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EFFECT OF STORAGE TIME ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN BLOOD BAGS CONTAINING CPDA-1 ANTICOAGULANT DURING 30 DAYS OF STORAGE

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Abstract:

Background/aim: *Blood is used for transfusion purpose in transfusion medicine. Blood is stored at proper temperature using anticoagulants for maximum validity. But certain progressive changes occur in biochemical and hematological parameters of blood, which can cause harm to the patients. Therefore, the purpose of this study was to determine the changes occur in hematological and biochemical parameters of blood during 30-days of storage.*

Materials and methods: *This prospective cohort study was conducted at Hayatabad Medical Complex Peshawar. We collected 450ml blood from 40 healthy voluntary donors in CPDA-1 anticoagulant. Collected blood was screened for transfusion transmissible infections. Fifty ml blood was transferred in another bag in a closed system. On 1st day, 15th day and 30th day of collection, hematological and biochemical parameters were determined and the data were analyzed through SPSS software using an ANOVA test.*

Results: *The data indicated that during storage significant changes occurs in WBC (P=.000), hemoglobin (P=.002), red blood cell count (P=.023), MCHC (P=.021) and platelets (P=.000) while in MCH (P=.216) and MCV (P=.693) no changes was observed. In biochemical parameters significant changes were observed in Na (P=.000), K (P=.000) and Cl (P=.023).*

Conclusion: *We concluded that during storage significant changes were observed in hematological parameters i.e. hemoglobin, RBC, red cell indices and platelet count. In biochemical parameters significant changes were observed in sodium, potassium and chlorides. Therefore, for cardiovascular and renal failure patients fresh blood would be recommended to avoid adverse transfusion reaction.*

Key words: *Storage time, changes during storing blood, CPDA-1, adverse transfusion reaction, hematologicl changes*

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INTRODUCTION:

Blood is a body fluid composed of RBCs, WBCs, platelets and plasma, which perform distinct functions in the body. The primary function of RBCs is to transport blood gases, nutrients, hormones and immune complexes. WBCs provide immunity and platelets help to prevent bleeding. Plasma provides different proteins and electrolytes [1]. Blood is used for transfusion purpose in blood banks. In blood bank, blood is mostly procure from voluntary donors and then separate it into its components to use it for therapeutic and prophylactic purposes [2]. Blood components are stored at their optimum temperature to increase its validity. During storage, certain progressive changes occur in blood components which could be harmful for the patients [3]. In 1915 the blood was stored for the first time along with the discovery of blood anticoagulant i.e. sodium citrate [4]. During storage FDA assure survival and efficacy of blood in blood bags containing fraction of red cell, i.e. 75%, for platelets 67% and plasma proteins 80% before preparation of blood. According to FDA cell lyse <1% in a storage bag and less than 25% of cell cleared from recipient within 24 hours of transfusion which is considered normal [5]. Transfused RBCs have shorter half-life. Twenty five present of transfused RBCs eliminated from the recipient's body circulation within 24 hours. This is because at the time of donation few RBCs are too old that complete their aging process. The other reason is storage condition which is more different from normal physiologic condition [6]. Hemolysis depends on numerous factors like storage solution, duration of storage, individual donor, mishandling of unit (freezing or heating or clinical use) and bacterial contamination causing osmotic rupture of cell membrane [7].

Hemoglobin, LDH, ammonia, lactate and potassium concentration increases with storage. Deformity in corpuscular shape, osmotic fragility, agreeability and intracellular viscosity occur [8]. Plasma is transfused for the acute treatment of angioedema and for the treatment of children who are deficient in VWF [9]. Level of electrolytes, especially potassium increases during storage time. Hyperkalemia can cause serious complications in renal failure patients. Arrhythmia is also caused by hyperkalemia. During storage, sodium level decreases below 135mEq/L causing hyponatremia. It may cause cellular oedema and complications in central nervous system like depression [10].

Phagocytic property of white cells can be lost within 4-6 hours of collection. Some lymphocytes remain functional after 3 weeks of storage [3]. Leukocytes cause adverse effects like febrile non- hemolytic transfusion reaction, nosocomial infection in trauma patients and transfusion related acute lung injury (TRALI) [8].

In order to reduce the adverse effect of transfusion, it is essential to determine the changes that occurred during the storage time. Therefore, this study was conducted to find out changes in hematological and biochemical parameters of blood during storage.

MATERIALS AND METHODS:

This prospective cohort study was conducted in the blood bank of Hayatabad Medical Complex Peshawar in 2018. Permission was granted from the head of the pathology department before collecting data. Forty voluntary donors were selected by using the simple random technique. All donors were male and were in 18 to 45 years of age. Informed consent was taken from the donors.

These donors have not taken antibiotic or aspirin form last 24 hours. In past 8 weeks donors have not donated blood. Donors had not done body tattooing or body piercing for past 12 months.

After fulfilling the donor selection criteria according to AABB standard [11], we used protective, uncontaminated gloves and sterile dry plastic syringes during phlebotomy. We collected 450 ml blood in blood bags containing 63 ml anticoagulant (CPDA-1) to prevent blood from clotting. The blood was screened for transfusion transmissible infections. Blood bags which were positive for TTI's were excluded from the study. Blood bags stored at room temperature for more than 8 hours were also excluded. From 450 ml blood we took 50 ml blood in another blood bag in a closed system. We stored this 50 ml blood at 2-8oC to maintain the viability of cells.

At the first day of collection we determined hematological and biochemical parameters of blood by using auto analysers. For hematology parameters we used CBC analyser Ceil-dyn Ruby that works on the principle of flow cytometry. For biochemical parameters we spun blood at 1500 rpm for 3 minutes to separate plasma. We used Roche Cobas 6000 for electrolytes that works on the principle of spectrophotometry and recorded the data.

After 15th and 30th of collection we again performed a complete blood count and biochemical parameters and recorded the data. For descriptive and inferential analysis of recorded data, we used Statistical tools, i.e. SPSS version 22 data analysis. ANOVA test was performed on data.

RESULTS:

We took 40 blood bags from voluntary blood donors. The age of donors were ranging from 18-45yrs and their corresponding blood groups were A +ive (15), AB +ive (6), B +ive (1), B -ive (1), B +ive (9), O +ive (8). Blood bag was stored for 30 days and evaluated on 1st day, 15th day and 30th day. According to descriptive statistics analysis the mean values of hematological parameters: RBC ($4.19*10^6$/UL±1.11), Hb (11.6g/dl±3.20), HCT (36.9%±10.94), MCV (91.9fL±5.71), MCH (28.7pg±1.74), MCHC (31.2g/dl±2.21), PLT ($1.90*10^3$±54.4), MPV (9.52Fl±2.36), WBC ($6.52*10^3$/UL±2.35), NEU ($2.83*10^3$/UL±1.51), LYM ($1.99*10^3$/UL±.94), MONO ($.52*10^3$/UL±.22), EOS ($.882*10^3$/UL±.41), BASO ($.455*10^3$/UL±2.0). 1st day mean values of blood components provide a baseline for the comparison with the mean values calculated after 15 and 30 days (Table 1).

Table 1 Analysis of hematological parameters of Whole blood during 30 days of storage

Parameters	Day 1	Day 15	Day 30	Total	F value	P value
RBC10^6ul	4.56±.677	3.64±1.53	4.37±.74	4.19±1.11	4.031	.023
Hbg/dl	12.9±1.81	9.66±4.22	12.25±1.97	11.63±3.20	6.761	.002
HCT%	42.17±5.47	30.66±14.24	37.89±8.27	36.91±10.94	6.399	.003
MCVfL	92.87±5.74	91.35±5.83	91.65±5.76	91.96±5.71	.369	.693
MCHpg	28.51±1.62	29.33±1.84	28.44±1.68	28.76±1.74	1.57	.216
MCHCg/dl	30.2±2.74	32.16±2.09	31.30±1.11	31.22±2.21	4.16	.021
PLT10^3/ul	2.42±46.22	1.75±43.82	1.54±24.98	1.90±54.4	26.2	.000
MPVfl	7.05±2.23	10.81±1.56	10.70±.49	9.52±2.36	34.0	.000
WBC10^3/ul	8.05±2.57	6.37±2.32	5.13±.86	6.52±2.35	9.58	.000
NEU10^3/ul	4.13±1.22	2.53±1.54	1.83±.54	2.83±1.51	19.0	.000
LYM10^3/ul	2.38±1.09	1.76±1.04	1.84±.49	1.99±.94	2.51	.091
MONO10^3/ul	.57±.19	.56±.29	.44±.16	.52±.22	2.11	.130
EOS10^3/ul	.87±.55	.94±.36	.82±.30	.88±.41	.381	.685
BASO10^3/ul	.10±.16	1.04±3.62	.21±.03	.45±2.0	1.14	.325

The mean values of biochemical parameters were: Na (1.60/mmol/l±8.65), K (14.6/mmol/l±8.64), Cl (66.4/mmol/l±8.27), Albumin (4.23g/dL±5.16) (Table 2).

Table 2 Analysis of biochemical parameters of blood during 30 days of storage

Parameters	Day 1	Day 15	Day 30	Total	F value	P value
Na/mmol/l	1.63±8.90	1.61±7.33	1.55±8.05	1.60±8.65	8.98	.000
K/mmol/l	5.12±3.95	14.5±2.49	24.1±4.48	14.6±8.64	259.7	.000
Cl/mmol/l	69.2±13.3	65.5±3.21	64.4±2.65	66.4±8.27	3.89	.023
Albumin g/dl	3.76±.255	3.72±.154	5.22±8.92	4.23±5.16	1.09	.338

DISCUSSION:

Our study shows that the normal values of hematological and biochemical components fluctuate during storage of blood. In vitro analysis of stored blood bags showed that momentous change occurred in RBCs, WBCs, platelets, sodium, potassium and chlorine. Normal erythrocyte count 4.5-5.9 10^6/µL decreases up to 4.19*10^6/µL during 30-days of storage. Recorded mean value of Hb and MCHC were 11.63g/dl and 31.22g/dl respectively, which is lower from the lower normal limit of Hb (14.0-18.0g/dL) and MCHC (33.4-35.6%). The normal level of potassium in blood is 3.5-5.0/mmol/l. Drastic increase occurred in potassium 14.61/mmol/l. Chloride ion decreases from 98-108/mmol/l to 66.41/mmol/l.

Significant changes occurred during storage time in RBC (P=.023). The level of red blood cells was decreased during storage time. As during storage up to certain level hemolysis of red cell occurs. Due to ATP depletion RBCs deformability decreases and its shape changes into echinocyte [5]. Furthermore, during storage RBCs loses its expression CD47, 10-65% and become susceptible for phagocytosis. Study conducted by Cohle in India determined that there were no significant changes occur in RBCs. Their mean value of RBCs was 4.75±0.49. They also determined that no significant change occurred in RBCs when stored for 5 days at room temperature [12]. A study performed in Nigeria on stored blood collected from 10 healthy donors. They determined that there were no notable change occur in RBCs (RBCs P=0.37) [3]. And this may be due to the low sample size they took. The drastic decrease occurs in Hemoglobin in the first 15 days (P=.002). Cohle and Adias concluded that no significant change occur in hemoglobin [12].

No significant changes were observed in MCV and MCH which is similar to the study conducted in Nigeria [1] and Texas. But according to Cohle significant change occur in MCV when stored at room temperature [12]. According to our study significant decrease occurs in platelets for 30 days of storage (P=.000). According to Adias no significant changes were observed in platelet count (P=0.195) and remarkable change occur in MPV (P= 0.000) [3] which is similar to our study. Cohle determined that platelets are non-significant [12]. But according various studies, presence of WBCs also affects the physiology of platelets as various WBC produce bio reactive substance that may lead to destruction of platelet which support our study [13]. Furthermore, as in Cohle's work the sample size was only 5 and they store the blood bags at room temperature which produce large platelets clumps, while we stored the blood at 2-8^{0}C.

Momentous change occurs in WBCs (P=0.000) during 30 days of storage time which is similar to the study conducted by Cohle in India (P<0.01) [12]. During storage WBCs release different bio reactive substances like histamine, cytokines which may affect recipients after transfusion. Neutrophils are significant (P=0.000) because of the degeneration of granulocytes [3].

Our study also showed that significant changes occurred in electrolytes i.e. Na (P=0.000), Cl (P=0.023) and K (P=0.000) during storage. Storage time significantly affects sodium, potassium and chlorine. Our results are similar with the study conducted in India by Verma in 2015 [7].

Clement et al also reported that potassium increases 21.4% on day 10 and 60.7% on day 15 [15]. During storage time potassium is constantly released from the cells. Therefore, patients having renal failure should be transfused either with fresh blood or washed blood with normal saline [16]. According to the study conducted by Adias [3] and Mane [14] significant changes occur in albumin. Our study determine that no significant change occur in albumin during storage.

In our study we processed 40 blood bags from volunteer donors and all the donors were male. Due to lack of awareness and culture constrain females are reluctant to donate blood voluntarily.

CONCLUSION:

Our study showed that variations occurred both in hematological and biochemical parameters of blood when stored in blood bags containing CPDA-1 at 2-8^{0}C for 30 days. Significant changes were observed in Red blood cells, hemoglobin, platelet count, sodium, potassium and chloride level.

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