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ORIGINAL ARTICLE

HEMATOLOGICAL CHANGES IN WHOLE BLOOD STORED IN BLOOD BAGS CONTAINING CPDA-1 OVER A PERIOD OF 28 DAYS

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ABSTRACT

Introduction: *Blood storage is needed to ensure a readily available and safe blood supply. During blood storage in blood bank, biochemical and physical properties of RBCs are altered because of storage conditions. In normal conditions in the body circulation, these do not occur as optimum pH, temperature, nutrient concentration and waste products removal are maintained. The aim of this study was to observe the changes in whole blood stored in blood bags containing CPDA-1 over a period of 28 days.*

Materials and Methods: *This was a hospital based observational study conducted in Blood Bank, National Medical College and Teaching Hospital (NMCTH), Birgunj. A total of 450 ml blood was drawn from 50 healthy volunteer donors into blood bags containing CPDA–1 anticoagulant and placed on the quarantine shelf of the blood bank refrigerator maintained at 2-8o centigrade. The blood bags were screened for HCV, HBsAg, syphillis and HIV. Ten milliliters blood was withdrawn from the blood bags on 1st, 7th, 14th, 21st, and 28th day and tested for hematological parameters using Yumizen H550 (HORIBA) fully automated hematology analyzer.*

Result: *At the end of 28 days, whole blood storage in CPDA-1 revealed significant decrease in WBC ($T = 4.79$, $p < 0.01$) and platelets count ($T=180.09$, $p < 0.01$). Hematocrit increased significantly after 21 days of storage ($T = 40.86$, $p < 0.01$). MCHC and MCH also demonstrated significant increase during the storage period ($T = 37.32$, $p < 0.01$) and ($T = 29.38$, $p < 0.01$), respectively.*

Conclusion: *WBC and platelets are significantly altered (decreased) when stored in blood bags containing CPDA- 1 after one week period. The study recommends whole blood transfusion before seventh day of donation in order to avoid the adverse outcome of hematological changes in stored blood. In case it has to be used beyond one week, then it should be leukodepleted before storage.*

Keywords: *Blood storage, CPDA–1, Whole blood.*

INTRODUCTION

The process of storing blood was first started in 1915 with the discovery of sodium citrate as blood anticoagulant.[1] Blood collection and storage systems licensed by the Food and Drug Administration allow red cells to be stored up to 42 days.[2] The ability of storing blood for a long time revolutionized blood transfusion practices and improved the practice of medicine and surgery. Storing blood also has pathological outcomes that are collectively known as the "storage lesions". The primary aim of red blood cell (RBC) transfusion is to improve oxygen delivery to the tissue.

Many evidence suggests that transfusion increases risk of serious complications and death in critically ill patients, especially in patients who are undergoing cardiac surgery. Recent research indicated that the RBC hypothermic storage lesion is the cause 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 finally increased morbidity and mortality.[3] Clinical implications which are known as RBC storage medium lesion are partly related to bio reactive substances released by leucocytes in the storage medium, such as histamine, lipids and cytokines which may exert direct effect on metabolic and physical changes due to the senescence, such as membrane reticulation, decrease in cell size, increase of cell density, alteration of cytoskeleton, enzymatic desilylation and phosphatidyl serine exposure. 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.[4] Blood is stored in a bag that contains preservative solution, which contains limited amounts of glucose in the form of dextrose, phoshphate and adenine to maintain ATP and 2,3- DPG levels. The preservatives provide fuel for energy-requiring processes that preserve cell membrane integrity and their functions. Due to this RBC pH decreases during storage and this increases phosphatase 3 enzyme activity, which results in 2,3 – diphosphoglycerol (DPG) degradation. 2, 3- DPG binds to deoxyhemoglobin and stabilizes it. This helps in oxygen transportation from the lungs to tissues by oxyhemoglobin. A decrease in 2,3-DPG results in increased oxygen affinity of hemoglobin and therefore less oxygen delivery to tissue.[5] Lactic acid accumulates in the blood bag during storage.[6] An important electrolyte change in stored blood is seen in potassium. During blood storage there is a constant and slow leakage of potassium (K) from cells into the surrounding plasma. In severe kidney disease, even a small amount of K fluctuations can be dangerous so relatively fresh or washed red cells are indicated.[7] Survival of transfused red blood cells (RBCs) is considered to be adequate when 70% of the RBCs are present in circulation after a period of 24 hours.[8] Storage conditions such as low ATP level cause morphological and physiological changes in RBC membrane.[9] Extensive remodeling of membrane and marked structural changes were seen in aging erythrocytes which were stored. Removal signs on their surface were seen among which are neoantigens on band 3 protein and phosphatidyl serine (PS).[10]

MATERIALS AND METHODS

This was a hospital based observational study conducted in Blood Bank, National Medical College and Teaching Hospital (NMCTH), Birgunj. A total 450 ml blood was drawn from 50 healthy volunteer donors and collected in a blood bag of 450 ml with 63 ml CPDA-1. The blood bags were screened for HCV, HBsAg, syphillis and HIV. Samples were collected at 1, 7, 14, 21 and 28 days and tested for hematological parameters.

Collection Procedure : Venous blood was collected from each of the donors with care and adequate safety precautions to avoid contamination from blood transmissible pathogens. Protective gloves were worn during collection , syringe were sterile and dry, blood collecting materials were discarded safely to avoid injury from needles and lancets. The blood bags were placed on the quarantine shelf of the blood bank refrigerator maintained at 2-8^{o} centigrade.

The hematological parameters were measured using autoanalyser. The Yumizen H550 (HORIBA) fully automated analyzer was used. Sabio 148, Biota ESR automated analyser was used to analyse ESR. The findings were tabulated and analyzed in Microsoft Excel and SPSS version 22.

RESULTS

Table 1: Hematological parameters during the storage period

Parameters	Day 1	Day 7	Day 14	Day 21	Day 28	t-value	p-value
WBC x10^9/ L	6.29±0.87	5.52±0.75	4.89±0.57	4.18±0.45	3.09±0.52	4.79	
LYM %	43.38±10.69	56.51±8.93	66.71±8.32	68.19±6.56	71.32±6.55	61.22	
GRAN%	34.10±3.80	10.28±2.20	7.65±1.37	6.54±1.30	71.32±6.55	12.96	
MID%	6.65±1.40	9.70±1.35	7.87±1.26	6.83±1.14	7.01±0.74	7.68	
RBCx10^{12}/L	5.01±0.57	5.50±0.53	5.17±1.05	5.15±1.02	4.57±1.16	7.08	
Hb g/dl	14±0.96	14.09±1.06	13.38±0.95	12.9±0.91	13.1±0.82	13.49	
HCT %	40.36±3.37	40.4±3.39	40.12±2.98	41.35±3.01	42.06±2.73	40.86	
MCV FL	81.92±6.22	81.49±5.07	78.96±4.13	79.49±3.85	81.37±3.02	80.65	
MCHC g/dl	36.98±3.68	37.01±3.54	36.95±3.50	37.44±3.65	38.23±3.67	37.32	<0.01
MCH pg	28.71±2.43	28.91±.45	29.14±2.55	29.97±2.58	30.19±2.25	29.38	
RDW %	13.80±0.91	14.22±0.82	14.89±0.87	15.42±0.81	15.91±0.78	14.85	
PLTx10^4/L	225.5±62.51	201.63±63.07	179.98±49.10	153.40±45.49	139.96±38.40	180.09	
MPV FL	9.81±1.40	10.28±1.21	11.37±1.13	11.80±1.06	12.39±1.05	11.13	
PDWC FL	14.26±2.44	14.65±2.19	14.92±2.06	15.78±1.94	16.12±1.77	15.15	
PCT %	0.25±0.35	0.25±0.02	0.21±0.03	0.29±0.05	0.26±0.5	0.25	
PLCR %	28.38±5.26	28.53±4.77	28.96±4.21	28.85±4.29	29.47±4.20	28.84	
ESR mm/hr	10.40±5.34	11.46±3.77	14.10±4.02	12.52±2.75	11.56±1.84	12.0	

Values are expressed as mean standard deviation.

The white blood cell count showed a decreasing trend after day 7 from 6.29 x 10^9/L±0.87 to 3.09 x 10^9/L±0.52 on day 28 indicating that they reduce significantly during this storage period. Platelets count demonstrated a significant decrease after day 7 and continued throughout the 28 days storage period (from 201.63 x 10^4/L ±63.07 to 139.96 x 10^4/L ±38.40). Red blood cells showed a mild decrease after 14 and 21 days of storage, however significant reduction was seen on day 28. Hematocrit showed a mild increase up to day 14 and then a significant increase was seen from day 21 to day 28. MCH demonstrated a mild increase throughout 28 days of storage.

DISCUSSION

This study shows that cellular changes take place during storage of blood. White blood cells count showed reduction after 7 days and further decreased significantly through 28 days of storage. These results show that white blood cells shows significant alteration by day seven.

These findings correlate with findings of a study done in Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Rivers State, Nigeria which demonstrated that at 28 days, there were significant changes in white blood cell differential and absolute counts.[11] Adetola et.al in 2020 conducted a study in which blood was stored for 35 days in CPDA–1. Significant reduction in white blood cells was seen. On day 1, it was 4.12x10^9/L±0.52 which was decreased to 2.90x10^9/L±0.48 on day 7.[12] Likewise, in our study WBCs count on day 1 was 6.29x10^9/L±0.87 and on day 7 the count was 5.52x10^9/L±0.75.

The findings of the present study necessitate white blood cells count monitoring during blood for transfusion storage in order to improve blood transfusion efficacy and safety.

In the present study, the red blood cells count changes were insignificant up to three weeks (21 days) of storage. Significant reduction was observed on day 28. These findings compare with findings from a previous study done in Bungoma country referral hospital, Kenya which showed statically significant changes in red blood cell count after 28 days of storage.[13] The findings of the

present study differs from the findings of a study done in Sanjay Gandhi Memorial Hospital, Rewa, India which demonstrated that red blood cells count showed no significant change during the 35 days storage period.[14]

In view of the findings of the present study, red blood cells count monitoring during blood for transfusion storage intending to improve blood transfusion efficacy is recommended.

Haemoglobin level estimation demonstrated a decrease on 21st day and then a slight increase on 28th day (from 12.9±0.91 to 13.1±0.82). The slight increase can be explained by the fact that during storage, the byproducts of glycolytic metabolism, lactic acid and proteins accrue, which in vivo are readily removed from the bloodstream, remain and give rise to physical changes and cell lysis releasing unbound haemoglobin into plasma.[15]

In this study, hematocrit demonstrates a mildly increasing trend with significant change noted from day 21 of the storage period and continues till day 28. These findings can be explained by the fact that the increase in hematocrit reflects the morphological alterations that take place during blood storage. The current study differs from the findings of a study done in Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria which showed a significant fall of hematocrit.[16]

In the present study, MCH and MCHC demonstrate significant increase from day 1 to day 28. These findings contrast the findings of a study done in Iran which demonstrated that MCH decreased during the storage period. [17] In view of the findings of the current study, MCH and MCHC monitoring during storage of blood for transfusion and use of fresh blood less than 28 days is recommended.

In the present study platelets demonstrates a significant decrease from day 7 and continues throughout the 28 days of storage. These findings can be explained by the fact that the cell loose viability owing to micro-aggregates development. [18] Findings of our study also compare with the results seen in a study done in L.N. Medical College and J.K. Hospital, Bhopal, India which demonstrated that platelets decreased significantly during the storage period.[19]

Cellular changes together with other parameters in stored blood for transfusion should be carefully monitored keeping in mind the patient to be transfused and the clinical indication of the blood.

CONCLUSION

WBC and platelets are significantly altered (decreased) when stored in blood bags containing CPDA- 1 after one week period. In view of the hematological changes seen it can be stated that long – preserved whole blood would be worthless as a clinical tool in the management of blood disorders. Whole blood before seventh day of donation is recommended as a better choice of transfusion but in case it has to be used beyond one week then it should be leukodepleted before storage.

REFERENCES

1. Rous P, Turner JR. The preservation of living red blood cells in vitro: I. Methods of preservation. The Journal of experimental medicine. 1916 Feb 1;23(2):219-37. [DOI]

2. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. New England Journal of Medicine. 2008 Mar 20;358(12):1229-39. [DOI]

3. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA et al. Evolution of adverse changes in stored RBCs. Proceedings of the National Academy of Sciences. 2007 Oct 23;104(43):17063-8. [DOI]

4. Hess JR. An update on solutions for red cell storage. Vox sanguinis. 2006 Jul;91(1):13-9. [DOI]

5. Högman CF. Preparation and preservation of red cells. Vox sanguinis. 1998 Jun;74(S2):177-87. [DOI]

6. Spiess BD, Gillies BS, Chandler W, Verrier E. Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. Journal of cardiothoracic and vascular anesthesia. 1995 Apr 1;9(2):168-73. [DOI]

7. Ono T, Kitaguchi K, Takehara M, Shiiba M, Hayami K. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. Clinical chemistry. 1981 Jan 1;27(1):35-8. [DOI]

8. Hamasaki N, Yamamoto M. Red blood cell function and blood storage. Vox sanguinis. 2000;79(4):191-7. [DOI]

9. Greenwalt TJ, Bryan DJ, Dumaswala UJ. Erythrocyte membrane vesiculation and changes in membrane composition during storage in citrate-phosphate-dextrose-adenine-1. Vox sanguinis. 1984 Oct;47(4):261-70. [DOI]

10. Bosman GJ, Lasonder E, Groenen-Döpp YA, Willekens FL, Werre JM, Novotný VM. Comparative proteomics of erythrocyte aging in vivo and in vitro. Journal of proteomics. 2010 Jan 3;73(3):396-402. [DOI]

11. Adias TC, Moore-Igwe B, Jeremiah ZA. Storage related haematological and biochemical changes of CPDA-1 whole blood in a resource limited setting. Journal of Blood Disorders & Transfusion. 2012;3(3):124. [DOI]

12. Adetola AA, Olooto WE, Olaniyi OD, Onayemi AA, Adeleke AD. Assessment of biochemical and haematological changes that occur in blood stored with CPDA-1 as an anticoagulant in a Tertiary Hospital in Nigeria. Journal of Medicine and Biomedical Research. 2020;19(1):13-22. [Full Text]

13. Marabi PM, Musyoki SK, Amayo A. Evaluation of cellular changes in blood stored for transfusion at Bungoma County Referral Hospital, Kenya. The Pan African Medical Journal. 2021;38. [DOI]

14. Batham P, Nayak R. Evaluation of haematological parameter in stored CPDA-1 whole blood. International Journal of Applied Research. 2018;4(11):220-3.

15. Arif SH, Yadav N, Rehman S, Mehdi G. Study of hemolysis during storage of blood in the blood bank of a tertiary health care centre. Indian Journal of Hematology and Blood Transfusion. 2017 Dec;33(4):598-602. [DOI]

16. Oluyombo A, Oluyombo O, Uchegbu OO, Adegbamigbe O, Ayodele OE. Quantitative assessment of erythrocytes and leucocytes in CPD-A stored blood. Biomed Res. 2013 Oct 1;24(4):503-8. [Full Text]

17. Ghezelbash B, Azarkeivan A, Pourfathollah AA, Deyhim M, Hajati E, Goodarzi A. Comparative evaluation of biochemical and hematological parameters of pre-storage leukoreduction during RBC storage. International Journal of Hematology-Oncology and Stem Cell Research. 2018 Jan 1;12(1):35. [Full Text]

18. Ahmed SG, Orakah JA. Cellular changes in stored whole blood and the implication on efficacy of transfusion therapy in Nigeria. International Journal of Biomedical and Health Sciences. 2021 Jun 11;5(4). [Full Text]

19. Bhargava P, Gupta R, Khare V. Cpda-1 stored blood induced effect on hematological and biochemical parameter up to 28 days. Rec Adv Path Lab Med. 2016;2(3&4):8-12.