ORIGINAL RESEARCH

Evaluation of Morphological and functional changes in blood stored for transfusion at GMERS Medical college, Gandhinagar

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ABSTRACT

Introduction: During the storage of transfusion blood, it can undergo cellular changes that could be the reason behind the risk of using prolonged stored blood. Therefore, it's important to monitor the cellular changes that may reduce its survival and function. The objective is to access the morphological and functional changes occur in blood stored for transfusion at GMERS Medical College, Gandhinagar.

Methods: A single center, prospective study design involving 51 randomly selected donor blood units in citrate phosphate dextrose adenine (CPDA-1) anticoagulant was employed; cellular changes were evaluated for 35 days. The changes were tested using the Haematology analyzer (Hemax 330). Results were regarded as significant at P<0.05. Results were presented in tables and charts.

Results: At the end of the 35 days blood storage at blood bank conditions, WBC, & platelets counts decreased significantly (P=0.0004 & <0.0001). The Hb, RDW, MCV & HCT increased significantly (P <0.0001, <0.0001, <0.0001, <0.0001)

Conclusion: Platelets, WBC and indices are significantly altered in stored blood especially when stored over two weeks based on most of the cellular components analyzed in this study. The study, therefore, recommends the utilization of fresh blood to avoid the adverse outcome of cellular changes of reserved blood.

Keywords: Blood transfusion, cellular changes, storage.

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INTRODUCTION

Blood is a composite tissue constituting cell and non-cell elements that perform multiple roles [1]. The non-cell elements comprise the plasma and its derivatives. The cell components are made up of WBCs, PLTs, and RBCs [1]. Blood to be transfused is kept for up to 35 to 42 days at 2-6°C in preservatives such as citrate phosphate dextrose adenine (CPDA) [2].

During storage, blood experiences a sequence of cellular changes which minimize their lifespan and purpose [3]. The deterioration in blood and cellular constituents happen almost immediately it is removed from the donor and recipients in need of transfusions rely on the

blood and blood components safety and potency [4]. To minimize the dangers linked with blood transfusion many steps were taken like advanced anticoagulants, additive solutions, red blood cell membrane stabilizers, preservatives, and blood bags quality [5]. Even with these developments, several changes in blood stored for transfusion have been encountered and referred to as 'red blood cell storage lesions'.

Even though RBCs might be stored at 2-6°C for up to 42 days before transfusion, less is understood of how changes to RBCs in the course of storage might alter their attachment properties [6]. During blood storage at 2-6°C, glycolysis is retarded and, as acid pile up, the amount of ATP reduces and the structure of the RBC is bit-by-bit changed from disc-shaped to echinocytic shapes [7].

The release of unbound Heamoglobin following red blood cells lyses during blood storage and its effect on the intravascular nitric oxide metabolism after transfusion has been considered a predominant role [8]. Studies have denoted that transfusion of long-stored blood is linked with a rise in plasma unbound hemoglobin and hunting of nitric oxide in vitro [9]. In line with this discovery, elevated unbound hemoglobin levels in patients with chronic and severe hemolysis have been connected with reduced nitric oxide bioavailability within the micro-capillary bed, reduced organ perfusion, and raised organ injury [10].

With prolonged storage, there is a shortage of ATP, then the pumps may not be able to maintain the ionic homeostasis of the red blood cell, leading to changes in shape and mean cell volume (MCV), Hematocrit (HCT), mean cell Heamoglobin (MCH) and mean cell Heamoglobin concentration (MCHC).

However, the accessibility of blood storage increases the question of to what extend blood components can and should be stored and to what extent are they safe and potent [11].

Elongated blood storage increases mortality, serious infections, and multi-organ failure after transfusion; however, the causes of these remain unknown [12].

According to records in Bungoma County Referral Hospital, a monthly average of 2-3% of patients reacts to transfused blood especially aged blood (>20 days). Blood toxicity has been speculated to be a result of changes as blood ages which are not monitored during storage [13].

MATERIAL AND METHOD

The present study is prospective study conducted in the Blood Bank, Dept. of Pathology, GMERS Medical College, Gandhinagar, in April-May - 2022. The study was conducted on 51 blood units of blood bank, GMERS Medical College, Gandhinagar. Blood collection bags -350 ml & 450 ml capacity containing CPDA-1 as anticoagulant were used, which were kept in refrigerator at 4-6°C. After elaborate medical history, necessary physical examination and haemoglobin estimation, the healthy adult donors were accepted and registered. Next phlebotomy was performed after taking written consent from the donor. Appropriate aseptic preparation of donor arm was done to ensure sterility of the area during phlebotomy.

Volume of blood collected was proportionate to the volume of anticoagulant. Quadruple pack system consisting of single main bag with three attached satellite bags, made of polyvinyl chloride with Di-(2-ethylhexyl) phthalate (DEHP) as plasticizer, was used to prepare and store packed red cells. Whole blood was collected in the anticoagulant solution of citrate, phosphate, dextrose and adenine in volume of 49 ml for 350 ml of blood & 63 ml for 450 ml blood in primary bag.

The dates of collection and expiry, ABO and Rh (D) group were also written on the label of bag. Donor blood was tested for transfusion transmissible diseases and was screened for hepatitis B surface antigen, anti–HCV antibody, anti-HIV 1 and HIV 2, syphilis and malaria as per guideline of DGHS.

Samples were collected from 51 units of Red Cell Concentrate blood bag, just after separation of whole blood was completed, by sterile sampling procedure in a laminar airflow

cabinet (Jove's automation). The sample was collected in dipotassium ethylene Diamine tetra–acetic acid (EDTA) vacutainer for morphological examination at Day 0 & Day 10, for WBC count, RBC count, HGB level, MCV, HCT, and Platelet count. Blood units were then stored in blood bank refrigerators at 2-6°C temperature for 42 days with repeat sampling at 10^{th} day to test for above mentioned same parameters. The laboratory results were recorded as excel sheet form of this study. Cellular changes (Including WBC count, and platelet count, HCT, and MCV) and haemoglobin changes were tested using hematology analyzer (Hemax 330).

For Morphological Assessment of Red Cells
Blood films were stained with H & E stain and red cell morphology was assessed by light microscopy.

RESULT
51 consecutive samples were analyzed; in the duration of 2 months.
To show the trends of the cellular changes in progressing storage period of blood, the mean of white blood cell (WBC) counts, red cell distribution width (RDW), haemoglobin (HGB) level, platelet counts, Hematocrit and MCV were measured at baseline on Day 0 and then at Day 10.

WBC Count and platelet count:
In the present study total 51 units of blood bags were analyzed. Mean of WBC count on day 0 was 7.02 and on day 10 were 6.81. The standard deviation falls within the limit of 0.15. The WBC count is in decreasing trend during 10 days storage period.
Observed value of Platelet count was also in decreasing trend during 10 days of storage. Mean of WBC count on day 0 was 306.45 and on day 10 were 299.31. The standard deviation falls within the limit of 5.05.

Table 1: Result of parameters (WBC Count and platelet count) on Day 0 and Day 10

Parameters	Day 0 (Mean)	Day 10 (Mean)	Standard Deviation	p- value
WBC Count	7.02	6.81	0.15	0.0004
platelet count	306.45	299.31	5.05	<0.001

Haemoglobin (HGB) level, red cell distribution width (RDW), Hematocrit (HCT) and Mean Corpuscular Volume (MCV)

In the present study Mean of haemoglobin on day 0 was 18.180 and on day 10 were 19.145. The standard deviation falls within the limit of 0.682. The haemoglobin is in slightly increasing trend during 10 days of storage period.
Observed value of Red cell distribution width (RDW) was also in slightly increasing trend during 10 days of storage. Mean of Red cell distribution width (RDW) on day 0 was 12.35686 and on day 10 14.14706. The standard deviation falls within the limit of 1.265.

Table 2: Haemoglobin (HGB) level, red cell distribution width (RDW), Hematocrit (HCT) and Mean Corpuscular Volume (MCV)

Parameters	Day 0 (Mean)	Day 10 (Mean)	Standard Deviation	p- value
Haemoglobin (HGB) level	18.18039	19.1451	0.68215	<0.001
Red cell distribution width (RDW)	12.35686	14.14706	1.26586	<0.001
Hematocrit (HCT)	54.54118	57.43529	2.04645	<0.001
Mean Corpuscular Volume (MCV)	90.4402	92.26667	1.29151	<0.001

Observed value of Hematocrit (HCT) was also in slightly increasing trend during 10 days of storage. Mean of Hematocrit (HCT) on day 0 was 54.54118 and on day 10 were 57.43529. The standard deviation falls within the limit of 2.05.

Observed value of Mean Corpuscular Volume (MCV) was also in increasing trend during 10 days of storage. Mean of Mean Corpuscular Volume (MCV) on day 0 was 90.4402 and on day 10 were 92.2667. The standard deviation falls within the limit of 1.29.

DISCUSSION

The present study has shown that there are morphological and functional changes during the storage period. The white blood cells count show significant reduction through 10 days of storage. These results indicate significantly altered WBCs during storage.

Factors that contribute to the WBC reduction during blood for transfusion storage could be loss of viability because of ATP depletion. The clinical significance of this finding is that stored blood for transfusion could be particularly ineffective as a clinical tool in the management of Aplastic anemia and other leucopenia patients[14].

These findings do compare with findings of a study done in Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Rivers State, Nigeria [15] Aminu Kano Teaching Hospital, Kano, Nigeria [16], Veterinary Transfusion Research Laboratory, 85 University of Milan, Italy, which were showed that there was a statistically significant drop in WBC count after storage[17]. Another study done in L. N. Medical College and J. K. Hospital, Bhopal, India which demonstrated that WBC count constantly decreased throughout the 28 days storage period also concurred with these findings [23].

In the current study, haemoglobin level estimation demonstrates a significant increase throughout the blood storage period. The slight increase in Heamoglobin level 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 lyses releasing unbound haemoglobin into plasma [18]. Unbound haemoglobin may trigger vasoconstructive, pro-oxidative, and pro-inflammatory events that have transfusion-related harm to the transfused patient [9]. Present study is comparable with a study done by Phidelis Maruti Marabi et al, which showed that the haemoglobin amount increased in the course of the 35 days of reservation [5]. However, study done in the Department of Pathology, S.S. Medical College Rewa, and India demonstrated that haemoglobin concentration gradually decreased during the 35 day storage period, which is contrast to our study [14].

In the present study, HCT demonstrates a slightly increasing trend with the significant change being noted during period of storage time. The current findings can be explained by the fact that the increase in Hematocrit reflects the morphological alterations that take place during blood storage (Bosman *et al*., 2008). The clinical significance of these findings is that increased morphological alteration minimizes the potency of transfused blood by increasing the speed of elimination of transfused cells by the macrophage [2]. These results compare with study done by São João Hospital, Porto, Portugal which demonstrated that HCT increased from day 0 to day 14 and remained stable afterward [5]. Our findings also compare with findings from another study done in Doha, Qatar that demonstrated a significant HCT increase after 35 days of blood storage [22]. However, the current study contrasts findings of a study done conducted in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria which demonstrated an insignificant change of HCT throughout the 28 days storage period [15]. In the view of the findings from the present study, HCT monitoring during blood storage and its use within 14 days is recommended to improve blood transfusion effectiveness.

In the current study, MCV demonstrate significant slight increase in value during 10 days of storage period. The changes in MCV appear to be the result of a deregulated mechanism in volume of cell, which causes the increase in the volume of the RBCs result in increasing hypochromia and anisocytosis[5]. These results compare with those findings documented in a study done in Phidelis Maruti Marabi et al, which showed that the MCV changes were increased during the 35 days of storage. However, the results contrast the findings of a study done in Iran which demonstrated that MCH decreased during the storage period [19].

In the present study, Platelets demonstrates a decrease during 10 days of storage which is comparable with Phidelis Maruti Marabi et al. The clinical significance of findings is that this may expose patients to possible decreases in platelets effectiveness as well as likely increases in adverse incidences in addition to transfusion-related sepsis [21]. These results are in comparable with study done in Aminu Kano Teaching Hospital, Kano, Nigeria which demonstrated a constant decrease of platelets throughout the 35 days storage period (Ahmed, 2008). Our findings also comparable with study done in L. N. Medical College and J. K. Hospital, Bhopal, India which demonstrated that platelet decreased significantly during the storage period [20]. Our findings also compare with the findings documented in a study done in Nigeria which demonstrated 86.2% platelet count fall from day 0 to day 28 storage times [16]. Our findings, however, contrast the findings of a study done in Nigeria which demonstrated insignificant platelet count variance throughout the 28 days storage period [15]. Regarding the findings from the present study, platelet count monitoring in stored blood to improve blood transfusion efficacy and safety is recommended. Overall, together with other parameters, cellular changes in stored blood for transfusion should be keenly monitored putting into consideration the patient to be transfused, and the clinical indication of the blood.

CONCLUSIONS

Platelets, WBC and indices are significantly altered in stored blood especially when stored over two weeks based on most of the cellular components analyzed in this study.

The clinical consequence of this long-reserved blood is distinctly worthless as a clinical tool in the management of blood disorders. Fresh blood of not more than two weeks is recommended for transfusion. However blood components may vary depending on the level of changes in each blood parameter or indices.

In present study, comparison of day 0 versus day at above mentioned interval reveals changes in leucocytes & platelets with storage. The release of various chemicals and enzymes, especially proteases from the leucocytes contributes significantly to an increase in red cell

hemolysis during storage. Therefore, various leucocyte reduction filters are commercially available to decrease the rate of hemolysis in stored red cell units.
With storage, rapid degeneration of leucocytes could lead to immunomodulation related to blood transfusion. Hence, whole blood should be leucodepleted before storage if it must be used beyond one week.

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