decreased pH of the medium as shown by Mukherjee *et al* [15]. Since lactic acid (the major determinant of falling pH) progressively accumulates as a by-product of the ongoing glycolysis, the resultant decreased pH might be conducive to increased rate of precipitation of UA, thus contributing to the slump in its level in the extracellular fluid beyond the first week. (ii) Another possible contributing reason for the decrease in UA levels, we suggest, might be utilization of UA to counter progressive oxidative stress inside stored blood bags. It has been reported earlier that oxidative stress in blood bags increases with storage time [22] and also that during exertion of its antioxidant properties UA is converted to allantoin [23]. We can further hypothesize that these factors may be acting in tandem. The two possible reasons that we were able to suggest explain the increase of UA at one week and ensuing decrease but not its subsequent rise on further storage.

It might be highlighted that due to accumulation of unwanted changes in stored blood over time, age of blood bags has been a concern in specific cases of blood transfusion: transfusion of packed RBC older than 7 days may contribute to haemorrhagic disorders in critically ill patients [24], blood < 14 days old is considered to be beneficial in transfusion for beta thalassaemia patients [25], whereas blood stored for >14 days has been associated with post-operative complications and decreased survival in patients of cardiac surgery [26] and splanchnic hypoxia [27].

Knowing the age of blood bags is important as they have a definite shelf-life and certain clinical situations, as described above, prefer fresh stored blood (seven to 14 days old). "First-in-first-out" (FIFO) policy is the usual practice for release of blood bags from the blood bank. It is obvious that a properly maintained chronological record will remain the cornerstone for determining the age of blood units as it is the easiest and most accurate way. We reckon that the observations of our study are primarily of theoretical interest but nevertheless would like to furnish our study with a possible practical use. Some observations that we make in respect to the weekly percentage increase in K, UA (which show the most remarkable changes) and the age of blood bags are as follows:

- (1) One week old blood (very fresh blood) was clearly distinguishable (from older blood) biochemically as a seven day old blood showed a large weekly increase (from day one), of K levels, of around 300% and this was further complimented with a concurrent weekly increase in uric acid levels.

- (2) Two to three week old blood were quite indistinguishable from each other as both showed a much lower increase in K (not beyond 75%) from the previous week and a simultaneous weekly decrease in UA levels.
- (3) Four week old blood might show a weekly increase in UA although similar to the previous two weeks it showed a very low increase in K (not beyond 75%)

Thus two samples separated by a period of one week can be employed to guess the age of a blood bag. Considering the fact that blood-banks are manually controlled to different extents and also that this commodity might be invaluable or scarce at times (especially in case of rare blood groups), such an extension of knowledge may prove to be useful in atypical situations.

As blood bag contents can be conveniently and safely extracted at any time of their shelf life for biochemical evaluation by "sterile connecting device", it would be desirable to conduct similar further studies on a larger number of parameters for the full shelf-life (five weeks) of CPDA-1 blood units to further scrutinize their dynamics in blood bags.

The major limitations of our study are (i) exclusion of the last week of storage (ii) the small sample size and (iii) non-inclusion of glucose and pH measurement of the extracellular fluid of blood bags as these parameters were employed in the explanation offered for our observation.

## CONCLUSION

In CPDA blood bags, UA and Alb concentrations show a rise from baseline at one week, subsequent fall and then rise beyond three weeks of storage. The percentage weekly rise in K levels is the greatest in the first week whereas UA shows an increase in the same at the end of one week and beyond two weeks of storage. These levels, especially those of K and UA can be employed to determine the approximate age of these blood bags.

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