Research Article

CPDA-1 Stored Blood Induced Effect on Hematological and Biochemical Parameter up to 28 Days

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Abstract

Introduction: When blood is stored outside the body, some hematological and biochemical changes take place resulting in reduced red blood cells survival which is an important drawback when transfused into the circulation of a recipient.

Objective: The stability of hematological parameters like RBC count, WBC count, differential count, platelet count, MCV, MCH, MCHC and biochemical parameters like S. Sodium, S. Potassium, S. Chloride and albumin during extended storage at 4°C for up to 28 days was evaluated.

Materials and Methods: The present research was conducted in L.N. Medical College and J.K. Hospital, Bhopal, in collaboration with blood bank 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). The blood was kept for 28 days and samples were evaluated on days 1, 7, 14, 21 and 28.

Results: Among the hematological parameters, there was a constant decline in WBC and platelet counts from day 0 to 28. RBC count, Hb, MCV, HCT showed increasing values; MCH was almost constant, while MCHC decreased. PDW increased while PCT increased till 4th day and then decreased. Neutrophils, Eosinophils, Monocytes decreased, Basophils remained constant while lymphocytes increased. Among the biochemical parameters, values of S. Sodium decreased, S. Chloride decreased till 3rd day, increased on 4th day and then again decreased on 5th day. S. Potassium and albumin showed increasing values.

Conclusion: Extended storage of blood in blood banks leads to changes in biochemical and hematological parameters of stored blood. RBC stored for a period of time at 4°C loses viability. Some may undergo spontaneous hemolysis while in storage; others lose the ability to survive in the recipient's circulation following transfusion. The structural and biochemical changes that RBCs go through during storage are likely to contribute to adverse transfusion effects.

Keywords: CPDA-1, Blood bank, Storage, Hematological and biochemical parameters.

Introduction

Preservation and long-term storage of red blood cells (RBCs) is needed to ensure a readily available, safe blood supply for transfusion medicine. Blood collection and storage systems licensed by the Food and Drug Administration allow red cells to be stored up to 42 days, while the median duration of storage of transfused red cell units in the United State is 15 days. Some studies have suggested that the risk of complications after transfusion increases when transfused blood has been stored for long periods [1].

In 4°C liquid storage, the biochemical and mechanical properties of red blood cells (RBCs) deteriorate progressively. When blood is stored in the blood bank, biochemistry

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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 [2].

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 [3].

Although accurate evaluation of hemolysis in RBC units has relevance to transfusion recipient safety, it is also an important quality indicator of blood manufacturing processes. Hemolysis is a very important parameter for assessing the quality of stored RBCs. Hemolysis of red cell units occurs during processing for component separation and also due to repeated handling during storage, issue and transport before transfusion to the patient.

The extent of hemolysis, however, does not exceed the permissible threshold for hemolysis up to day 28 of storage [4].

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]. The citrate prevents coagulation by binding or chelating to calcium, phosphate acts as a buffer hence, maintains the pH of the blood. Dextrose serves as substrate for the blood cells, while adenine maintains high ATP level in the RBC [7].This study was done to determine effects of blood storage on hematological and biochemical parameters during different periods of time (at 5 storage periods from zero time up to 28 days) in both sexes using CPDA1 solution as preservative.

Materials and Methods

The present research was conducted in L. N. Medical College and J. K. Hospital, Bhopal, in collaboration of blood bank 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. 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.

The recommended quantity of blood (437 mL) was obtained from all donors in this study by antecubital venepuncture, added to special blood bags containing 63 mL of CPDA1 anticoagulant solution, and given to the blood bank to be stored. A blood sample of 50 mL from each blood bag was taken. Each sample was divided into 5 portions, each portion of 7 mL of blood was added into plain test tubes. One of these tubes was analyzed immediately, which was regarded as control or at zero time. The other 4 tubes were kept in the blood bank refrigerator at 2–8°C to be analyzed later on at 7 day, 14 day, 21 day and 28 day intervals. Each sample was analyzed for hematochemical and biochemical parameters such as RBC count, WBC Count, Hb, HCT, MCV, MCH, MCHC, PLT, PCT, PDW, granulocytes, monocytres and lymphocytes. These were studied using Mindray 5 part automated analyzer and biochemical parameters. Serum Na, K, and Cl were studied using Roche electrolyte analyzer while albumin was studied using Siemens biochemistry analyzer.

Results

The evaluation of the effect of blood storage on both hematological and biochemical parameters was carried out using citrate phosphate dextrose adenine (CPDA-1), anticoagulated blood was drawn from thirty healthy volunteer donors and placed on the quarantine shelf of the blood bank refrigerator. The blood was kept for 28 days and samples were evaluated on days 1, 7, 14, 21 and 28. The following results were obtained.

Among the hematological parameters, there was a constant decline in WBC and platelet counts from day 0 to 28. RBC count, Hb, MCV, HCT showed increasing values. MCH was almost constant, while MCHC decreased. PDW increased while PCT increased till 4th day and then decreased. Neutrophils, eosinophils, monocytes decreased, basophils remained constant while lymphocytes increased.

Among the biochemical parameters, values of S. Sodium decreased, S. Chloride decreased till 3rd day, increased on 4th day and then again decreased on 5th day. S. Potassium and albumin showed increasing values.

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Table 1.

Hematological Parameter	Day 0	Day 7	Day 14	Day 21	Day 28
WBC	7.82	6.58	6.23	6.64	5.42
RBC	4.00	3.96	4.06	4.24	4.26
Hb	12.5	12.13	12.33	12.8	13.01
Platelet	1.84	1.58	1.32	1.28	1.17
Neutrophil	55	57	48	42	38
Lymphocyte	29	34	43	51	55
Eosinophil	5	4	5	4	4
Monocyte	6	4	4	4	3
Basophil	1	1	1	1	1
MCV	86.9	85.5	89.5	92.6	95.5
MCH	30.7	30.1	30.4	30.6	30.6
MCHC	35.3	34.9	33.9	33.0	32.1
HCT	34.8	35	36.2	39.0	40.4
PCT	0.14	0.15	0.12	0.19	0.11
PDW	16.5	16.4	16.9	17.4	17.7

Table 2.

Biochemical Parameter	Day 1	Day 7	Day 14	Day 21	Day 28
S. Sodium	151	145.5	140.4	139.1	134.1
S. Potassium	2.96	12.5	High	High	High
S. Chloride	84.4	83	83.2	84.5	82.8
S. Albumin	3.81	3.78	3.8	3.9	4.2

Discussion

Several changes were observed during storage of whole blood in blood bank. Some parameters were found to be decreased while some RBC stored for a period of time at 4°C lost 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) [6,8,9].

Red blood cell (RBC) storage lesion has recently been recognized as an important issue facing transfusion medicine. The issue has attracted numerous studies to determine the potential risks associated with transfusion of RBCs stored over a longer period of time and the underlying mechanisms responsible. Several major projects are ongoing and clinical trials and laboratory studies have already shown that long-stored red blood cells have harmful effects.

The structural and biochemical changes that RBCs go through during storage are likely to contribute to adverse transfusion effects. A definitive determination of the potential risks associated with transfusion of RBCs stored for longer periods of time, however, is still elusive not only because the responsible mechanisms have not yet been identified, but also because some facts are not clear. For example, it is unknown why and how up to 30% of long-stored RBCs rapidly disappear from circulation within 24 hours after transfusion.

The number of intact RBCs that actually remain in a long-stored RBC unit before transfusion is also unknown and merits further research. A human RBC has a lifespan of approximately 120 days. Under normal circumstances, approximately 2.4 million new RBCs are produced per second with the concomitant removal of a similar number of senescent RBCs from the circulation. Therefore, human blood contains RBCs that range from 0 to 120 days of age, which is equivalent to a unit of freshly drawn RBCs. Young RBCs can survive for a long period of time after transfusion, but senescent RBCs are rapidly eliminated from the circulation. Therefore, to evaluate the survival time of blood-banked RBCs after transfusion, it is important to determine the proportions of young and old RBCs in the blood-banked RBC unit as well as assess how the proportions and the cells' properties change during storage [10].

In the study by Bailey and Bove [11], mean plasma hemoglobin concentration increased over a period of 28 days, the WBC showed a progressive drop, the hematocrit value remained essentially constant, the MCV remained constant and the MCHC remained basically unchanged. Mean plasma potassium in their study increased while mean plasma sodium decreased. These findings corresponded with the findings of our study except for MCV value which showed a gradual increase in our study.

In our study, WBC count is reduced subsequently from day 1 to day 28. The mechanism of leucocyte depletion during whole blood storage may include loss of cell viability due to ATP depletion. Moreover, leucocytes are also consumed in the formation of microaggregates, which are conglomerates of leucocytes, platelets, fibrin, cold insoluble globulin and cellular debris formed during storage [12]. This finding is similar to finding done by Ahmed et al. in 2008 [13]. Our study revealed a progressive fall in all types of leucocyte; however, the pattern of changes observed in the serial differential count would suggest that granulocytes were more labile than the mononuclear cells comprising the easinophil and monocytes.

WBC count with automated differential count is stable at 4°C for at least 24 hours, or even to 72 hours, with significant differences depending on the type of automated blood cell analyzer. In the study by Zini, in particular, the monocyte count tended to increase, while eosinophil and lymphocyte counts tended to decrease over time; neutrophil count was stable up to 72 hours with most last-generation instruments [14]. In our study, neutrophils, eosinophils, monocytes decreased, basophils remained constant while lymphocytes increased. This finding was similar to finding done by Ahmed et al. in 2008 [13].

Present study shows increasing number of lymphocytes over the days revealed a specific survival advantage of lymphocytes in stored whole blood, which will imply that stored whole blood carries the risk of graft versus host disease if viable donor lymphocytes get engrafted in immunodeficient recipients and premature neonates [15].

There is substantial evidence from in vitro studies documenting the change that hematological parameters undergo changes during storage. When changes observed in the hematological parameters were categorized, based on whether the initial days mean values were maintained when compared with other days (below the lowest normal value), normal (within the normal range), or high (above the highest normal value), some of the hematological parameters analyzed decreased or increased [16].

In the study by Adias et al., potassium increased within the period of 7 days and continued subsequently. The only important electrolyte change in stored blood is that of potassium. During blood storage, there is a slow but constant leakage of potassium from cells into surrounding plasma.

The leakage of potassium from cells into surrounding plasma may be responsible for the drastic progression in potassium increase in this study. Sodium on the contrary reduced, suggesting that sodium in stored whole blood may produce adverse effect after transfusion. The increase in potassium value and reduction in sodium value simply indicates the preference of component therapy to whole blood transfusion [16]. These changes in serum sodium and serum potassium matched with the findings of our study. No significant changes in chloride and albumin were observed.

Conclusion

Hemolysis of the red cells that occurs during component processing and storage of red cell units has serious clinical implications for the transfused patients. Detecting excessive hemolysis is important to minimize transfusion of bacterially contaminated RBC units. Also an elevated potassium and free hemoglobin itself may cause significant complications in some patients. The extent of hemolysis in blood components is an important indicator of cellular integrity and a quality parameter. The structural and biochemical changes that RBCs go through during storage are likely to contribute to adverse transfusion effects.

A definitive determination of the potential risks associated with transfusion of RBCs stored for longer periods of time, however, is still elusive not only because the responsible mechanisms have not yet been identified, but also because some facts are not clear. For example, it is unknown why and how up to 30% of long-stored RBCs rapidly disappear from circulation within 24 hours after transfusion. The number of intact RBCs that actually remain in a long-stored RBC unit before transfusion is also unknown and merits further research.

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