ResearchTJH-2018-0081.R2

Submitted: 28 February 2018

Accepted: 28 May 2018

Bringing PRBC to the point of combat injury: are we there yet?

Authors:

[1]Aytekin UNLU, Associated Professor, Health Science Univercity, Gulhane Training and Research Hospital, Department of General Surgery; Etlik, Ankara, Turkey.

[2]Soner YILMAZ, MD, Health Science Univercity, Gulhane Training and Research Hospital, Regional Blood and Training Center; Etlik, Ankara, Turkey.

[3]Ozlem YALCIN, Associated Professor, Koc University School of Medicine, Hemorheology, Hemodynamics and Vascular Biology Laboratory,; Sariyer, Istanbul, Turkey.

[4]Metin UYANIK, MD, Corlu State Hospital, Department of Biochemistry, Tekirdag, Turkey

[5]Patrizio PETRONE, Associated Professor, Department of Surgery, NYU Winthrop Hospital; Mineola, L.I., New York. USA. New York Medical College; Valhalla, New York, USA. University of Las Palmas de Gran Canaria; Canary Islands, Spain.

[6]Rıza Aytac CETINKAYA, Assistant Professor, Health Science Univercity, Sultan Abdulhamid Han Training and Education Hospital, Department of Infectious Disease, Istanbul, Turkey.

[7]Ibrahim EKER, Assistant Professor, Afyon Kocatepe University, Faculty of Medicine, Department of Pediatric Hematology. Afyon,Turkey.

[1]Murat URKAN, MD, Health Science Univercity, Gulhane Training and Research Hospital, Department of General Surgery; Etlik, Ankara, Turkey.

[8]Taner OZGURTAS, Professor, Health Science Univercity, Gulhane Training and Research Hospital, Department of Biochemistry; Etlik, Ankara, Turkey.

[9] İsmail Yasar AVCI, Professor, Health Science Univercity, Gulhane Training and Research Hospital, Department of Infectious Disease and Clinical Microbiology; Etlik, Ankara, Turkey.

[1]Nazif ZEYBEK, Professor, Health Science Univercity, Gulhane Training and Research Hospital, Department of General Surgery; Etlik, Ankara, Turkey.

Corresponding author:

Dr. Soner Yılmaz, drsoneryilmaz@gmail.com

Tel: 0312 304 4902

Fax: 0312 304 3402

Running Head:

Simulated shear stress caused PRBC hemolysis.

Abstract

Introduction: Hemorrhage is the leading cause of injury related pre-hospital mortality. We investigated worst case scenarios and possible requirements of Turkish Military. As we plan to use blood resources during casualty transport, the impact of transport related mechanical stress on PRBC (packed red blood cell) were analyzed.

Material and Methods: The in vitro experiment was performed in the environmental test laboratories of ASELSAN®. Operational vibrations of potential casualty transport mediums such as Sikorsky Helicopters, Kirpi® Armoured Vehicle and NATO vibration standardsoftware MIL-STD-810G were recorded. The most powerful mechanical stress, which was created by the NATO standard, was applied to 15 units of fresh (≤ 7 days) and 10 units of old (>7 day) PRBC in a blood cooler box. The vibrations were simulated by TDS v895 Medium-Force Shaker Device. On site blood samples were analyzed at 0, 6th and 24th hours for biochemical and biomechanical analyses.

Results: The mean age of fresh and old PRBCs was 4.9 (SD ± 2.2) and 32.8 (SD ± 11.8) days, respectively. Six-hour mechanical damage of fresh PRBC was demonstrated by increased erythrocyte fragmentation rates

(p=0.015), hemolysis rates (p=0.003), supernatant potassium levels (p=0.003) and decreased hematocrit levels (p=0.015). Old PRBC hemolysis rates (p=0.015), supernatant potassium levels (p=0.015), supernatant Hb (p=0.015) were increased and Htc levels were decreased (p=0.015) within 6 hours. Two (%13) units of fresh and none of the old PRBC were eligible for transfusion after 6 hours of mechanical stress.

Conclusion: When the austere combat environment was simulated for 24 hours, fresh and old PRBC hemolysis rates were above the quality criteria. Currently, a technology to overcome this mechanical damage does not seem to exist. In the light of the above data, a new national project is being performed.

Key words: Combat trauma; Blood transport; Prehospital transfusion; Hemolysis.

Introduction

During the last century, 90% of combat related deaths have occurred in the pre-hospital period (PHP), which only decreased to 75-87% in the recent military conflicts [1,2,3,4,5,6,7]. PHP mortality may be stratified as non-survivable (75%) and potentially survivable (25%) from a medical perspective [5,6,7]. Majority of potentially survivable PHP deaths (90%) were attributed to hemorrhage [7].

The Department of War Surgery under the University of Health Sciences has regarded the above data from recent military conflicts an essential field of medical research. In order to decrease the preventable injury-related deaths in the PHP, acquiring the capability of *en-route* blood transfusion by transporting packed red blood cells (PRBC) to the point of injury has proved valuable [8].

In a worst case scenario, prolonged transportation of the already limited PRBC resources by army tactical ambulances and helicopters may be required for casualty evacuation missions. However, movement of these vehicles creates mechanical vibrations with different amplitudes and frequencies, which exert mechanicalstress on PRBC. We investigated the biochemical and biomechanical parameters of PRBC exposed to vibration for 24 hours.

Methods

The study was designed as a *within subjects, in vitro experiment*, and was approved by the Yeditepe University Clinical Research Ethics Committee (24/11/2016-682). The study was conducted at the Environmental Test Laboratory of ASELSAN Company (Macunkoy, Ankara, Turkey).

Preparation of PRBC

All consenting volunteers that meet the criteria of the 2016 Turkish National Blood and Blood Products Guide for blood donations were eligible for the study. PRBC were prepared by Gulhane Regional Blood and Training Center from whole blood as described by Cetinkaya et al [9]. The mean volume of PRBC was 220 ± 40 mL. We named ≤ 7 days blood as fresh and > 7 days blood as old PRBC. The mean age of fresh and old PRBC was 4.9 (SD ± 2.19) and 32.8 (SD ± 11.8), respectively. All PRBC were placed in MT-25E cooler box and transported to Aselsan Laboratories in 15 minutes. Tinytag View 2 (TV-4510, UK) temperature data logger was placed in the cooler box and records showed the temperature remained between 3.2ºC and 3.9ºC.

Vibration Analysis and Simulation of Vibration

The PRBC were exposed to 24 hours of simulated shake by LDS v895 Medium-Force Shaker Device (BRÜEL & KJÆR Inc., Denmark). The first step was recording vibration profiles of potential PRBC carriers such as Sikorsky Blackhawk Helicopters (S70) (routine flight) (Lockheed Martin, USA) and KIRPI Multipurpose Armored Vehicle (rough terrain, 30 km/h speed) (BMC Inc., Turkey). Vibrations were recorded by G-Sensor Pro v3.0.5 application for android devices.

Currently, durability of all military equipment against vibrations is tested using Test Method 514.6 (MIL-STD-810G, US ArmyTest and Evaluation Command, 2008) software program. Vibrations of Test Method 514.6 were also recorded. Root mean square (rms) acceleration values of three different vibration environments were calculated. Test Method 514.6 had the highest value and chosen for testing PRBC for 24 hours. (Table 1)

Blood Sample Analyses

Hematocrit, pH, supernatant hemoglobin, supernatant osmolality, supernatant potassium, 2,3-diphosphoglycerate (2,3-DPG), ATP (adenosine 5′-triphosphate), osmotic fragility, drythrocyte deformability, erythrocyte fragmentation were measured at 0, 6 and 24 hours.

One milliliter of blood was collected and immediately analyzed for hematocrit and pH measurement via the IRMA Truepoint Blood Gas Analyzer (ITC, System Version 7.1, USA). Supernatant osmolality, potassium (mmol/L), and hemoglobin (g/dL) were measured using Radiometer ABL 800 (Radiometer Trading, Copenhagen, Denmark).

ATP was assayed using ATP Assay Kit (ab83366; Abcam, Cambridge, MA) and 2,3-DPG was assayed using a human 2,3-DPG enzyme-linked immunosorbent assay (ELISA) kit (CK- E11265, Eastbiopharm). Osmotic

fragility test was calculated using the Parpart method [10]. Presence of hemolysis at 0.45%– 0.55%, >0.55%, and ≤0.30% PubMed NaCl concentrations was defined as normal, increased, and decreased osmotic fragility, respectively. Supernatant hemoglobin values were measured using Drabkin's method [11].

Erythrocyte deformability was determined using Optical Rotational Cell Analyzer (LORCA) (RR Mechatronics, Netherland). Elongation Index (EI) were calculated during the application of 10 steps of shear stress (SS), in the range of 0.3 to 50 Pa; RBC deformability was expressed as EI-SS curves. These EI-SS data were characterized by the maximum EI at infinite SS (EI_{max}) and the SSneeded to achieve one-half of this maximum ($SS_{1/2}$); the $SS_{1/2}/EI_{max}$ ratio was calculated as a normalized measure of $SS_{1/2}$. $SS_{1/2}/EI_{max}$ is inversely related to RBC deformability such that a lower value indicates better deformability.

Red Blood Cell fragmentation was determined using a cell counter system Multisizer 3 (Beckman Coulter, USA).

Statistical Analysis

In order to determine the number of fresh and old PRBC, we conducted *a priori* power analysis. PRBC in a blood cooler box were exposed to Test Method 514.6 vibrations for 6 hours and tested for hemolysis percent and potassium levels. Sample size analysis was performed using Guc Analizi (Power Analysis) application (Savante Mobile Apps, Google Play). Sample size power was set at 80%. Analysis showed that 15 units of fresh and 10 units of old PRBC were required for the study.

All data were analyzed using SPSS v.22 (IBM Corp; Armonk, New York, USA). Friedman test was used to analyze the difference within each group. Upon finding a statistically significant difference, analyses between comparison groups were performed using Bonferroni corrected Wilcoxon Signed Ranks test. Mann-Whitney U test was performed for analyzing the differences in biochemical and biomechanical values between the fresh and old PRBC. As Friedman and Wilcoxon tests were performed for statistical analyses, we used median (min/max) values for comparative analyses and descriptive purposes. Level of statistical significance was set at 0.05.

Results

Analyses between 0 vs. 6, 6 vs. 24, and 0 vs. 24 hours were defined as Comparison 1, 2 and 3 respectively.

Analysis of Fresh PRBC

There were no statistically significant differences in erythrocyte deformability parameters; EI_{max} and $SS_{1/2}$ values ($p=0.14$, and $p=0.36$, respectively) (Figure

1). However, statistically significant erythrocyte fragmentation occurred in comparison 1 [1.72(1.13/2.43 vs. 2.29(1.36/3.15), p=0.015], which continued to increase without statistical significance in comparison 2 [2.29(1.36/3.15) vs. 2.24(1.69/4.96), p=0.09] (Figure 2). Similarly, hemolysis of erythrocytes was statistically significantly increased in comparison 1 [0.37(0.19/0.80) vs. 1.49(0.47/5.09), p=0.003], which continued to increase in comparison 2 with a borderline statistically significance [1.49(0.47/5.09) vs. 1.74(0.78/5.21), p=0.04] (Table 2). Unsurprisingly, the hemolysis percent was also statistically significantly increased in comparison 3 [0.37(0.19/0.80) vs. 1.74(0.78/5.21), p=0.003] (Figure 3). Only two fresh PRBC at the 6th hour and one PRBC at the 24th hour had hemolysis percentages less than 0.8%.

The 24 hours of simulated shear stress was found to statistically significantly affect pH levels (p = 0.001) (Table 2). However, the statistical significance was due to differences in Comparison 1 [6.9(6.9/7.08) vs. 6.8(6.8/7.0), p = 0.003], whereas comparisons 2 [6.8(6.8/7.0 vs. 6.8(6.7/7.1), p=1] and 3 [6.9(6.9/7.08) vs. 6.8(6.7/7.1), p=0.05] were not statistically significantly different.

ATP levels decreased significantly in comparison 1 [90.2(60.88/189.8) vs. 70.4(38.2/102.9), p=0.003] and comparison 2 [70.4(38.2/102.9), vs. 84.6(63.9/166.8), p=0.04]. Likewise, 2,3-DPG levels also decreased statistically significantly in comparisons 1 [1.06(0.9/1.9) vs. 0.86(0.65/1.35), p=0.003], comparisons 2 [0.86(0.65/1.35)vs. 0.64(0.53/0.84), p=0.003] and comparison 3 [1.06(0.9/1.9) vs. 0.64(0.53/0.84), p=0.003]. However, despite the statistically insignificant increase in osmotic fragility in comparison 1 [0.4(0.35/0.40) vs. 0.40(0.4/0.45), p=0.25], continued mechanical agitation consequently resulted in a statistically significant increase in comparison 2 [0.40(0.4/0.45) vs. 0,45(0.4/0.45), p<0.001] and comparison 3 [0.4(0.35/0.40) vs. 0,45(0.4/0.45), p<0.001]. The supernatant hemoglobin levels were not significantly increased in comparison 1 [0.08(0.02/0.17) vs. 0.07(0.02–0.17), p=0.08] and comparison 2 [0.07(0.02–0.17) vs. 0.11(0.02/0.3), p=0.09]. However, the difference was statistically significant in comparison 3 [0.08(0.02/0.17) vs. 0.11(0.02/0.3), p=0.009]. Supernatant osmolality was statistically significantly decreased in comparison 1 [284(248/307) vs. 265.4(234/292), p=0.003], comparison 2[265.4(234/292) vs. 259(227.7/293), p=0.003] and comparison 3 [284(248/307) vs. 259(227.7/293), p=0.003]. Naturally, mechanical agitation significantly caused an increase in supernatant potassium in

comparison 1 [21.8(10.2/33.12) vs. 32.9(21/40.7), p=0.003], comparison 2 [32.9(21/40.7) vs. 37.7(27/44.5), p=0.003], and comparison 3 [21.8(10.2/33.12) vs. 37.7(27/44.5), p=0.003]. As a result of fresh PRBC hemolysis, hematocrit levels were significantly decreased in comparison 1 [62.4(50/79.1) vs 48(38.3/80), p=0.015] and comparison 3 [62.4(50/79.1) vs. 46.4(36/60.4), p=0.015](Table 2).

Analysis of Old PRBC

No statistically significant differences in EI_{max} and $SS_{1/2}$ values were found in any of the comparisons (p>0.05) (Figure 1).Fragmentation rates of old PRBC continued to increase without significance, in comparison 1 [3.01(1.3/4.39) vs. 2.53(1.62/5.69), p>0.05] and comparison 2 [2.53(1.62/5.69) vs. 3.20(1.3/6.71), p>0.05] (Figure 2). Old PRBC's Median (Min/Max) hemolysis percent at 0-hour, 6-hour and 24-hour analyses were0.7(0.26/0.8), 2.47(1/6.44) and 2.95(1.31/10.3), respectively, and the increases were statistically significant (p<0.05) (Table 2). Hemolysis percentages of all blood bags were above 0.8% after 6 hours of simulation

ATP and osmotic fragility values showed no significant changes in any of the comparisons (p>0.05). However, there were significant differences in supernatant osmolality values in comparison 1 [240.3(191/274.1) vs. 235(189.4/268), p=0.015], comparison 2 [235(189.4/268) vs. 233.9(185/266), p=0.015] and comparison 3 [240.3(191/274.1) vs. 233.9(185/266), p=0.015]. Supernatant potassium values in comparison 1 [38.6(28.3/48.6) vs. 42.8(32.9/53.5), p=0.015], comparison 2 [42.8(32.9/53.5) vs. 43.5(33.5/56.2) and comparison 3 [38.6(28.3/48.6) vs. 43.5(33.5/56.2), p=0.015] were also statistically significant. Likewise, 2,3 DPG values in comparison 1 [1.16(0.84/1.58) vs. 0.74(0.6/1), p=0.015], comparison 2 [0.74(0.6/1) vs. 0.61(0.53/0.76), p=0.015] and comparison 3 [1.16(0.84/1.58) vs. 0.61(0.53/0.76), p=0.015] were statistically significantly different. Supernatant Hb levels increased significantly in comparison 2 [0.07(0.02–0.11) vs. 0.19(0.12/0.44), p=0.013], while differences comparisons 1 [0.10(0.03/0.2) vs. 0.07(0.02–0.11), p=0.2] and 3 [0.10(0.03/0.2) vs. 0.19(0.12/0.44), p=0.06] were statistically insignificant. The hematocrit levels were also statistically significantly decreased in comparison 1 [58(44.7/70.2)vs. 45.9(34.3/56.6), p=0.015] and comparison 3 [58(44.7/70.2) vs. 43.6(34/49.5), p=0.015] (Table 2).

Analyses of Differences between Fresh and Old PRBC

2,3 DPG levels between the fresh and old PRBC were not statistically significantly different ($p>0.05$). Fresh PRBC ATP levels at 0-hour analysis was significantly higher ($p<0.001$), However, the 6-hour and 24-hour analyses were not statistically significant ($p>0.05$). Supernatant hemoglobin levels or hemolysis percent between the fresh and old PRBC were not statistically significantly different ($p>0.05$). When compared, supernatant K levels of old PRBC was statistically significantly higher than the fresh PRBC in all three successive analyses ($p<0.001$, $p<0.001$, $p=0.005$, respectively). The other biochemical analyzes has been shown on Table 2. EI_{max} ($p=0.003$, $p=0.004$, and $p=0.004$, respectively), and $SS_{1/2}$ ($p=0.031$, $p=0.036$, and $p=0.004$, respectively) values of fresh PRBC were significantly better in all comparisons. Fragmentation rates of fresh erythrocytes were significantly lower at 0-hour analysis; however, the difference was not significant at 6-hour and 24-hour analyses (Table 2).

Discussion

In order to prevent hemorrhage-related early mortality, strategies that comprise crystalloid use, a high ratio of fresh frozen plasma, and platelets to PRBC transfusion protocols have been developed [12]. Malsby et al reported the initial military experience of *en-route* blood product transfusion to combat trauma casualties. Transfusions were started aboard upon receiving the casualties from the point of injury. More interestingly, clinical indications for transfusion were appreciated and executed by well-trained flight medics. They concluded that flight medic-initiated transfusions were safe and effective and studies to determine the effect of PHP transfusion on outcomes were required [13]. Brown et al performed an outcomes study. and they showed that PHP PRBC's transfusion was associated with significant 24-hour and 30-day reduction in mortality rates. They alsoshowed that trauma induced coagulopathy was reduced by 88% [8].

In order to take the transfusion capability closer to the point of injury, some practical questions about blood logistics needed answers for future planning: *"If PRBC could be kept at 4 ºC, would their quality be maintained after prolonged transport times?"* Otani et al investigated whether a helicopter flight affected the quality and shelf-life of RBC. Seven days after donation, five units of PRBC were packed into a blood cooler box and transported in a helicopter for 4 hours. Then they were stored again and their quality was evaluated 7, 14, 21, and 42 days after donation. Only supernatant hemoglobin and hemolysis rate were slightly increased 42 days after donation. Supernatant potassium, hematocrit, pH, and 2,3-DPG levels at 42 days remained unchanged [14].

Boscarino et al exposed 20 units of pooled PRBC (7 days old) in a golden hour container to a 30,000 feet parachute descent, followed by carrying the container in a rucksack for 12 hours in a 48 ºC and 9% humidity environment. They investigated the biochemical (pH, lactate, potassium, ATP) and biomechanical parameters (EI_{max}, half EI_{max}, Percent Hemolysis, and Morphology) and found no significant impact on markers of RBC stress [15].

In our worst-case scenario, the blood supplies are limited and the forward deployed fresh and old blood products will be subject to continuous shear forces due to perpetual tactical evacuation missions. As the above-mentioned studies' simulated conditions that showed no resemblance to our envisioned combat environment, we have designed a 24-hour simulation study.

Our study was performed in "within limits" temperature settings, as hemolysis would increase linearly with temperature [16]. When blood is collected in a bag with limited amounts of dextrose, phosphate, and adenine to maintain ATP and 2,3-DPG levels, erythrocytes metabolize these preservatives to maintain their integrity. Lactate level increases progressively in the blood bag, which decreases the pH and 2,3-DPG levels during the storage period [17,18]. Decreased ATP levels reduce the deformability of the cells and cellular homeostasis [19]. Approximately 25% of ATP content, over 90% of 2,3-DPG is lost in a unit of PRBC after 42-day storage[20]. As this study lacks control groups, the decrease in erythrocyte metabolism related parameters cannot be solely attributed to shear stress. The pH levels of fresh PRBC decreased significantly in the first 6 hours.

Storage temperatures at 1 ºC to 4 ºC slow the RBC metabolism and decrease the energy demand. However, storage at 4 ºCimpairs the ATP-dependent potassium pump, resulting in potassium leakage. The extracellular potassium concentration increases approximately 1 mEq/L per day, until the intracellular and extracellular potassium ion reaches equilibrium. Potassium loading may be of clinical importance in massive transfusion patients [21]. After 24 hours, the supernatant potassium level of fresh and old PRBC was significantly 71% and 87% higher than the expected value.

According to the European Directorate for the Quality of Medicine- Healthcare of the Council of Europe (EDQM) criteria and the North American Blood Quality Licensure, acceptable level of hemolysis has been set at 0.8% and 1%, respectively [22].The EDQM hemolysis criterion has been approved by the Turkish National Blood and Blood Products Guide (2016) [23]. After 6 and 24-hour shear stress, only 2 (13%) and 1 (6.6%) of fresh blood packs were eligible for transfusion, respectively. None of the old PRBC was found eligible at the 6^{th} hour test. Mechanical agitation significantly increased hemolysis and fragmentation values of fresh and old PRBC at 6-hour and

24-hour analyses. Decrease in hematocrit levels were observed in fresh and old PRBC at 6-hour analysis.

Storage induced red blood cell damage increases osmotic fragility, especially after 5 weeks [24]. Increased osmotic fragility was evident in old PRBC. However, mechanical stress significantly increased osmotic fragility values of fresh PRBC, especially after 6 hours of simulation.

Erythrocytes may deform under a wide range of mechanical stresses and LORCA is capable of measuring the deformation, which is usually presented as maximum elongation index (EI_{max}) and half maximum elongation index ($SS_{1/2}$). Boscarino et al exposed PRBC to parachute descent and 12 hours of simulated soldier patrol and found no shear stress related differences [15]. In our vigorous and longer duration study, EImax and $SS_{1/2}$ values showed no significant changes. Unsurprisingly, fresh erythrocyte deformability values were significantly better throughout the simulation.

The current study is not without limitations. Our primary concern was creating control groups would not represent real life environment. Dividing each donor's blood into two equal volumes and the blood sampling would further decrease the blood volume. The duration of the experiment was set at 24 hours due to the convenience of the simulator.

Conclusion

Under the simulated conditions, we were unable to demonstrate the feasibility and safety of carrying PRBC. Given the demonstrated benefits of transfusion in the prehospital period, our efforts shall not be hindered by the initial experience, and new projects are underway.

Acknowledgement

We are grateful to the Turkish Red Crescent and its precious staff, Dr. Murat Güler, Dr. Hatice Öztürk and Dr. Sebahattin Yılmaz (director of Gulhane Blood Center) for their precious support. Special thanks to Prof. Mehmet Akgün for root mean square calculations of the three dimensional vibrations, and to Prof. Mine Yarım Yüksel for enlightening every difficult step of the project. Finally, we also would like to thank POINT Ltd. Inc. for helping us with on site blood sample analyses.

REFERENCES

1- Bellamy RF, Maningas PA, Vayer JS. Epidemiology of trauma: military experience. Ann Emerg Med. 1986;15:1384-8 PubMed .

2- Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. Mil Med. 1984;149:55-62 PubMed .

3- Champion HR, et al. Improved characterization of combat injury. J Trauma. 2010;68:1139-50 PubMed .

4- Mabry RL, et al. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. J Trauma. 2000;49:515-29 PubMed .

5- Holcomb JB, et al. Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001-2004. Ann Surg. 2007;245:986-91 PubMed .

6- Kelly J.F, et al. Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003Y2004 versus 2006. J Trauma. 2008;64(suppl 2):S21-7.

7- Eastridge et al. Death on the battlefield (2001-2011) Implications for future combat casualty care. J trauma Acute Care Surgery. 2012;73:S431-7.

8- Brown JB, Cohen MJ, Minei JP, Maier RV, West MA, Billiar TR, Peitzman AB, Moore EE, Cuschieri J, Sperry JL. Pre Trauma Center red blood cell transfusion is associated with reduced mortality and coagulopathy in severely injured patients with blunt trauma. Ann Surg 2015;261:997-1005 PubMed .

9- Cetinkaya RA, Yilmaz S, Eker I, Ünlü A, Uyanik M, Tapan S, Pekoğlu A, Pekel A, Ertas Z, Gürsel O, Muşabak UH, Yilmaz S, Avcı IY, Çetin AT, Eyigün CP. In vitro efficacy of frozen erythrocytes: implementation on new blood stores to alleviate resource shortage (issue revisited). Turkish Journal of Medical Sci. 2015;45(3):638-43.

10- Parpart AK, Lorenz BL, Parpart ER, Gregg JT, Chase AM. The Osmotic Resistance (Fragility) of human Red Cells. J Clin Invest. 1947; 26:636-40 PubMed .

11- Han V, Serrano K, Devine DV. A comparative study of common techniques used to measure haemolysis in stored red cell concentrates. Vox Sang 2009;98(2):116 PubMed -23.

12- Bhananker SM, Ramaiah R. Trends in trauma transfusion. Int J Crit Illn Inj Sci. 2011;1(1):51-6.

13- Malsby RF, Quesada J, Powel-Dunford N, Kinoshita R, Kurtz J, Gehlen W, Adams C, Martin D, Shackleford S. Prehospital blood product transfusion by U.S. army MEDEVAC during combat operations in Afghanistan: A process improvement initiative. Mil Med. 2013;178(7):785 PubMed -91.

14- Otani Taiichi, Oki Ken-ichi, Akino Mitsuaki, Tamura S, Yuki N, Homma C, Ikeda H, Sumita S. Effects of helicopter transport on red blood cell components. Blood Transf 2012: 10: 78-86 PubMed .

15- Boscarino C, Tien H, Acker J, Callum J, Hansen AL, Engels P, Glassberg E, Nathens A. Feasibility and transport of packed red blood cells into Special Forces operational conditions. J Trauma Acute Care Surg 2014;76:1013-9.

16- Richieri GV, Mel HC. Temperature effects on osmotic fragility and the erythrocyte membrane. Biochim Biophys Acta. 198528;813(1):41-50.

17- Hogman CF. Preservation and preservation of red cells. Vox Sang 1998;74(Suppl 2):177-87.

18- 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. J Cardiothoracic Vascular Anesthesia 1995;9:168-73.

19- Kinoshita A. Simulation of human erythrocyte metabolism. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013.

[https://www.ncbi.nlm.nih.gov/books/NBK6563/ (Accessed in:17-02-208)]

20- Zubair AC. Clinical impact of blood storage lesions. Am. J. Hematol. 2010;85:117-22.

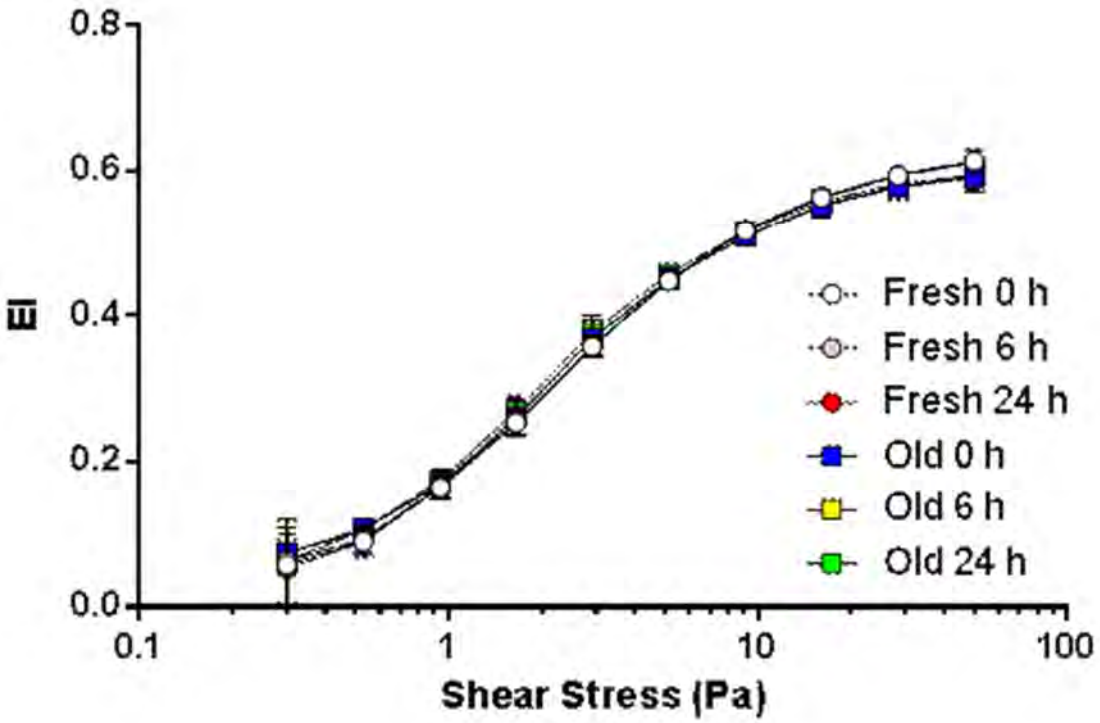
21- Sowemimo-Coker SO. Red blood cell hemolysis during processing. Transfus Med Rev. 2002;16(1):46 PubMed -60.

22- Principles of blood component processing. Guide to the preparation, use and quality assurance of blood components, Recommendation No. R (95) 15. EDQM 2015; pp139-142.

23- Ulusal Kan ve Kan Bileşenleri Hazırlama, Kullanım ve Kalite Güvencesi Rehberi. [Available: http://www.shgm.saglik.gov.tr/TR,9968/ulusal-kan-ve-kan-bilesenleri-hazirlama-kullanim-ve-kalite-guvencesi-rehberi.html (Accessed in:17-02-208)].

24- Barshtein G, Gural A, Manny N, Zelig O, Yedgar S, Arbell D. Storage-Induced Damage to Red Blood Cell Mechanical Properties can be only partially reversed by rejuvenation. Transfus Med Hemother. 2014 Jun; 41(3): 197-204.

Figure 1: Elongation indexes (EI) of fresh and old samples measured at shear stresses between 0.3 and 50 Pa.



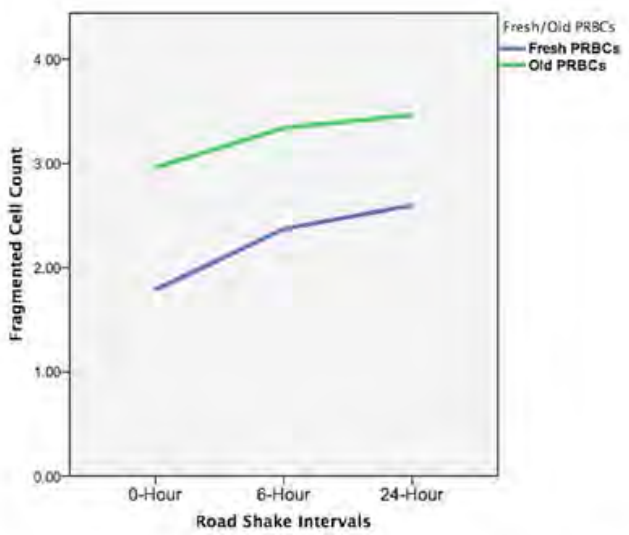


Figure 2: Cell counts between 15-40 fL (decreased cell volume due to fragmentation) of fresh and old samples measured at Multisizer 3 (Beckman & Coulter, CA, USA).

Figure 2: Cell counts between 15-40 fL (decreased cell volume due to fragmentation) of fresh and old samples measured at Multisizer 3 (Beckman & Coulter, CA, USA).

209x296mm (72 x 72 DPI)

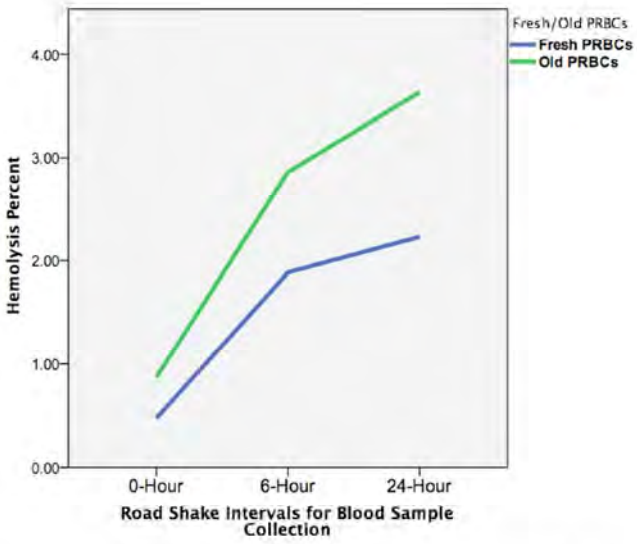


Figure 3: Differences in hemolysis percentages between fresh and old PRBC during road shake simulation.

Figure 3: Differences in hemolysis percentages between fresh and old PRBC during road shake simulation.

Table 1: Acceleration (a) root mean square (rms) calculations in x, y and z directions of three different vibration recordings.

Vibration Medium	*	*	*	*
Blackhawk Helicopter (S70)	1.007	2.032	9.621	9.885
KIRPI Armored Vehicle	7.622	4.510	5.605	10.481
MIL-STD-810G Test Method 514.6	12.187	9.402	15.554	21.883

* $a_{t,rms:}$ Total acceleration root mean square

Table 2: Effect of simulated Test Method 514.6 (MIL-STD-810G) shake on biochemical and biomechanical values of fresh and old PRBC stored in blood cooler box.

Parameters	Fresh PRBCs (14 Units) [Median (Min / Max)]				
	Before shake	After 6 Hours of shake	After 24 hours of shake	*p value*	Befor
pH	6.9(6.9/7.08)	6.8(6.8/7.0)	6.8(6.7/7.1)	0.001	6.4(6.
Supernatant Osmolality (mOsm/kg)	284(248/307)	265.4(234/292)	259(227.7/293)	<0.001	240.3(1
Supernatant K^+(mmol/L)	21.8(10.2/33.12)	32.9(21/40.7)	37.7(27/44.5)	<0.001	38.6(2
Supernatant Hb (g/dL)	0.08(0.02/0.17)	0.07(0.02–0.17)	0.11(0.02/0.3)	0.74(0.6/1)	0.61(0
ATP (pmol/μL)	90.2(60.68/189.8)	70.4(38.2/102.9)	84.6(63.9/166.8)	0.001	60.9(4
Fragmentation	1.72(1.13/2.43)	2.29(1.36/3.15)	2.24(1.69/4.96)	0.008	3.01(1
Hemolysis (%)	0.37(0.19/0.80)	1.49(0.47/5.09)	1.74(0.78/5.21)	<0.001	0.7(0