Research Article

Effect of Blood Storage on Complete Biochemistry

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Abstract

Background: Prolonged storage of blood leads to alteration in RBCs biochemistry which may lose viability with time.

Aim: This study was planned to observe biochemical changes on stored blood on 19 different analytes.

Material and Methods: The study was conducted on blood donated by 30 healthy volunteer donors. Effect of storage was analyzed at 0, 3, 7, 14 and 21 days interval. Biochemical parameters were measured using Randox suzuka autoanalyzer and Combiline ISE analyzer.

Results: Significant changes were observed in serum phosphorus, SGOT, serum protein, LDH, pH, serum chloride, ionized calcium, serum sodium, potassium and bicarbonate levels ($p < 0.05$ for ionized calcium, serum protein and $p < 0.001$ for rest of the parameters). On the other hand there was no impact of storage time on rest of the parameters.

Conclusion: Prolonged contact of plasma with RBCs results in exchange of contents between plasma and red cells which leads to changes in analyte concentrations as well as dilution. RBC stored for a period of time at 4°C loose viability. Some may undergo spontaneous hemolysis while in storage; others lose the ability to survive in the recipient's circulation following transfusion. Inspite of storing blood with CPDA, the storage time has a negative impact on the biochemical composition of RBCs. Therefore, it is better to give patients fresh blood with less than 7 days of storage in order to decrease the levels of non-viable red blood cells.

Keywords: Hemolysis; Blood; Transfusion

Introduction

In 4°C liquid storage, the biochemical and mechanical properties of red blood cells (RBCs) deteriorate progressively. When blood is stored in blood bank, biochemistry and physical properties of RBCs are altered because of storage conditions. These are referred to as storage lesions. Under normal conditions in the body's circulation, these do not occur as optimum temperature, pH, nutrient concentration and waste product removal are maintained [1].

Hemolysis can produce three sorts of effects: the release of erythrocytic constituents can result in some increased values for serum; there is some dilution, resulting in decreased values; and hemoglobin may interfere directly, e.g. in the colorimetric quantitation of constituents. Caraway reported that erythrocytes contain about 160-fold as much lactate dehydrogenase, 67-fold as much acid phenyl phosphatase, 20-fold as much aspartate aminotransferase, and 23-fold as much potassium as does plasma [2]. Visible hemolysis is commonly defined as an extracellular hemoglobin concentration of 0.3 g/L (4.65 mol/L), resulting in a detectable pink-to-red hue of serum or plasma with a visible appearance in specimens containing as low as 0.5% hemolysate [3].

This study was planned to observe biochemical changes on stored blood on 19 different analytes at an interval of 0, 3, 7, 14 and 21 days.

Material and Methods

The present research was conducted in Pt B D Sharma PGIMS, Rohtak in collaboration of biochemistry department with blood transfusion department of our institute. 450 ml of blood was drawn from 30 healthy volunteer donors into citrate phosphate dextrose adenine (CPDA-1) anticoagulant (63 ml). 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]. All subjects were serologically examined for hepatitis B virus, hepatitis C virus and HIV before blood donation. 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 anticoagulant which is sufficient to anticoagulate and ensure 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 blood sample from each blood bag was taken for study purpose and stored in plain bags. Rest of the blood was used for

transfusion purpose. Effect of storage was analyzed at 0, 3, 7, 14 and 21 days interval by withdrawing 8 ml blood each time from the bag. The sample analyzed on 0 day served as control. Biochemical parameters were measured using Randox suzuka autoanalyzer and Combiline. Alkaline phosphatase (ALP) (IFCC method with aminomethyl-propanol buffer), alanine aminotransferase (ALT) (IFCC method without pyridoxal phosphate activation), aspartate aminotransferase (AST) (IFCC method without pyridoxal phosphate activation), creatinine (Jaffe kinetic method), lactate dehydrogenase (LDH) (DGKC method), inorganic phosphorus (UV Molybdate method), urea (urease method), total protein (biuret method), cholesterol (Enzymatic endpoint method), triglycerides (GPO method), HDL-cholesterol (Enzymatic method) and uric acid (uricase method) were assayed on an automated clinical chemistry autoanalyzer (Randox suzuka), according to the manufacturer's specifications and using proprietary reagents. Sodium, calcium, chloride, potassium and pH were measured on Combiline Ion Selective Electrode analyzer by an indirect method using ion-selective electrodes.

Normal ranges of different parameters used in this study are as follows: urea (10-50 mg/d/L), creatinine (0.7-1.3 mg/dL), uric acid (3-7 mg/dL), phosphorus (2.5-4.5 mg/dL), ionized calcium (1.1-1.35 mmol/L), AST (upto 40 U/L), ALT (upto 40 U/L), ALP (30-120 U/L), total proteins (6-8 g/L), cholesterol (130-230 mg/dL), triglycerides (60-160 mg/dL), HDL cholesterol (30-60 mg/dL), blood sugar (70-110 mg/dL), LDH (235-470 U/L), sodium (135-155 mEq/L), potassium (3.5-5.5 mEq/L), chloride (96-112 mEq/L) and pH (7.38-7.42).

Standard statistical methods were used to determine the mean, and standard deviation. Student t-test was used to find the effect of blood storage on its hematochemical parameters. The blood at zero time is considered as a control. All values were quoted as the mean±SD. The difference between observations was considered significant at $p < 0.05$. Correlation analysis was used to find the relationship between hematochemical parameters and period of storage.

Results

Out of 30 donors 25 were males and 5 were females. There ages ranged between 21-40 years (mean age 24.2 years) with corresponding blood groups of 6 O+, 15 B+, 4 A+, 1 AB+, 1 O-, 2 A- and1 B-. The various biochemical parameters analyzed are shown in Table1.

Significant changes were observed in serum phosphorus, AST, serum protein, LDH, pH, serum chloride, ionized calcium, serum sodium, potassium and bicarbonate levels ($p < 0.05$ for ionized calcium, serum protein and $p < 0.001$ for rest of the parameters). At the end of 21st day there was 34.91%, 94.38%, 9.12%, 265.59%, 25%, 522% increase in serum phosphorus, AST, serum protein, calcium and potassium levels respectively and 4%, 2%, 9%, 79% decrease in pH, serum chloride, sodium and bicarbonate levels respectively. On the other hand there was no impact of storage time on rest of the parameters.

Parameter	Day 0 N=20	Day 3 N=20	Day 7 N=20	Day 14 N=20	Day 21 N=20	pvalue
UREA (mg/dL)	24.7 ± 4.82	23.1 ± 4.87	25.86 ± 5.85	23.3 ± 2.79	23.2 ± 1.3	0.176
CREATININE (mg/dL)	1.06 ± 0.07	1.08 ± 0.08	1.04 ± 0.08	1.03 ± 0.10	1.04 ± 0.10	0.153
URIC ACID (mg/dL)	5.22 ± 0.81	5.39 ± 0.86	5.29 ± 0.93	5.1 ± 1.05	5.1 ± 1.8	0.747
PHOSPHORUS (mg/dL)	11.86 ± 0.62	12.26 ± 0.86	13.4 ± 1.15	15.0 ± 0.76	16.0 ± 0.88	0.000**
AST (mg/dL)	23.15 ± 6.25	24.0 ± 8.61	29.7 ± 9.32	41.1 ± 10.36	45 ± 6.7	0.000**
ALT (mg/dL)	41.45 ± 20.88	41.25 ± 23.7	37 ± 22.4	47 ± 28.36	49 ± 23.55	0.312
PROTEIN (g/dL)	6.14 ± 0.24	6.3 ± 0.24	6.27 ± 0.30	6.41 ± 0.22	6.7 ± 0.77	0.003*
TRIGLYCERIDES (mg/dL)	175.2 ± 61.63	162.1 ± 57.32	147.26 ± 58.7	154 ± 54.07	152 ± 16.2	0.449
CHOLESTEROL (mg/dL)	147.15 ± 25.47	141.4 ± 24.72	141.8 ± 23	137.3 ± 23.2	135 ± 14.7	0.599
HDL-C (mg/dL)	31.6 ± 6.69	29.85 ± 6.58	29.6 ± 6.59	37.2 ± 28.8	35 ± 20.8	0.122
LDL-C (mg/dL)	80.55 ± 21.2	77.65 ± 15.52	82.7 ± 17.4	78.6 ± 14.6	79.2 ± 15.0	0.398
VLDL-C (mg/dL)	35.00 ± 12.21	32.55 ± 11.47	29.5 ± 11.7	31.5 ± 10.78	29.5 ± 12.2	0.505
LDH (mg/dL)	205.15 ± 32.7	293.0 ± 51.61	516.7 ± 153.7	699.4 ± 78.6	750 ± 23.2	0.000**
Ph	7.03 ± .035	6.97 ± 0.04	6.86 ± 0.07	6.84 ± 0.15	6.75 ± 0.04	0.000**
Calcium (mg/dL)	0.04 ± 0.005	0.045 ± 0.006	0.048 ± 0.04	0.05 ± 0.01	0.05 ± 0.008	0.011*
Chloride (mg/dL)	87.75 ± 2.55	92.20 ± 2.41	97.4 ± 6.62	94.4 ± 2.45	85.9 ± 3.30	0.000**
Sodium (mEq/L)	158.8 ± 2.03	153.2 ± 2.84	146.7 ± 6.07	140.2 ± 4.66	144.75 ± 4.94	0.000**
Potassium (mEq/L)	3.26 ± 0.25	6.78 ± 0.82	10.1 ± 1.28	16.56 ± 2.19	20.3 ± 3.62	0.000**

HCO3 (mg/dL)	17.6 ± 1.24	16.78 ± 1.98	13.09 ± 2.76	6.25 ± 3.41	4.79 ± 1.34	0.000[**]

*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.

Table 1: Table showing biochemical parameters at day 0, 3, 7, 14 and 21 of storage.

Discussion

Several changes were observed during storage of whole blood in blood bank. Some parameters were found to be decreased while some showed increased values. In some analytes no change was observed. RBC stored for a period of time at 4°C loose viability. Some may undergo spontaneous hemolysis while in storage; others lose the ability to survive in the recipient's circulation following transfusion. Whole blood was stored in CPDA-1 bags. This anticoagulant present in the collection bag is composed of Citrate (chelates ionized calcium that prevents coagulation), Dextrose (a source of energy for the red blood cells), phosphate containing anticoagulants (lower acidity than other anticoagulants without phosphate and have a higher concentration of 2,3 DPG and red cell phosphate) and Adenine (ATP content and post-transfusion viability of red cells regenerated by addition of adenine) [4-6].

Prolonged contact of plasma with RBCs results in exchange of contents between plasma and red cells which leads to changes in analyte concentrations as well as dilution. Potassium levels increased within the period of 3 days and the increase continued subsequently. There was a significant rise in K+ concentration (p<0.001) from day 1 to day 21 of storage. Latham et al. and Bailey et al. also observed decline in concentration of plasma glucose and bicarbonate and increase in potassium, lactate, LDH, ammonia, and hemoglobin concentration with storage [7,8].

In blood bags the glucose concentration is limited and as glucose is utilized, there is a concomitant ATP (adenosine triphosphate) depletion and decrease in red cell viability [9]. So, energy required for operating ATPase pump in RBCs decreases with time. Under physiological conditions, it pumps three sodium ions out of the cell for every two potassium ions pumped in. Inhibition of sodium pump leads to hyperkalemia and hyponatremia as observed in present study. Adias et al. also observed hyperkalemia in their study but they didn't find any significant change in Na+ [10]. Hemolysis also leads to hyperkalemia. During storage there is slow and constant release of potassium ions from cells into surrounding plasma which may be responsible for dramatic increase in potassium in this study. At low temperature there occurs cold-induced blockade of ATP. This leads to extracellular release of K+ and entry of Na+ ions into RBCs [10,11].

Anaerobic glycolysis results in increased concentration of lactic acid which may have caused a decrease in pH as observed in this study. Significant increase in LDH may also be because of this reason. Hemolysis results in release of LDH in plasma. LDH best reflects the degree of hemolysis by its increased activity [2]. Castro et al. has also identified total hemoglobin concentration, bilirubin level, lactate dehydrogenase, and the arginine:ornithine ratio to be markers of hemolysis [12].

At 0 day pH was within normal range which decreased to 6.75 at the end of 21st day. Each 0.1 unit of pH change results in a 0.4 mmol/L change in the serum potassium level. Potassium levels are increased by acidosis and decreased by alkalosis [13]. So, this also leads to hyperkalemia. This dramatic increase in potassium levels is dangerous for recipient's body and more dangerous if transfused to a patient with severe kidney disease. This can be prevented by using potassium adsorption filters [11] prior to transfusion or by transfusing blood with 0-3 day's storage only.

Ionized calcium levels were found to be significantly increased during storage duration from 0 to 21st day, although the levels were very low (below normal range). This low level of ionized calcium can be explained because of citrate present in CPDA-1 bags, the role of which is to chelate calcium ions to prevent coagulation. Ionized calcium binds to negatively charged sites on protein molecules, competing with hydrogen ions for the same binding sites on albumin and other calcium-binding proteins. This binding is pH dependent and alters the level of ionized calcium in the blood. Alkalosis, promotes increased protein binding, which decreases free calcium levels. Acidosis, on the other hand, decreases protein binding, resulting in increased free calcium levels [9]. So, increase in levels with storage time may be because of acidosis.

Chloride levels increased initially up to 7 days thereafter a significant decrease was seen. Chloride, calcium and sodium are low molecular compounds and on storage, these enter erythrocytes under the influence of their concentration gradients, namely 19:1 for sodium, 128:1 for calcium and 1.5:1 for chloride resulting in decreased levels in plasma [14]. Phosphorus levels were found to be significantly increased (p<0.001) with storage duration, from 11.86 ± 0.62 mg/dL at 0 day to 16 ± 0.88 mg/dL at 21st day. Whole blood contains many phosphatases which cause hydrolysis of phosphate esters resulting in increase in inorganic phosphate levels [14]. In addition CPDA also has phosphates in it resulting in increased levels [4].

Bicarbonate levels were found to be significantly reduced at the end of study (p<0.001). This is usually due to buffering of excess lactate production by cells with anaerobic metabolism. Chloride-bicarbonate shift results in inward movement of chloride and outward movement of bicarbonate ions with subsequent buffering of H+ ion (from lactic acid) by bicarbonate with production of CO_2 gas leading to increased chloride and decreased bicarbonate levels [15]. The blood bags used for storage are made up of polyvinyl chloride (PVC) with plasticizer, di-(2-ethylhexyl) phthalate (DEHP). These bags are easily permeable to CO_2. Moderate hemolysis also decreases bicarbonate levels.

Significant changes were observed in AST levels (p<0.001) which can be because of hemolysis as observed in other studies. Koseoglu et al. studied the effects of hemolysis interferences on routine biochemistry parameters. Hemolysis interference affected LDH and AST almost at undetectable hemolysis by visual inspection (plasma hemoglobin <0.5 g/L) while clinically meaningful variations of potassium and total bilirubin were observed in moderately hemolyzed samples (hemoglobin >1 g/L). ALT, cholesterol, gamma glutamyltransferase (GGT), and inorganic phosphate concentrations were not interfered up to severely hemolyzed levels (hemoglobin: 2.5-4.5 g/L) [16]. RBCs contain 20 fold as high concentration of AST as

plasma, so, even mild hemolysis produces significant alterations in AST. We found 51% increase from day 0 to day 21 in AST levels. The disproportionate correlation with AST, and not ALT, is consistent with higher concentrations of AST than ALT in red blood cells released during intravascular hemolysis [17].

Hemoglobin (Hb) strongly absorbs light at 540 nm. Hemolysis therefore increases absorption in this wavelength range and causes an apparent increase in the concentration of analytes measured in this range. Significant increase in protein concentration is because of addition and optical interference. Additional interference is because of intracellular leakage of total proteins. False elevated protein levels were also observed in study by Roman et al. [18]. Quantitative estimation of total protein was done by biuret method in which absorbance is measured at 546 nm. Hb itself is a protein with same absorbance range [19] but the method was said to be not significantly affected till Hb concentration of 250 mg/dL and we did not measure Hb concentration. So we cannot say whether the increase in total protein was because of addition or optical interference but both can account for this increase.

Conclusion

Inspite of storing blood with CPDA, the storage time has a negative impact on the biochemical composition of RBCs. Therefore, it is better to give patients fresh blood with less than 7 days of storage in order to decrease the levels of non-viable red blood cells. Further studies are needed to support this evidence.

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