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Original Research Paper

Transfusion Medicine

A STUDY OF STORAGE RELATED CHANGES IN WHOLE BLOOD IN A BLOOD BANK OF A TERTIARY CARE CENTRE

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ABSTRACT

Introduction: The Food and Drug Administration has licensed the collection and storage of Whole blood and its components. The duration of storage of whole blood in India is 35 days in CPDA- 1 blood bag at 4 0 C in blood bank refrigerators. At different duration of storage whole blood shows change in levels of many hematological and biochemical parameters. **Aims and Objectives:** To study change in hematological and biochemical parameters in stored CPDA-1 whole blood at different duration of storage. **Material and Method:** This was a prospective study on 50 healthy voluntary donors in the blood bank of a tertiary care centre of Madhya Pradesh. 350 ml of blood was collected in CPDA-1 Blood bags. About 50 ml blood sample was taken from each blood bag and divided into four portions, One tube was assessed immediately ,the other 3 tubes were kept at 4-6 0 C and assessed later on day 14 , day 28 and day 35 respectively. Each sample was analyzed for various haematological and biochemical parameters by using autoanalyser. **Result:** The values of MCV on day one was 82 fl, progressively becoming 122 fl on day 35th. ANOVA (F- test) $P < 0.0001$ and was highly significant. Mean hemoglobin on day one was 11.36 gm/dl gradually falling up to 10.7 gm/dl on last storage day showing slight reduction. It was observed that there was rapid deterioration in WBC count. By ANOVA (F-test) $P < 0.0001$, is highly significant. The mean values of platelet count on day one was 229.5 x 109 /L, and decline to 8.48 x 109 /L on last storage day. By ANOVA (F-test) $P < 0.0001$ was highly significant. The mean values of potassium increased from 3.49 meq/L on first day to 17.18 meq/L on 35th day. **Conclusion:** CPDA-1 whole blood can be used up to the last acceptable storage day in patients having low R.B.C. count as RBC and Hb level show slight change during storage. In thrombocytopenic patients, use fresh whole blood (less than 7 days) and platelet transfusion will be more useful than whole blood.

KEYWORDS: Hematological, Biochemical Changes, Stored Blood

INTRODUCTION

Whole blood comprises of cellular and non-cellular components and essential for the maintenance of life. The Food and Drug Administration has licensed the collection and storage of Whole blood and its components. Red blood cells can be stored up to 42 days; however the duration of storage of transfused red cell units in India is 35 days. Rous P and Turner, 1916 [1] found, citrate and dextrose mixed RBCs could be stored in a refrigerated environment for many days, Further it is shown to be re-transfused. Now a days, blood component therapy is practiced in the developed countries as a single unit of blood can be utilized in more than one patient. In many developing nations including India, blood fractionation technology at many blood banks is still not fully developed. Hence whole blood stored at 4^{0} C is used frequently and has remained the most common blood product used in many hospitals. The concentrations of leucocytes and platelets decrease with the duration of storage. RBCs oxygen delivery decreases with storage. Allogenic RBCs infusion may harm some patients. It is found that transfusion increases the risk of complications and death in critically ill patients, especially those undergoing cardiac surgery {Koch et al. 2008} [2]. RBCs show reduced potassium, 2, 3-diphosphoglycerate (2, 3-DPG), Adenosine Triphosphate (ATP) stores, becoming more rigid and shows reduced oxygen delivery. WBCs loose phagocytic features within 4–6 hrs and after 24 hrs of storage become nonfunctional. But their antigenic property retained and are capable of sensitizing the recipient may result in nonhaemolytic febrile transfusion reactions. Few lymphocytes may remain viable after 3 weeks of storage. This Study will be helpful to study the limitations of stored whole blood as a source of leucocytes and platelets in transfusion therapy. Red cells during storage metabolize glucose and produce lactic acid and pyruvic acid causing fall in pH and the rate of glycolysis got reduced. This reduction in glycolysis, causing depletion of adenosine triphosphate (ATP) in Red cells. The red cell storage can be prolonged by addition of exogenous adenine and inorganic phosphate, which improve the cell ability to regenerate ATP. Red cell lose potassium and gain sodium during storage, because the Na-K gradient is normally maintained by Na-K ATPase that does not work properly at 4^{0} C [3]. The leakage of potassium from red blood cells also promoted by Gamma-irradiation of blood components done for prevention of transfusion associated graft-versus-host disease (GvHD). Hall TL et al recommended transfusion of fresh packed RBCs or saline washed packed RBCs to minimize the electrolyte disturbances associated problems [4].

AIMS AND OBJECTIVES

1. To study change in haematological parameters in stored CPDA-1 whole blood.
2. To study change in biochemical parameters in CPDA-1 stored whole blood at 4 C.
3. To compare haematological parameters of fresh blood with CPDA-1 stored whole blood on last acceptable day.

MATERIAL AND METHOD

This study was a prospective study on 50 healthy voluntary donors in the blood bank of a tertiary care centre of Madhya Pradesh. All subjects were serologically examined for hepatitis B virus, HCV, HIV I and II, Syphilis and Malaria parasite found negative. 350 ml of blood was collected in CPDA-1 Blood bags. About 50 ml blood sample was taken from each blood bag and divided into four portions, each portion kept into plain test tubes. One tube was assessed immediately and recorded as day 1. The other 3 tubes were kept at 4-6 ^{0}C in blood bank refrigerator and assessed later on day 14, day 28 and day 35 respectively. Each sample was analyzed for, serum sodium and potassium, by using AVL 9180 electrolyte analyser. Haematological Parameters was measured by fully automatic haematology analyser.

RESULTS

Table 1: Change in RBC Associated Hematological Parameters

Day of storage	RBC count (x10^{12}/L)	Haemoglobin (gm/Dl)	MCV (fl)	Haematocrit (%)

1st	4.54	11.36	82.1	41.24
14th	4.36	11.06	91.3	40.22
28th	4.34	10.75	110.9	39.81
35th	4.27	10.72	122.46	39.54

Table 2: Change in WBC Associated Haematological Parameters

Day of storage	WBC count ($x10^9$/L)	Granulocytes count ($x10^9$/L)	Lymphocytes count (x109/L)
1st	6.7	4.9	2.7
14th	3.8	1.6	1.5
28th	1.5	0.8	0.7
35th	0.6	0.3	0.3

Table 3: Change in Platelet Counts

Day of storage	Platelet count (x109/L)
1st	229.5
14th	118.2
28th	37.6
35th	8.4

Table 4: Comparison of Haematological Parameters (Fresh Blood Vs 35thDay Stored Blood)

S. No.	Parameters	Fresh blood	35th day stored blood	P value
1	RBC (x1012/L)	4.5	4.2	<0.0001
2	Hb (gm/Dl)	11.3	10.7	<0.0001
3	MCV (fl)	82.1	122.4	<0.0001
4	WBC(x109/L)	6.7	0.6	<0.0001
5	Platelet (x109/L)	229.5	8.4	<0.0001

Table 5: Change in Serum Sodium Level

Serial no.	Day of storage	Value (meq/L)
1	1st	136.7
2	14th	135.4
3	28th	134.7
4	35th	134.0

Table 6: Change in Serum Potassium Level

Serial no.	Day of Storage	Value (meq/L)
1	1st	3.4
2	14th	8.2
3	28th	14.2
4	35th	17.1

DISCUSSION

The mean values of Red cell count showing no significant changes, Teddy C Adias et al. 2012 [5]. The mean value of Hb from day one till the last storage day, showing slight reduction, ANOVA (F- test) P < 0.0003 and it is significant. In this study, mean hemoglobin on day one was 11.36 gm/dl gradually falling up to 10.7 gm/dl on last storage day showing slight reduction, due to hemolysis that might occurs during storage. The results was similar to Donahne et al. [6] The erythrocyte hemolysis can be due to old erythrocytes , improper storage or blood bags not mixed periodically causing reduced 2, 3-diphosphoglycerate . Fall in Hb was still within the acceptable normal range as found by Ahmed Y et al. [7] in his study 2009. The value of MCV on day one was 82 fl, progressively becoming 122 fl on day 35th. ANOVA (F- test) P < 0.0001 and was highly significant. Similar result found by Cohl SD et al. 1981 [8] and D.N. Baily 2003 [8]. The values of HCT showing slight reduction, However by ANOVA (F- Test) P< 0.0001and it is highly significant. The gradual, steady fall in hematocrit during storage was due to depletion of red cell ATP. The decrease in hematocrit (PCV) continued during storage, can be due to erythrocytes hemolysis. {Ahmed Y et al 2009 and D.N. Bailey et al 2003} [9,7]. ATP depletion causes loss of membrane function, reduced cell viability and rising levels of potassium with free hemoglobin in the plasma of stored whole blood (Sagir G Ahmed et al 2009) [10]. The adenine of the anticoagulant CPDA-1, provide a substrate for the synthesis of ATP, prolonging the shelf life of stored blood to 35 days. {Sagir G Ahmed et al 2009} [10] It was observed that there was rapid deterioration in WBC count. By ANOVA (F-test) P< 0.0001, is highly significant. The leucocyte depletion might be due to loss of cell viability due to ATP depletion. leucocytes are also consumed in the formation of conglomerate of leucocytes, platelets, fibrin, coldinsoluble globulin and cellular debris formed during storage, similar to the study of Sagir G Ahmed et al. 2009 [10], suggested that the usefulness of stored whole blood as a source of leucocytes and/or platelets was limited to the first 2days. The serial differential count suggests that granulocytes were more labile than the lymphocytes and monocytes. The stored whole blood would be ineffective in the management of aplastic anemia and other leucopenic cases, since the main problem in these cases is neutropenia. The specific survival advantage of lymphocytes in stored whole blood, carries the risk of graft-versus- host disease, in immuno-deficient recipients and premature neonates {Sagir G Ahmed et al. 2009} [10] . By statistical analysis ANOVA(F-test) P value of granulocyte < 0.0001 and P value of lymphocyte < 0.0001, both are significant while P value of monocyte < 0.3085, non significant.

The mean values of platelet count on day one was 229.5 x 109 /L, and decline to 8.48 x 109 /L on last storage day. By ANOVA (F-test) P< 0.0001 is highly significant. In similarity to leucocytes the fall in platelet count might be due to loss of cell viability due to ATP depletion along with platelet consumption in micro aggregates formation. Similar to Sagir G. Ahmed et al 2009 [10] in his study. Paired t- test shows significant changes in RBCs, WBCs, MCV, platelet and haemoglobin from day one to last acceptable storage days.

The mean values of potassium increased from 3.49 mEq/L on first day to 17.18 mEq/L on 35th day. This rise correlates with the other studies [11, 12]. Wallas C.H 2003 found that sodium and potassium did not change if red blood cells with normal ATP content , stored at 20 to 24^0 C. This shows that in the red blood cell during blood bank storage, it is the temperature that inhibits membrane ATPase, leading to cations leak in and out of the red blood cells. The leakage of potassium from cells into plasma may be the reason for the progression in potassium level. During blood storage the leakage of potassium from cells into plasma, is because the Na+-K+ gradient is maintained by Na+-K+ ATPase which does not function properly at 4^0 C. In severe kidney disease even fresh or washed red cells are indicated. In our study sodium level is slightly reduced, on day 1 serum sodium level was 136.7 mEq/L, reduced to 134.0 mEq/L on 35th day suggest that it may cause adverse effect after transfusion. The increase in potassium and slight reduction in sodium suggest the preference of component therapy over whole blood transfusion [11].

CONCLUSION

From the above study it is concluded that ,CPDA-1 whole blood can be used up to the last acceptable storage day in patients having low R.B.C. count that is 35th day as RBC and Hb level show slight change during storage. During storage, there was rapid fall in leucocyte count, may result in Postoperative risk of bacterial infection therefore in patient having major surgery fresh blood (less than 7 days) indicated. In thrombocytopenia patients, use fresh whole blood (less than 7 days) and platelet transfusion will be more useful than whole blood. In cases of Renal diseases, Liver diseases, Neonatal exchange transfusion and major surgical procedure, we should use fresh whole blood to avoid ill effect of high serum potassium level.

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