ORIGINAL RESEARCH PAPER

Pathology

HAEMATOLOGICAL CHANGES OF CPDA-1 STORED WHOLE BLOOD

KEY WORDS: Whole blood, Hematological, Biochemical, CDPA1

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ABSTRACT

Whole blood is still commonly transfused in developing countries including India where blood components are not easily available.The haematological and biochemical changes associated with the storage of blood in our blood banks have been reported. Blood (350 ml) was drawn from 65 healthy volunteer donors into CPDA-1 anticoagulant and placed on the quarantine shelf of the blood bank refrigerator maintained at 2-6°C. Blood bags were screened for HCV, HBsAg, Syphilis, Malaria and HIV 1&2 and were confirmed negative. Samples were collected at 1, 7, 14, 21, 28 and 35 days and tested for hematological and biochemical.

INTRODUCTION

Preservation and long term storage of Red Blood Cells (RBCs) is needed to ensure a readily available, safe blood supply for transfusion medicine. Some studies have suggested that the risk of complications after transfusion increases when transfused blood has been stored for long periods [1]. During storage, in fact, preserved blood cells undergo progressive structural and functional changes that may reduce red cell function and viability after transfusion [1]. Storage has a negative effect on RBC oxygen delivery [2] and emerging evidence suggests that allogenic RBC infusion may actually harm some recipients. Considerable evidence suggests that transfusion increases the risk of serious complications and death in critically ill patients, especially in patients who are undergoing cardiac surgery. Current research indicated that the RBC hypothermic storage lesion is responsible for the association of blood transfusion with an increased length of stay in the hospital, impaired tissue oxygen use, pro-inflammatory and immunomodulatory effects, increased infections, multiple organ system failure, and ultimately increased morbidity and mortality [3]. Clinical implications, collectively known as the RBC storage medium lesion, is in part related to bioreactive substances released by leucocytes in the storage medium, such as histamine, lipids, and cyto-kines, which may exert direct effect on metabolic and physical changes associated with the senescence, such as membrane reticulation, decrease in cell size, increase of cell density, alteration of cytoskeleton, enzymatic desilylation, and phosphatidylserine exposure, RBCs lose potassium 2,3-diphosphoglycerate (2, 3-DPG), Adenosine Triphos-phate (ATP) stores, lipids and membrane, while becoming more rigid and demonstrating reduced oxygen off-loading [4]. Moreover, stored units become more acidic and the suspending fluid has higher concentrations of free hemoglobin and biologically active lipids, and contains greater quantities of negatively charged microvesicles with pro- inflammatory and procoagulant activity [4]. Platelets circulate longer when stored at room temperature and are more activated and able to form clot more effectively when stored at 4°C [5]. White cells lose their phagocytic property within 4-6 hrs of collection and become non-functional after 24 hrs of storage [6]. It is important to remember they do not lose their antigenic property and are capable of sensitizing the recipient to produce non-hemolytic febrile transfusion reactions. Few lymphocytes may remain viable even after 3 weeks of storage.

MATERIALS AND METHODS

This study was conducted Blood Bank, Anugrah Narayan Magadh Medical College, Gaya. The Blood (350 ml) was drawn from ten healthy volunteer donors into Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant and placed on the quarantine shelf of the blood bank refrigerator. The donors were 65 in number; they had their ages ranging from 23 to 38 years . The donors were all male and tested negative for: HCV, HbsAg , Syphilis, Malaria and HIV 1 & 2.

Blood collection and storage:

Blood bag of 350 ml ± 10% which contains CPDA-1 was used. 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. Most blood collection bags (adult) contain 49 ml CPDA anticoagulant which is sufficient to anticoagulant and ensure the viability of blood cells in 350 ml ± 10% blood for up to 35 days when the blood is stored at 2-6°C [11].

Procedures Hematological parameters: These parameters were measured using sysmax xp100 autoanalyser. It enumerates 20 parameters with 3-part differentiation of WBC.

RESULTS:

The evaluation of the effect of blood storage on both hematological parameters was carried out using Citrate Phosphate Dextrose Adenine (CPDA -1) anticoagulant and blood was kept for 35 days and samples were evaluated on days 1, 7, 14, 21, 28 and 35 days.

Hematological Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
WBC's count Mean Value x 10^9/L	6.78	5.08	3.80	2.59	1.56	0.64
Granulocyte count Mean Value x10^9/L	4.97	2.78	1.65	1.21	0.81	0.33
Lymphocyte count Mean Value x10^9/L	2.75	2.19	1.52	1.11	0.77	0.33
RBC's count Mean Value x 10^{12}/L	4.54	4.33	4.36	4.35	4.34	4.27
HB g/dl	11.36	11.07	11.06	10.90	10.75	10.72
HCT %	41.24	40.66	40.22	40.00	39.81	39.54
MCV fl	90.84	93.90	92.25	91.73	91.73	92.60
MCHC g/dl	27.55	27.23	27.50	27.25	27.00	27.11
MCH pg	25.02	25.57	25.37	25.06	24.77	25.11
Plate late count Mean Value x 10^9/L	229.52	169.36	118.2	74.64	37.64	8.48

Mean values of hematological and biochemical parameters At the end of the study period as shown in (Table 1), the mean values of some hematological parameters were as follows: WBC (3.40×10^9/L), Hb(10..97 g/dl), PCV (40.24%), Lymphocytes (1.44×10^9/L), Granulo-cytes (1.95×10^9/L), MCHC (27.27 g/dl), MCV (91.17 fl), MCH (25.15 pg), Platelet (106.30×10^9/l.

DISCUSSION

Several changes were observed during storage of whole blood in blood bank. Whole blood was stored in CPDA-1 bags. This anticoagulant present in the collection bag is composed of citrate (chalets 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) [.Red blood cell (RBC) storage lesion has recently been recognized as an important issue facing transfusion medicine. 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 from0 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 haemoglobin 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 haemoglobin 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 leukocyte 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].

There is substantial evidence from in vitro studies documenting the change that haematological parameters undergo changes during storage. When changes observed in the haematological 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 haematological parameters analyzed decreased or increased [16].

CONCLUSION

From the present study it is concluded that in the patients having only low erythrocyte count, CPDA-1 whole blood can be used up to the last acceptable storage day that is 35th day because RBC and Hb level show only slight change during storage. During storage, we observed that there was rapid deterioration in leucocyte count. Postoperative risk of bacterial infection increases in patient having major surgery so fresh blood (less than 7 days) indicated. In thrombocytopenia patients, we should use fresh whole blood as far as possible (less than 7 days) and platelet transfusion should be preferred over whole blood. In our study, mean platelet values also revealed progressive decline in count, during the period of storage. In similarity to leucocytes the fall in platelets levels may be related to loss of cell viability due to ATP depletion as well as platelet consumption due to micro aggregates formation. Similar type of result found by Sagir G. Ahmed *et al* 200910 in his study.

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