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Evaluation of haematological parameter in stored CPDA-1 whole blood

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Abstract

Introduction: Blood has always been considered essential for the maintenance of life. The preservation of human red blood cells (RBCs) outside the body is a rather remarkable phenomenon when you think about it. The aim of this study is to measure the trend in the levels of haematological and biochemical parameters in whole blood from the day of donation till the last day of acceptable storage. This will eventually highlight the potentials and limitations of stored whole blood as a source of leucocytes and/or platelets in transfusion therapy for patients with leucopenia and/ or thrombocytopenia.

Material and Method: This is a prospective study carried out in the Department of Pathology, S.S. Medical College Rewa on 50 healthy voluntary donors included were those who donated blood in the blood bank of Sanjay Gandhi Memorial Hospital, Rewa (M.P). Haematological parameters were measured in each donor's blood bag immediately and on 7th, 14th, 21st 28th and 35th day intervals using auto analyser.

Result: The mean values of Red cell count showing no significant changes. By statistical analysis ANOVA (F- test) $P < 0.1472$ and it is non significant. In present study, mean hemoglobin levels on day one was 11.36 gm/dl gradually falling till the last storage day up to 10.7 gm/dl showing only slight reduction. The mean values of MCV were on day one is 82.19 fl, while progressively becoming 122.46 fl on day 35th. By statistical analysis ANOVA (F- test) $P < 0.0001$ and it is highly significant. The mean values of HCT showing slight reduction only during storage, However by statistical analysis ANOVA (F- Test) $P < 0.004$ and it is significant. The mean values of WBC on day 1 were compared to other storage day. It was observed that there was rapid deterioration in WBC count. By statistical analysis ANOVA (F-test) $P < 0.0001$ and it is highly significant. The mean values of granulocyte count on day one were compared to other storage day. It was observed that there was rapid deterioration in granulocyte. By statistical analysis ANOVA (F- test) $P < 0.0001$ and it is highly significant. The mean values of lymphocyte reduced during storage. By statistical analysis ANOVA (F-test) $P < 0.0001$ and it is highly significant.

Conclusion: From the present study it is concluded that, CPDA-1 whole blood can be used up to the last acceptable storage day that is 35th day in case of low RBC count. 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.

Keywords: Haematological parameter, whole blood, CPDA

Introduction

Blood has always been considered essential for the maintenance of life. The preservation of human red blood cells (RBCs) outside the body is a rather remarkable phenomenon when you think about it. Blood collection and storage system is licensed by the Food and Drug Administration. They allow red cells to be stored up to 42 days, while the median duration of storage of transfused red cell units in India is 35 days. Whole blood is a complex tissue, comprising cellular and non-cellular components that serve diverse function. The non-cellular component consist of the plasma and its derivatives. The cellular components consist of leucocytes, platelets and red blood cells. Red blood cell (RBC) are ideally suited to their primary function i.e., transport of oxygen from lungs to the tissues and carbon dioxide from the tissues to lungs. In 1916, Rous P and Turner [1] demonstrated that RBCs mixed with citrate and dextrose could be stored in a refrigerated state for several days and successfully re-transfused to rabbit (Klein and Anstee, 2005) [2].

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Component therapy has a distributive advantage since a single unit of blood can be fractionated to the benefit of more than one patient. Because of its efficacy, blood component therapy has become the established modality in modern transfusion medicine in the developed countries where whole blood is now rarely used. However, in many developing nations including India {Rewa(M.P.)}, blood fractionation technology is still at its infancy and restricted to a few health care outlets at tertiary levels. It is therefore not surprising that whole blood stored at 4°C is still frequently used and has remained the most commonly used blood product in many hospitals. Stored whole blood has several limitations as a source of leucocytes and platelets since the concentrations of leucocytes and platelets fall as the length of storage increases. Storage has a negative effect on RBCs oxygen delivery and emerging evidence suggests that allogenic RBCs 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 {Koch *et al.* 2008} [3]. RBCs lose potassium, 2, 3-diphosphoglycerate (2, 3-DPG), Adenosine Triphosphate (ATP) stores, lipids and membrane, while becoming more rigid and demonstrating reduced oxygen off-loading. White cells lose their phagocytic property within 4–6 hrs of collection and become non-functional after 24 hrs of storage. It is important to remember that they do not lose their antigenic property and are capable of sensitizing the recipient to produce non-haemolytic febrile transfusion reactions. Few lymphocytes may remain viable even after 3 weeks of storage. The aim of this study is to measure the trend in the levels of haematological and biochemical parameters in whole blood from the day of donation till the last day of acceptable storage. This will eventually highlight the potentials and limitations of stored whole blood as a source of leucocytes and/or platelets in transfusion therapy for patients with leucopenia and/ or thrombocytopenia.

Aims and Objectives

1. To study various haematological parameters change in stored CPDA-1 whole blood.
2. Comparison of haematological parameters between CPDA-1 stored whole blood (on 35th day) and fresh blood.

Storage Days	RBC's count Mean Value x 10 ¹² /L	Hemoglobin Mean Value gm/dl	MCV Mean Value fl	Hematocrit Mean Value %
Day1 st	4.54	11.36	82.19	41.24
Day7 th	4.33	11.07	86.6	40.66
Day14 th	4.36	11.06	91.39	40.22
Day21 st	4.35	10.90	99.83	40.00
Day28 th	4.34	10.75	110.94	39.81
Day35 th	4.27	10.72	122.46	39.54

The mean values of Red cell count showing no significant changes. By statistical analysis ANOVA (F- test) $P < 0.1472$ and it is non significant.

In present study, Red Blood Cell (RBCs) count showing no significant changes. The results agree with the study of Teddy C Adias *et al.* 2012 [4].

The mean value of Hb from day one till the last storage day, showing slight reduction only, However by statistical analysis ANOVA (F- Test) $P < 0.0003$ and it is significant.

Material and Methods

The present study was carried out to assess the quality assurance for CPDA-1 stored whole blood. This study is performed in order to establish the safety of CPDA- 1 stored whole blood in different types of clinical cases by showing the gradual changes in various haematological parameters.

This is a prospective study carried out in the Department of Pathology, S.S. Medical College Rewa on 50 healthy voluntary donors included were those who donated blood in the blood bank of Sanjay Gandhi Memorial Hospital, Rewa (M.P.).

Male's ages were ranged between 18 to 45 years with a mean of 28.4 years while the female's ages were ranged between 23 to 45 years with a mean of 32 years. All subjects were serologically examined for hepatitis B virus, HCV, HIV I and II, Syphilis and Malaria parasite and found negative.

Blood Collection and Storage

- **Blood bag:** Blood bags contain 49 ml CPDA-1 anticoagulant used for collection of 350 ml blood. CPDA solution stored whole blood for 35 days. The citrate prevents coagulation by binding or chelation to calcium, phosphate act as a buffer hence, maintains the pH of blood. Dextrose serves as substrate for the blood cells, while adenine maintains high ATP level in the RBC.
- A blood sample of about 50 ml is taken from each blood bag and blood bags are carefully stored in a quarantine shelf of the blood bank refrigerator maintained at 4 -6°C. Each sample was divided in to 6 portion, each portion consisted of 7ml of blood was added in to plain test tube. One of these tubes was analyzed immediately, which was regarded as day 1. The other five tubes were kept in the blood bank refrigerator at 4-6°C to be analysed later on at 7th day, 14th day, 21st day, 28th day, and 35th day intervals. Each sample was analysed for haematological parameters. +++

Testing Procedures

Haematological Parameters-These parameters will be measured by using 'Auto analyser'.

ERMA PCE 210 (N) fully automatic haematology analyser used

Observations & Discussion

In present study, mean hemoglobin levels on day one was 11.36 gm/dl gradually falling till the last storage day up to 10.7 gm/dl showing only slight reduction. This might be due to hemolysis that occurs during storage. The results were in agreement with the results of Donahne *et al.* [5] The erythrocyte hemolysis can be attributed to several causes including: old erythrocytes age hemolysis (ranged between 100-120 days), improper storage of blood (higher than 80C in blood bank refrigerator) or blood bags not mixed periodically leading to decreased 2, 3-diphosphoglycerate

which is very important to preserve RBC and maintains physiological functions. Although hemoglobin decrease was significant during storage, however its concentration in the blood was still within the acceptable normal range value. Similar type result found by Ahmed Y *et al.* [6] in his study 2009.

The mean values of MCV were on day one is 82.19 fl, while progressively becoming 122.46 fl on day 35th. By statistical analysis ANOVA (F- test) $P < 0.0001$ and it is highly significant.

In present study, mean MCV value on day one of donation was 83.6 fl while progressively become 122.5 fl on 35th day. Similar result also found by two other authors Cohl SD *et al.* 1981 [7] and D.N. Baily 2003 [8] in his study.

The mean values of HCT showing slight reduction only during storage,

However by statistical analysis ANOVA (F- Test) $P < 0.004$ and it is significant.

Storage Days	WBC's count Mean Value $\times 10^9/L$	Granulocyte count Mean Value $\times 10^9/L$	Lymphocyte count Mean Value $\times 10^9/L$
Day1 st	6.78	4.97	2.75
Day7 th	5.08	2.78	2.19
Day14 th	3.80	1.65	1.52
Day21 st	2.59	1.21	1.11
Day28 th	1.56	0.81	0.77
Day35 th	0.64	0.30	0.33

The mean values of WBC on day 1 were compared to other storage day. It was observed that there was rapid deterioration in WBC count. By statistical analysis ANOVA (F-test) $P < 0.0001$ and it is highly significant.

In present study, leucocyte count fell more drastically, during the storage period. 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 micro-aggregates, which are conglomerate of leucocytes, platelets, fibrin, cold-insoluble globulin and cellular debris formed during storage. This is similar to the study of Sagir G Ahmed *et al.* 2009 [10]. Sagir G. Ahmed *et al.* 2009 [10] conducted a study, A unit of whole blood was collected (in CPDA-1 bag) from a Nigerian donor. Serial blood counts were determined from day of donation till expiry (day-35). The values before refrigeration were: hematocrit 40.1%, leucocyte $6.7 \times 10^9/l$ and platelets $253 \times 10^9/l$, which fell during 40C storage to 35.1%, $0.2 \times 10^9/l$ and $35 \times 10^9/l$ on day -35. The leucocyte and platelet counts fell below the critical values of $3 \times 10^9/l$ and $100 \times 10^9/l$, which corresponded to the lower limits for leucocyte and platelet counts in Nigerians, after the 2nd day of storage. This study suggested that the potential usefulness of stored whole blood as a source of leucocytes and/or platelets was limited to the first 2days after collection.

The mean values of granulocyte count on day one were compared to other storage day. It was observed that there was rapid deterioration in granulocyte. By statistical analysis ANOVA (F- test) $P < 0.0001$ and it is highly significant.

The mean values of lymphocyte reduced during storage. By statistical analysis ANOVA (F-test) $P < 0.0001$ and it is highly significant. Present study, revealed a progressive fall

The mean hematocrit level is showing slow and steady fall during the entire storage period. This gradual but steady fall in hematocrit during storage was an expected finding that was due to depletion of red cell ATP. Hematocrit at day 7, started to decrease. The decrease in hematocrit (PCV) continued during storage. These results were in agreement with results of Bensinger *et al.* [9] The cause of decrease in hematocrit can be attributed to erythrocytes hemolysis. {Ahmed Y *et al* 2009 and D.N. Bailey *et al* 2003} [10, 8]. Previous studies had linked ATP depletion to loss of membrane function, reduced cell viability and rising levels of potassium along with free hemoglobin in the plasma of stored whole blood (Sagir G Ahmed *et al* 2009) [10]. The adenine component of the anticoagulant CPDA-1 was strategically added to provide a substrate for the synthesis ATP, hence prolonging the shelf life of stored blood to 35 days, which is longer than with non-adenine containing anticoagulants {Sagir G Ahmed *et al* 2009} [10].

in all type of leucocytes, however the pattern of changes observed in the serial differential count would suggest that granulocyte were more labile than the mononuclear cells comprising the lymphocytes and monocytes. The clinical significance of this observation is that stored whole blood would be particularly ineffective as a clinical tool in the management of aplastic anemia and other leucopenic patients, since the most critical entity in these cases is almost always neutropenia. Further more, this data 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 immuno-deficient recipients and premature neonates {Sagir G Ahmed *et al.* 2009} [10]. This is particularly important within the context of the current HIV pandemic, which is strongly associated with anemia and frequent transfusions.

The fall in Leucocyte count was associated with reversal of granulocyte to lymphocyte ratio as revealed by relative rise in proportion of lymphocyte in the serial differential counts. The differential monocyte count showed a steady fall that was not as severe as that seen in granulocyte. By statistical analysis ANOVA(F-test) P value of granulocyte < 0.0001 and P value of lymphocyte < 0.0001 , both are significant but P value of monocyte < 0.3085 , non significant.

Storage Day	Storage Day	Storage Day	Storage Day
Day1 st	Day1 st	Day1 st	Day1 st
Day7 th	Day7 th	Day7 th	Day7 th
Day14 th	Day14 th	Day14 th	Day14 th
Day21 st	Day21 st	Day21 st	Day21 st
Day28 th	Day28 th	Day28 th	Day28 th
Day35 th	Day35 th	Day35 th	Day35 th

Storage Days	Plate late count Mean Value x 10 ⁹ /L
Day1 st	229.52
Day7 th	169.26
Day14 th	118.2
Day21 st	74.00
Day28 th	37.64
Day35 th	8.48

The mean values of platelet count on day one is 229.5 x 10⁹/L, while progressively decline 8.48 x 10⁹/L on last

storage day. By statistical analysis ANOVA (F-test) $P < 0.0001$ and it is highly significant.

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.

S. No.	Haematological Parameters	Fresh Blood	Last day of Acceptable storage days	P value
1	WBC x10 ⁹ /L	6.78	0.64	$P < 0.0001$
2	RBC x10 ¹² /L	4.54	4.27	$P < 0.0001$
3	Hb gm/dl	11.36	10.73	$P < 0.0001$
4	MCV fl	82.19	122.46	$P < 0.0001$
5	Plt x10 ⁹ /L	229.5	8.48	$P < 0.0001$
6	HCT %	41.24	39.54	$P < 0.0001$
7	Granulocyte x10 ⁹ /L	4.97	0.31	$P < 0.0001$
8	Lymphocyte x10 ⁹ /L	2.75	0.34	$P < 0.0001$

Above table shows significant changes in WBCs, MCV, platelet, granulocyte and lymphocyte count from day one to last acceptable storage days while slight reduction in other parameters studied, however statistical analysis (Paired t-test) shows significant changes in all of the above haematological parameters.

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.

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