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Comparative Study on Hematological and Biochemical Parameters of Blood Units Stored in CPDA, CPD-SAMG and Leukoreduced Bags

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Abstract: ***Red cell units stored in the blood bank for the purpose of further transfusion. To ensure safety and therapeutic value, one need to follow instructions and standard operating procedures of a blood bank. Storage beyond stipulated temperatures could lead to storage lesions, these changes occur both in red cells and the suspending medium. These storage changes increase with the duration of storage of red cell units. This study aims at evaluation of in vitro effect of storage on hematological and biochemical parameters. Objectives: To determine changes in complete blood count, electrolytes (Na+, K+) and LDH changes in stored blood units on day 0,1, 7, 14, 21 and 28. Materials And Methods: Prospective study was conducted in the blood bank, department of pathology and department of biochemistry, MAMC Agroha, Hisar. A total of 100 blood units were studied over a period of one year for storage lesions of blood. Results: Prestorage leukoreduced red cell units showed significantly lower plasma potassium levels as compared to non-leukoreduced red cell units. It was observed that the fall in hematocrit with storage is least when blood is preserved in CPD-SAGM bags with Integral leukoreduction filters. It was also observed that rise in LDH level is least in Integral bag (indicates less hemolysis) as compared to single CPDA and triple CPD-SAGM bags. Conclusion: leukoreduction for specific indications can be undertaken to improve the quality of stored blood.***

Keywords: Biochemical, hematological, CPDA, CPD-SAGM, leukoreduction

1. Introduction

The development of blood storage systems has allowed donation and transfusion to be separated in time and space. This separation has allowed regionalization of donor services with subsequent increase in quality and availability of blood products. Storing blood for longer times has both its advantages and disadvantages. Storage of blood allows to maintain inventory, processing and testing and reduces transfusion associated graft versus host diseases. However our increasing understanding of RBC physiology and experience with RBC transfusion enable us to appreciate a growing list of storage lesions of Red Blood Cells (1).

During refrigeration RBC's undergo numerous physiology-chemical changes collectively referred to as RBC storage lesions which affect the quality, function and in-vivo survival of transfused RBC's(2). Hemolysis may occur in a red blood cell unit during blood collection, transportation, preservation and or different stages of handling in the blood bank(3,4).The conditions and ambient temperature under which outdoor blood units are collected and transported in Indian settings differ from that in the west. In India there are no definite guidelines for acceptable limits of hemolysis of red cell units. The US FDA has recommended 1% hemolysis at the end of storage period. European guidelines are more stringent that allows only 0.8% hemolysis in red cells for transfusion. Changes in red cells units are relevant to transfusion recipient safety (5).

WBCs are exposed to acidic conditions of storage and refrigerated, they respond with activation and cytokines production before they die within 24 hours of blood donation and storage which are responsible for transfusion related adverse reactions in the recipients(6).

Storage has negative effect on RBC oxygen delivery and allogenic RBC infusion may actually cause harm to some recipients and even death in critically ill and in those undergoing cardiac surgery. Units with high potassium should be used carefully in cardiac patients, neonates, renal failure, massively transfused patients. Hyperkalemia associated cardiac deaths have been reported in setting of massive or rapid transfusions(7,8).

RBC hypothermia storage lesion is responsible for association of blood transfusion with an increased length of stay in the hospital, impaired tissue oxygen use, proinflammatory and immunomodulatory effects, increased infections, multiple organ system failure and ultimately increased mortality(8).

2. Materials and Methods

Prospective study was conducted in the blood bank, department of pathology and department of biochemistry, MAMC Agroha, Hisar. A total of 100 blood units will be studied over a period of one year for storage lesions of blood. Blood (450ml) is drawn from blood donors into CPD-SAGM bags and 350 ml blood is drawn into CPDA-1 bags. Collection is as per NACO guidelines and prescribed departmental SOP. Single bags with capacity 350 ml are used for donors weighing >45 kg. Triple and quadruple bags are used for donors weighing >60 kg.

Data is collected from the below investigation reports of biochemical and hematological parameters of blood units stored for 28 days.

1) Complete blood count. Measured using fully automated cell counter (sysmex xs1000)
2) Electrolytes: Na^+, K^+. Measured using NOVA biomedical electrolyte 1 analyzer (ion selective electrode).
3) Serum LDH. Measured using erba mannheim CHEM-7 (DGKC method, kinetic).

Blood collection is done by phlebotomy method. Blood units are tested negative for human immunodeficiency virus, hepatitis B surface antigen, hepatitis C virus, venereal disease research laboratory andmalaria parasite. Blood processing is done using centrifugation principle. Prepared red cell units are put on quarantine shelf of blood storing refrigerator. Samples were collected after gentle mixing by inversion, by a sterile sampling procedure in a laminar airflow cabinet. The blood bag tubing was uniformly, carefully stripped and refilled to collect representative samples are to be analysed for various parameters and results.

Inclusion criteria: Blood donors who qualifies for donation as standard operating procedure for blood donor selection of the blood bank MAIMRE, Agroha.
Written informed consent, Age 18-65 years, Weight > 45 kg, Hb>12.5g/dl.

Exclusion criteria: Blood donors deferred from blood donation as standard operating procedure for blood donor selection of the blood bank MAIMRE, Agroha.

3. Results

Blood collection was done in three types of blood bags. The types and number stored blood bags which were studied are as follows:

a) Single CPDA bags - 40
b) Triple CPD-SAGM - 40
c) Integral CPD-SAGM - 20

Blood was collected from donors ranging from the age group of 18-60 years of age. The donors were screened as per DGHS criteria. The collection and processing were done in the blood bank, department of pathology, Maharaja Agrasen Medical College, Agroha, Hisar.

The stored red cell units were assessed for various quality parameters by obtaining sample representatives of the bags on day 0 of collection, day 1 after processing to packed red cells and on a subsequent day 7,14, 21, and 28 from triple and integral bags. For single bags, sample representatives were collected on day 0, and subsequently on days 7,14,21 and 28, since these bags stored whole blood and involved no processing. Samples were obtained in EDTA vials to assess the hematological parameters and in plain vials for biochemical parameters. The blood sample was centrifuged at 1000 rpm for 10 minutes and supernatant plasma was removed for processing and testing. Parameters: Plasma Sodium, Plasma Potassium, LDH, Haematocrit, Total Leukocyte Count. Results are depicted in the tables 1-5

Table 1: Plasma levels of sodium (mEq/l) in various bags on different days.

Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	F value	ANOVA p value
CPDA (n=40)							
143.70 ±2.79		136.62±8.38	131.17±7.94	128.23±7.61	126.13±6.84	86.64	<.001
CPD-SAGM (n=40)							
142.40±5.77	145.23±10.56	137.50±6.07	128.03±8.71	116.40±10.04	115.68±10.86	47.31	<.001
INTEGRAL (n=20)							
144.0±2.71	148.05±5.57	141.65±6.94	132.70±7.89	129.80±9.11	126.55±10.41	13.83	<.001

Table 2: Plasma levels of potassium (mEq/l) in various bags on different days.

Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	F value	ANOVA p value
CPDA (n=40)							
4.955 ±1.07		13.24 ±5.74	18.93 ±6.40	25.02 ±6.84	34.32 ±8.98	112.92	<.001
CPD-SAGM (n=40)							
5.3072 ±1.31	11.89 ±7.33	18.38 ±8.84	22.46 ±8.53	30.64 ±9.13	34.92 ±9.75	88.86	<.001
INTEGRAL (n=20)							
4.82 ±1.33	11.99 ±2.79	15.15 ±4.47	18.89 ±7.28	22.47 ±7.01	29.16 ±9.49	24.11	<.001

Table 3: Plasma levels of LDH (U/L) in various bags on different days of storage

Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	F value	ANOVA p value
CPDA (n=40)							
1086.08 ±667.26		1343.40 ±587.24	1589.10 ±529.21	1892.80 ±648.28	2392.50 ±709.69	23.04	<.001
CPD-SAGM (n=40)							
819.00 ±517.67	1180.53 ±782.35	1452.38 ±588.01	1984.08 ±668.76	2298.62 ±651.25	2492.78 ±772.10	28.91	<.001
INTEGRAL (n=20)							
657.55 ±202.68	668.00 ±242.44	740.90 ±395.28	892.70 ±488.01	918.90 ±543.95	935.20 ±560.09	18.28	<.001

Table 4: Levels of Haematocrit (%) in various bags on different days of storage

Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	F value	ANOVA p value
CPDA (n=40)							
41.39±5.02		37.14±10.80	34.24±12.57	32.16±11.13	31.242±9.93	2.41	<.05
CPD-SAGM (n=40)							
37.70±6.16	48.53±8.52	45.61±13.07	43.00±12.64	41.20±9.79	39.60±13.90	20.91	<.05
INTEGRAL (n=20)							
36.82±6.33	48.76±13.11	46.52±9.16	42.40±8.84	45.88±11.09	45.32±10.67	8.76	>.05

Table 5: Levels of TLC($x10^3$/dl) in various bags on different days of storage

Day 0	Day 1	Day 7	Day 14	Day 21	Day 28	F value	ANOVA p value
CPDA (n=40)							
6.37±1.72		6.08±1.84	5.34±1.58	5.10 ±1.64	4.76±1.61	23.56	<.001
CPD-SAGM (n=40)							
9.05±2.12	8.52±3.06	7.73±3.16	8.11±4.63	7.40 ±3.58	6.81±2.79	22.06	>.05
INTEGRAL (n=20)							
6.71±1.60	0.13±0.23	0.05±0.08	0.04±0.07	0.06 ±0.11	0.02 ±0.05	74.11	<.001

Note: *Values are represented as mean ± SD.*

The most salient biochemical changes occurring in stored red cell units:In the present study, we found that the plasma potassium levels increased during the 28 day storage period in all three groups (p <0.001). Since the triple CPD-SAGM bags and integral CPD-SAGM bags were subjected to processing, this finding shows the effect of processing in addition to storage on these red cell units. This observation shows that gradual hemolysis of red cells takes place with an increase in the storage period of red cell units.

The effect of leukoreduction on plasma potassium levels was also studied during storage.

1) For integral bags, the highest mean potassium was found on day 28 and the rise was maximum between day 21 to day 28 of storage. The marked increase in potassium concentration following processing from day 0 to day 1.
2) For triple CPD-SAGM bags, the highest mean potassium was found on day 28 and the rise was maximum between day 14 to day 21 of storage. The marked increase in potassium concentration following processing from day 0 to day 1.
3) For CPDA single bags with whole blood, the highest mean potassium was found on day 28 and the rise was maximum between days 21 to 28(p<0.001).

Significant mean difference (p<.001) was found between potassium levels of leukoreduced integral bags, triple bags, and single CPDA bags from days 1 to day 28 with the mean for triple bags and single CPDA bags being greater than integral bags.

The most salient hematological changes occurring in stored blood units:

1) There is no significant difference (p>.05) in hematocrit for integral CPD-SAGM bag with leukoreduction filter from the day of processing up to day 28. Overall fall in hematocrit is less as compared to single CPDA bags and triple CPD-SAGMbags.
2) There is a significant decline (p<.05) in hematocrit for triple CPD-SAGM bags throughout the period of storage.
3) There is a significant decline (p<.05) in hematocrit for single CPDA bags throughout the period of storage.

The most salient changes in total leucocyte count occurring in stored blood units:

1) Integral CPD-SAGM bags with leukoreduction filter show a significant decline(p<.001) in leucocyte count throughout the period of storage. There is marked decrease following processing from day 0 to day 1.
2) Triple CPD-SAGM bags show no significant change in TLC over 28 days of storage.

Single CPDA bags show a significant decline in TLC over 28 days of storage.

4. Discussion

Blood banking attempts to bring the potentially life-saving benefits of transfusion to the patients who need them by making blood components available, safe, effective, and cheap. Blood banks try to maximize delivering getting blood from the right donors to the right patients on time. The easiest way to assure the timely availability of blood is to have an appropriate inventory on the shelf at all times.

A major remaining problem associated with red cell storage is that viability is very different from one donor to another. Being able to identify those donors whose cells store well is potentially useful for recipients such as children with thalassemia or sickle cell anemia who can become iron overloaded from repeat transfusions.

In this study, there was a significant rise in plasma potassium levels throughout the period of storage were in concordance to the Sawant et al(5).who had reported a significant increase in plasma potassium in red cell units over 28 days of storage for all the three types of blood bags. Similar findings for plasma potassium levels in red cell units were reported by Michael et al(9). They studied the plasma potassium concentrations, unit weights, and hematocrit of 20 units of stored whole blood. 27 units of stored packed red cells, and 20 units of packed cells prepared from stored whole blood of various ages during the 21-day storage period. They found that the plasma potassium levels increased in all three groups with an increase in the period of storage.

In this study, potassium increased within a period of 7 days and continued subsequently. The only important electrolyte change in stored blood is that of potassium. This is in concordance to the Adias and Moore-Igwein study(10). During blood storage, there is a slow but constant leakage of potassium from cells into surrounding plasma. In severe kidney disease, even a small amount of potassium fluctuations can be dangerous, and relatively fresh or washed red cells are indicated. Potassium loss is recognized to be secondary to the changes in metabolic activity with cooling. 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 an adverse effect after transfusion. The increase in potassium value and reduction in sodium value simply indicates the preference of leukoreduced blood transfusion.

There is a significant decline in hematocrit and TLC in single CPDA bags over the storage period in our study were in concordance to the Adias and Moore-Igwe(10) in their study on storage-related hematological and biochemical changes of CPDA-1 whole blood have observed changes in the hematological parameters that were categorized based on whether the initial days mean values were maintained when compared with other days, some of the hematological parameters analyzed decreased in hematocrit and TLC. Our study also agrees with the work done by Queen et al(11).found the mean values of WBC on day 1 was compared to day 7, it was observed that there was a overall deterioration in WBC. These changes in white blood cells are most likely due to the sum effects of the loss of individual cell characteristics specifically degeneration that is known to occur as the cell ages. Clinical implications collectively known as the RBC storage lesion is in part related to bio-reactive substances released by leucocytes in the storage medium, such as histamine, lipids, and cytokines, which may exert direct effects on recipients, but many others are related to metabolic activity with the senescence, such as membrane vesiculation, decrease in cell size, increase of cell density, alteration cytoskeleton, enzymatic desilylation, and phosphatidylserine exposure. Other hematological parameters remained fairly stable during this study period, hence may be considered acceptable for clinical utility.

Rapid degeneration of leukocytes could lead to immunomodulation related to blood transfusion. Whole blood should be leukodepleted before storage if it must be used beyond one week. In this study, there is a relatively lesser rise in LDH in blood stored in leukoreduced integral bags compared to single CPDA bags and triple CPD-SAGM bags Similar effects of leukoreduction have been observed by other authors in different studies. Heaton et al.(12) found significantly lower hemolysis ($p < 0.05$) and plasma potassium levels in the leukoreduced group compared to the non-leukoreduced group after 42 days of storage. Leucoreduced units were found to show approximately one-third hemolysis as compared to the non-leukoreduced group. Gyongyossy-Issa et al.(13)LR-RBCs prepared by any of three methods filtered at 4°C, were less hemolysed during storage than non-filtered concentrates4°C leukoreduction is beneficial for RBCs and does not cause hemolysis or enhance fragility. Most studies ascribe improved red cell survival of leukoreduced units, to the lack of enzymes, cytokines and free radicals derived from leucocytes in such units. Studies have shown that the presence of leucocytes thus can damage the membrane of red cells resulting in potassium leakage, accelerate glycolysis, and compromised ATP preservation. The deleterious effects of leucocytes on the quality of blood had lead to the leukoreduction of red cell units.

Benefits of leukoreduction include the prevention of febrile non-hemolytic transfusion reactions, the transmission of leucotropic viruses, and platelet refractoriness. Hence, leukoreduction offers along with the above-mentioned benefits, the improved quality of stored blood. Many developed nations like Canada, France, and Britain have adopted universal leukoreduction. However, it may not be practically feasible to implement universal leukoreduction in a developing country like ours; hence selective leukoreduction for specific indications can be undertaken to improve the quality of stored blood. Blood collection centers and hospital transfusion services will remain largely as they are in the immediate future. There are no alternatives.

5. Conclusion

When red cell units are stored in the blood bank, due to inherent red cell storage lesions, changes occur both in red cells and the suspending medium. These storage changes increase with the duration of storage of red cell units.

a) Prestorage leukoreduced red cell units showed significantly lower plasma potassium levels as compared to non-leukoreduced red cell units. Hence, such leukoreduced units should be used to further improve the quality of stored blood.
b) It can be concluded that the fall in hematocrit with storage is least when blood is preserved in CPD-SAGM bags with Integral leukoreduction filters.
c) It can be concluded that the rise in LDH level is least in Integral bag as compared to single CPDA and triple CPD-SAGM bags. LDH is abundant in RBCs and its raised plasma level is an indicator of red cell hemolysis in stored blood.

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