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Original Article

Transfusion Medicine Section

Effect of Blood Storage on Biochemical Parameters Assessed at Periodic Intervals in CPDA1 Blood Bags

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ABSTRACT

Introduction: Blood when stored at 4°C, effectuates alterations in biochemical and mechanical properties of red blood cells because of storage conditions, which are called as storage lesions. Red blood cells lose their viability over a period of time even after storing blood with Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant.

Aim: To observe the changes in biochemical parameters namely Glucose, Urea, Creatinine, Total protein, Aspartate Transaminase (AST), Albumin, Sodium, Potassium and Chloride in stored blood.

Materials and Methods: The present study was a prospective study conducted on blood samples donated by 50 healthy voluntary donors. Biochemical parameters namely Glucose, Urea, Creatininie, Total protein, AST, Albumin, Sodium, Potassium and Chloride were estimated in stored blood collected in CPDA1 blood bags on 0,3,7,14 and 21st day. All the data were analysed using SPSS 22.0 software. Student's t-test was used to find the effect of blood storage on its biochemical parameters. Correlation analysis was used to find the relationship between biochemical parameters and period of storage.

Results: Significant changes were observed in serum AST, Total protein, Albumin, Urea, Chloride, and Potassium levels (p-value <0.05). Rest of the biochemical parameters did not show any significant change over the period of storage time.

Conclusion: In the present study, authors observed that there were significant changes in some of the biochemical parameters namely serum AST, Total protein, Albumin, Urea, Chloride, and Potassium levels (p-value<0.05) assessed at periodic intervals. The changes might be due to spontaneous haemolysis or non viable red blood cells caused due to storage. Therefore, it is better to transfuse blood as early as possible in order to prevent a negative impact on the biochemical composition of red blood cells. Also, usage of CPDA2 or SAGM anticoagulant solutions may to some extent minimise this issue. The further contrivance of newer anticoagulant solution in blood bags can be focused upon to reduce the impact of storage.

Keywords: Red blood cell, Haemolysis, Transfusion

INTRODUCTION

Blood is an indispensable life-saving drug used in saving the life of critically ill patients. Blood is stored at 4°C with an appropriate anticoagulant solution. In the process, red blood cells deteriorate progressively to effectuate alterations in biochemical and mechanical properties which are called as storage lesions [1]. Red blood cells lose their viability over a period of time leading to haemolysis and negative impact on different biochemical analytes [2]. Under normal conditions in the body's circulation, these do not occur as optimum temperature, pH, nutrient concentration and waste product removal are well maintained [1].

Erythrocyte rupture and release of intracellular contents leading to haemodilution or concentration of haemoglobin are some of the mechanisms by which haemolysis affects the concentration of different biochemical parameters [3].

Due to storage of blood at 4°C, RBC's lose their viability either due to prolonged contact with plasma or inability of the cells to survive in recipient's circulation following transfusion. Due to this, there are several changes in biochemical parameters [4,5].

Donated blood is stored in CPDA-1 blood bags. This anticoagulant is composed of Citrate (chelates ionised calcium that prevents coagulation), Dextrose (a source of energy for the red blood cells), phosphate-containing anticoagulants (lower acidity than other anticoagulants without phosphate have a higher concentration of 2,3 BPG and red cell phosphate) and Adenine (ATP content and post-transfusion viability of red cells regenerated by addition of adenine) [6].

Review of literature shows very few studies that correlate the usage of CPDA-1 blood bags and the effect of prolonged contact of plasma with red blood cells resulting in changes in analyte concentration due to the ongoing metabolism of cellular constituents [7-9].

Thus the present study was designed to study the effect of blood storage on biochemical parameters assessed at periodic intervals.

MATERIALS AND METHODS

The present prospective study conducted at the Department of Biochemistry and Department of Pathology (Blood bank) at The Oxford Medical College Hospital and Research Centre, Bangalore, India from December 2017 to February 2018. The study protocol was approved by Institutional Ethical Committee before the commencement of the study. All the healthy voluntary donors aged 18-55 years were included in the study. The exclusion criteria were; donors suffering from hypertension, diabetes and tuberculosis. Donors, not voluntary willing to take part in the study and insufficient blood bags (less than 450 mL of blood) was deferred.

A 450 mL of blood was drawn from 50 healthy volunteer donors into CPDA-1 anticoagulant (63 mL) containing blood bags. Blood was collected with adequate safety precautions to avoid contamination and infection. Blood donors were screened as per regulations of drugs and cosmetics rules, Government of India [4]. Blood bags were carefully stored in a quarantine shelf in the blood bank at 2-4°C.

Citrate-phosphate dextrose adenine solution was developed in 1968 and shown to permit whole-blood storage for 5 weeks [5]. Most blood collection bags (adult) contain 63 mL CPDA-1 anticoagulant which is sufficient to anticoagulant and ensures the viability of blood cells in 450 mL±10% blood for up to 28-35 days when the blood is stored at 2-8°C [6]. A 50 mL of the blood sample from each blood bag was taken for study purpose and stored in plain bags under all aseptic precautions without contamination

of the blood bags. Rest of the blood was used for transfusion purpose. Effect of storage was analysed at 0, 3, 7, 14 and 21 days interval by withdrawing 5-10 mL blood each time from the bag. The sample analysed on zero days served as control. Biochemical parameters were measured using Erba EM 360 autoanalyser and Diamond Prolyte electrolyte analyser.

Aspartate aminotransferase (AST) (IFCC method without pyridoxal phosphate activation), creatinine (Jaffe kinetic method), Glucose (GOD-POD method), Urea (Urease Glutamate dehydrogenase method), Total protein (Biuret method), Albumin (Bromocresol green method) were assayed using Erba EM 360 autoanalyser and electrolytes were assayed using Diamond Prolyte electrolyte analyser (Direct ISE method).

STATISTICAL ANALYSIS

Mean and standard deviation was calculated using SPSS version 22.0. All values are quoted as mean±SD. Student's t-test was used to find the effect of blood storage on its biochemical parameters. The difference between observations was considered significant at $p<0.05$. Correlation analysis was used to find the relationship between biochemical parameters and period of storage.

RESULTS

In the present study, all the 50 donors were males and there were no female donors. Their age ranged between 18-36 years (mean age 26.36 years) with corresponding blood groups of 24 O 'positive', 15 B 'positive', 8 A 'positive', 2 AB 'positive', 1 O 'Negative' respectively. The various biochemical parameters analysed are shown in [Table/Fig-1].

Significant changes were observed in serum AST, Total protein, Albumin, chloride, potassium and urea levels ($p<0.05$). Rest of the biochemical parameters like glucose, creatinine and sodium did not show any significant changes due to storage time. At the end of 21st day, there was 57.46%, 7.37%, 3.82%, 30.32%, 601% and 14.78% increase in serum aspartate transaminase (AST), Total protein, Albumin, chloride, potassium and urea levels respectively. Even though there was a decrease in levels of glucose, sodium and increase in creatinine levels, these were not statistically significant.

DISCUSSION

In the present study, we observed significant changes in biochemical parameters in whole blood stored in the blood bank. In blood bags the glucose concentration is limited and as energy is utilised there is concomitant ATP (Adenosine Tri-Phosphate) depletion and decrease in red cell viability [10]. So there is a depletion of energy and inhibition of sodium-potassium ATPase pump which leads to hyperkalemia and hyponatremia as observed in the present study [11,12].

There was a significant increase in potassium values from 0 to 21 days of storage which may be due to haemolysis and constant release of potassium ions from cells into surrounding plasma due to storage. This was supported by studies done by Verma M et al., and Tayal et al., [1,7] Adias TC et al., also observed hyperkalemia in their study but they did not find any significant change in Sodium which is in concordance with our study [11], whereas study was done by Verma M et al., observed statistically significant gradual decrease in sodium levels [1].

In the present study, we observed significant changes in AST levels which can be because of haemolysis as observed in other studies. Koseoglu M et al., studied the effects of haemolysis interferences on routine biochemistry parameters. The two parameters which were most affected by haemolysis interference was LDH and AST almost at undetectable haemolysis by visual inspection (plasma haemoglobin <0.5 g/L) while clinically meaningful variations of potassium and total bilirubin were observed in moderately haemolysed samples (haemoglobin >1 g/L) [13]. RBCs contain 20 fold as high concentration of AST as plasma, so even mild haemolysis produces significant alterations in AST. We found 57.46% increase from day 0 to day 21 in AST levels. The disproportionate correlation with AST is consistent with higher concentrations of AST in red blood cells released during intravascular haemolysis [14].

In the present study glucose levels decreased by 54% from 0 days to 21 days of storage which can be explained by ATP depletion over a period of time and consumption of glucose molecules for RBC'S metabolism [10], however statistically, the decrease in glucose levels was not significant in the present study. Latham JT et al., and Bailey DN and Bobe JR, observed decline in concentration of plasma glucose with storage [15,16]. Urea levels were significantly increased (14.78%) on 21 day when compared to 0 day in the present study but most of the studies did not observe statistically significant changes.

Creatinine levels were increased to an extent of 2.63% which was not significant and this increase might be due to interferences by pseudocreatinines as shown by Hein M et al., [17]. In the present study there was increase in chloride levels to an extent of 30.32% from 0 to 21 days of storage which is in contrast with other studies where there is an increase in first few days and followed by a decrease because it starts entering erythrocytes under the influence of its concentration gradient (1.5:1) [1,17].

Total protein and albumin both were significantly increased in the present study and this can be explained by the fact that Haemoglobin (Hb) strongly absorbs light at 540 nm and haemolysis therefore increases absorption in this wavelength range affecting the concentrations of different analytes which are measured in the above wavelength range. The rise in albumin and protein concentration is because of optical interference and intracellular leakage of total proteins. False elevated protein levels were also observed in study by Roman Y et al., [18]. Quantitative estimation of total protein was done by biuret method in which absorbance is measured at 546 nm and Hb which is a protein interferes because it also falls with same absorbance range [19]. However, the method was said to be not significantly interfering till Hb concentration of 250 mg/dL. Authors did not measure Hb

Parameter	Day 0 (N=50) mean±SD	Day 3 (N=50) mean±SD	Day 7 (N=50) mean±SD	Day 14 (N=50) mean±SD	Day 21 (N=50) mean±SD	p-value (p<0.05)
Glucose (mg/dL)	482.82±105.69	440.95±115.16	393.48±115.40	306.70±102.34	221.91±101.43	0.162
Urea (mg/dL)	20.22±7.05	19.79±4.51	20.95±4.58	22.55±4.83	23.21±4.84	0.004**
Creatinine (mg/dL)	1.14±0.12	1.14±0.23	1.13±0.14	1.18±0.14	1.17±0.16	0.393
Tprotein (g/dL)	5.83±0.48	5.74±0.50	5.92±0.46	6.10±0.50	6.26±0.56	0.017*
Albumin (g/dL)	3.40±0.20	3.37±0.24	3.39±0.20	3.46±0.29	3.53±0.25	0.002**
AST (IU/L)	29.39±8.43	21.59±5.70	25.55±7.09	37.22±8.86	46.28±13.03	0.004**
Sodium (mEQ/L)	149.34±2.66	144.19±6.81	139.07±8.75	135.88±8.32	132.06±8.03	0.131
Potassium (mEQ/L)	3.20±0.39	9.41±2.86	14.85±3.24	19.85±4.08	22.46±1.84	0.016*
Chloride (mEQ/L)	75.00±12.46	87.83±16.97	95.15±17.55	97.51±14.98	97.74±13.29	0.017*

[Table/Fig-1]: Biochemical parameters at day 0, 3, 7, 14 and 21 of storage.
*Significant difference from 0 day according to Dunnet test in each row; **Highly significant difference from 0 day according to Dunnet test in each row

concentration, so cannot say whether the increase in total protein and albumin was because of optical interference or intracellular leakage of total proteins but probably both can account for this increase in concentration.

LIMITATION

In the present study, authors did not measure the other important biochemical parameters such as calcium, phosphorous, bicarbonate pH etc to know the effect of storage on blood stored in CPDA1 blood bags which would have been helpful to find out the effect on red cells viability and its impact on storage time.

CONCLUSION

In the present study, despite using CPDA-1 blood bags authors observed that there were significant changes in serum AST, Total protein, Albumin, Urea, Chloride, and Potassium levels (p-value <0.05) assessed at periodic intervals. The changes might be due to spontaneous haemolysis or non-viable red blood cells caused due to storage. Therefore, it is better to transfuse blood as early as possible in order to prevent a negative impact on the biochemical composition of red blood cells. Also, usage of CPDA-2 or SAGM anticoagulant solutions may to some extent minimise this issue. The further contrivance of a newer anticoagulant solution in blood bags can be focused upon to reduce the impact of storage. This would result in better yield and help in improving patient outcome.

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