

The Influence of Blood Storage Time on the Potassium Level of Patients Receiving Packed Red Cell (PRC) Transfusion in Adam Malik General Hospital, Medan

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Abstract:-

Background: Packed Red Blood Cell (PRC) is a blood component obtained after most of the plasma is separated from Whole Blood (WB) by various methods and has a hematocrit value of 80%. During storage time, blood cells undergo metabolic changes, so in vitro storage must be considered in an effort to reduce changes that occur in blood cells, because the atmosphere is really different from in vivo.

Objective: This study aims to determine the effect of the long storage time of blood on the potassium levels of patients receiving Packed Red Cell (PRC) transfusion in Haji Adam Malik General Hospital, Medan.

Methods: Analytical study with a pretest-posttest group design to determine the effect of blood storage time on changes in blood potassium levels after Packed Red Cell (PRC) transfusion in Haji Adam Malik General Hospital, Medan.

Results: The pre-potassium value with blood storage time < 3 days, the average potassium value was 3.79 ± 0.54 , the mean potassium value on the blood bag was 4,14 and the post mean value found $4,25 \pm 0,55$, with the p value 0,81 means there're no significant difference between the pre potassium, bag potassium and post potassium. Meanwhile, the duration of blood storage > 3 days, the average potassium value was 4.01 ± 0.79 , the mean potassium value in the bag was 6.45 ± 0.51 and the average post potassium value was 7.75 ± 0.95 with a p value of 0.001, where there was a significant difference between pre potassium, bag potassium and post potassium. There was an increase in the value of pre-potassium levels after administration of a transfusion.

Conclusion: There is an effect of blood storage time on potassium levels, where there is a change in potassium levels in > 3 days of blood storage, while blood storage time of < 3 days we found a steady potassium levels.

Keywords: *Packed Red Blood Cell, Potassium, Storage Time*

I. INTRODUCTION

Blood transfusion is an important and routine medical treatment, especially in cases that require surgery such as trauma. This procedure is generally relatively safe, but the risk of the procedure still cannot be completely stopped. In addition, the blood transfusion procedure is also a difficult procedure. This is due to standard operating procedures related to the collection, storage, supply, and use of blood products as well as post-operative care, both with and without complications. Blood transfusion has become an important part of health services. One of the needs that is needed is when there are patients who are in an emergency (Mohamed Saleh R et al, 2018).

Packed Red Blood Cell (PRC) is a blood component obtained after most of the plasma is separated from Whole Blood (WB) by various methods and has a hematocrit value of 80%. Packed Red Blood Cells still contain leukocytes, platelets and a small amount of plasma. One RRC unit with a volume of 150-300 mL has a blood cell mass of about 100-200 mL. Erythrocyte transfusion aims to lift or lift blood to transport oxygen, therefore good quality erythrocytes are needed (Kaur P, Basu S, 2013).

Packaged red blood cells can be stored from the time of donation until they are transfused to the recipient. The purpose of PRC storage is to maintain the viability and function of erythrocytes by reducing the metabolic activity of cells. Packed red blood cells were stored at 2-6°C in certain media. Proper storage is one way to maintain the quality of erythrocytes. Current standards require that 24 hours after transfusion, more than 75% of blood cells are recovered in circulation (Marti, 2015). During storage, blood cells undergo metabolic changes, so in vitro storage must be considered in an effort to reduce changes that occur in stored blood cells, because the atmosphere is very different from in vivo. Changes that occur during storage are decreased levels of Adenosine 5'-triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG), decreased blood pH, increased potassium and lactate levels, changes in erythrocyte cell shape, loss of erythrocyte viability. and hemolysis (Lelubre C, 2013).

Biochemical changes that occur in PRC due to storage of erythrocytes, one of which is an increase in potassium levels in PRC plasma. The activity of the Na⁺/K⁺-ATPase pump is strongly influenced by temperature. The pump becomes active at 4°C causing potassium to leave the cell and sodium into the cell. During blood storage, potassium leaks into the plasma due to failure of the Na⁺/K⁺-ATPase pump, this process occurs slowly and continuously. Potassium levels in PRC plasma can increase by 0.5-1 mmol/L per day. Potassium leaks progressively from erythrocytes after three days of storage and extracellular levels may increase to 50 mmol/L. The peak plasma potassium level of PRC after two weeks of storage can reach 90 mmol/L (Madina UU, 2014).

Several studies were conducted to determine the potassium level in plasma PRC which was associated with storage time. Research conducted by Minnesota concluded that as potassium increased with storage time of PRC, the plasma potassium level of PRC increased by 77.0 mmol/L on day 14 of storage. In a study conducted by Monica 2015 it was said that PRC given to patients is better given before 7 days because the longer PRC is stored, there is a significant increase in potassium levels. (Monica, 2015). Ratcliffe et al. reported an increase in potassium and lactate levels, as well as an associated decrease in sodium and glucose during blood storage. Smith et al, analyzed that the potassium level of PRC increased with storage time, ie initially 7.3 mEq/L (1 mEq/L=1 mmol/L) and in the first week (0–7 days) of storage, the mean potassium level was 19 mEq/L, in the second week (8–14 days) 31.5 mEq/L and between 15–28 days of storage 39.9 mEq/L. In the study of Uvizl et al., in 2011 found changes in potassium levels in stored PRC, which increased gradually from 4.0 mmol/L on the first day to 40.5 mmol/L on the 35th day.

Hyperkalemia is the most common complication of stored blood transfusion. Cardiac arrest has been widely reported in transfusion-associated hyperkalemia. Several studies suggest that the clinical impact of storage injury becomes significant after two weeks of storage (Lelubre C, 2013). The British study by Koch CG in 2008 reported that in 6,002 cardiac surgery patients who received PRC transfusions for either 14 days or >14 days, there was a significant relationship between mortality and blood retention time (Koch CG, 2008). An Australian study by Yap CH in 2008 reported that long-stored transfusion of PRC units (more than two weeks) was associated with an increased risk of postoperative complications and mortality (Yap CH, 2008). The storage of transfused blood at Haji Adam Malik Hospital is a maximum of 7 days for PCR while whole blood is 1 month. Based on the above background and no research has been conducted on the comparison of PRC potassium levels based on storage time in the blood bank, the researchers are interested in conducting research on the effect of blood storage time on changes in blood potassium levels after packed red cell (PRC) transfusion at Haji Adam Malik Hospital Medan.

II. METHODS

This study used an analytical study with a pretest-posttest group design to determine the effect of blood storage time on changes in blood potassium levels after transfusion of packed red cell (PRC) type at Haji Adam Malik Hospital, Medan. This research was conducted in the central operating room, emergency operating room and Clinical Pathology Laboratory, Haji Adam Malik Hospital, Medan. The research time starts after ethical clearance is issued until the number of samples is met. The study population was all patients who underwent elective and emergency surgery requiring PRC blood transfusion at the Haji Adam Malik General Hospital Medan.

The research sample was patients who underwent elective surgery and emergency surgery that required PRC blood transfusion at the Haji Adam Malik General Hospital Medan who met the research criteria. The technique of obtaining samples was by consecutive sampling, namely looking for patients who met the inclusion and exclusion criteria until the required number of samples was met. After statistically calculated, all samples were divided into 2 groups, namely:

- Group A blood transfusion store < 3 days
- Group B blood transfusion store > 3 days

Inclusion criteria in this study were patients who underwent elective and emergency surgery requiring PRC blood transfusion, experienced grade 3-4 bleeding, aged 18-64 years, PS ASA 1-3, and normal potassium levels before surgery. While the exclusion criteria in this study were patients who refused to participate in the study, had a history of allergies to blood transfusions, patients with pregnancy, patients with kidney disorders or patients with a history of whole blood transfusions, platelets. Dropout Criteria The tests in this study were patients who experienced allergies during blood transfusion, cardiovascular and respiratory emergencies occurred in the form of: MAP <50 and persistent, pulse rate <60 or >150 beats per minute and persisted, arrhythmia occurred, respiratory rate <12 breaths per minute or apnea, oxygen saturation <90% and not increasing with oxygen administration and patients with bleeding greater than 30% EBV.

From the results of calculations using the sample size formula, the total sample size for both groups was 28 samples, the researchers rounded up to 30 samples, each group 15 samples. After obtaining approval from the Ethics Committee of the Faculty of Medicine, University of North Sumatra, General Hospital Haji Adam Malik Medan (RSUP HAM), research samples were taken at Haji Adam Malik Hospital Medan. Informed consent of volunteers who will help to oversee the process of giving the transfusion, the volunteer is an anesthesiologist (senior anesthesiologist PPDS who performs anesthesia and monitoring operations).

The sample population was divided into 2 groups, namely Group A stored blood transfusion < 3 days and group B stored blood transfusion > 3 days. Before the blood transfusion is given, it is first asked how long the blood has been stored to the UTD officer. Then, the potassium was

checked on the transfusion bag. On admission to the operating room, systolic blood pressure (SBP), diastolic blood pressure (DBP), Mean Arterial Pressure (MAP), Heart Rate (HR), oxygen saturation (SpO₂) before anesthesia was performed.

During the operation, fluid maintenance was given using 0.9% NaCl solution, every 5 minutes during the operation the systolic blood pressure (SBP), diastolic blood pressure (DBP), average arterial pressure, heart rate (HR) rate, oxygen saturation was recorded (SpO₂), and the amount of bleeding. If there is bleeding that requires blood according to the inclusion criteria, the patient is transfused with PRC. At the end of the operation, the amount of blood transfused was recorded and after 6-8 hours after the transfusion, the blood potassium level was checked.

After giving the transfusion, the side effects are monitored, if there are side effects, they are recorded. The

collected data was processed and analyzed statistically using statistical product and service solution (SPSS 25) windows program. For numerical data presented in the mean ± standard deviation and statistical tests to compare between statistically significant or significant.

III. RESULTS

This study used an analytical study with a pretest-posttest group design to determine the effect of blood storage time on changes in blood potassium levels after transfusion of packed red cell (PRC) type at Haji Adam Malik Hospital, Medan. The research sample in the study amounted to 30 people, although the study found the dropout criteria for 2 samples due to bleeding > 30%, but it did not interfere with the number of samples because the sample dropped out, then replaced with a new sample.

Characteristics	Subjects	P value
Age	46,60 ± 13.37	0,07
Gender		
Female	15 (50%)	0,06
Male	15 (50%)	
Systolic	105,50 ± 20,84	0,7
Diastolic	70,20 ± 12,08	0,10
MAP	83,08 ± 14,67	0,65
RR	23 ± 2,42	0,87
ASA II	26(86,7%)	0,001
ASA III	4 (13,3%)	

Table 1: Sample Characteristics

*Shapiro Willk

Based on table 1, it shows that the distribution of sample characteristics in this study has a mean age of 46.60 ± 13.37. Based on gender, the distribution of male is 15 (50%) and female is 15 (50%). Based on the hemodynamic picture, the mean systolic was 105.50 ± 20.84, diastolic was 70.20 ± 12.08, RR was 23 ± 2.42 and MAP was 83.08 ± 14.67. Asa in this patient was most commonly found in ASA II by 26 (86.7%) and ASA III 4 (13.3%). With p > 0.05, then based on age, gender, systolic, diastolic, MAP, RR the sample is said to be normally distributed or homogeneous.

Characteristics	Subject	P value
Digestive Surgery	7 (23,3%)	
Surgical Oncology	3 (10%)	
Neurosurgery	1 (3,3%)	
BTKV	1 (3,3%)	0,001
Obgyn	6 (20%)	
Orthopaedic	4 (13,3%)	
Urology	8 (26,7%)	

Table 2: Sample Characteristics by Type of Operation

Based on table 2 shows that the distribution of sample characteristics based on the type of surgery obtained the most types of surgery in urological surgery of 8 (26.7%), and the smallest type of surgery in BTKV surgery and neurosurgery 1 (3.3%) With p < 0.05, then it is not normally distributed or not homogeneous.

Characteristics	Blood Storage Time < 3 days	P Value
Patient's Potassium Before Transfusion	3,79 ± 0,54	0,10
Potassium in Bag	4,14 ± 0,59	0,57
Patient's Potassium After Transfusion	4,25 ± 0,55	0,54

Table 3: Potassium Levels Based on Blood Storage Duration <3 Days

*Shapiro Willk

Based on table 3, the duration of blood storage < 3 days, the potassium value before transfusion was 3.79 ± 0.54 , the mean potassium value in the bag was 4.14 ± 0.59 and the average potassium value after 4.25 ± 0.55 with a p value > 0.05 then the average potassium level in the sample is said to be normally distributed or homogeneous.

Characteristics	Blood Storage Time > 3 days	P Value
Patient's Potassium Before Transfusion	$4.01 \pm 0,79$	0,08
Potassium in Bag	$6,45 \pm 0,51$	0,26
Patient's Potassium After Transfusion	$7,75 \pm 0,95$	0,61

*Shapiro Willk

Based on table 4 for blood storage > 3 days, the mean value of potassium before transfusion was 4.01 ± 0.79 , the average potassium value in the bag was 6.45 ± 0.51 and the average potassium value was 7.75 ± 0.95 with a p value > 0.05 then the average potassium level in the sample is said to be normally distributed or homogeneous.

Characteristics	Patient's Potassium Before Transfusion	Patient's Potassium After Transfusion	P value
Blood storage time <3 days	$3,79 \pm 0,54$	$4,25 \pm 0,55$	0,72
Blood storage time >3 days	$4.01 \pm 0,79$	$7,75 \pm 0,95$	0,001

Table 5: Effect of Potassium Levels Based on Blood Storage Duration <3 Days and >3 Days

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Based on table 5 the duration of blood storage < 3 days, the potassium value before transfusion was 3.79 ± 0.54 and the mean value of potassium after 4.25 ± 0.55 with a p value of $0.72 > 0.05$, it can be concluded that there is no significant difference. between pre-transfusion and post-transfusion potassium levels. This is different from the storage time of blood > 3 days, the mean value of potassium before transfusion was 4.01 ± 0.79 and the value of potassium after the mean was 7.75 ± 0.95 with a p value of $0.001 < 0.05$. It could be concluded that there was a significant difference between potassium levels before transfusion and after transfusion, although the mean level of potassium after transfusion on blood storage duration > 3 days, the average was 7.75 ± 0.95 , there would be no side effects after transfusion

IV. DISCUSSIONS

In this study, we assessed the level of potassium after transfusion was associated with the length of storage of blood in H Adam Malik General Hospital Medan. Based on gender, the distribution of male 15 (50%) and female 15 (50%). Based on the hemodynamic picture, the mean systolic 105.50 ± 20.84 , diastolic 70.20 ± 12.08 , RR 23 ± 2.42 and MAP 83.08 ± 14.67 we can see that the MAP in this study is stable. Based on the duration of blood storage < 3 days, the average blood potassium level was within normal limits of ($3.79 \pm 0.54 : 4.25 \pm 0.55$). Meanwhile, for blood storage > 3 days, there was a significant difference where the mean potassium value was 4.01 ± 0.79 and the potassium value after average was 7.75 ± 0.95 with a p value of 0.001 which there was a significant difference between potassium before potassium after transfusion where there is an increase in the value of potassium levels after transfusion. This is in accordance with a study conducted by Minnesota which concluded that potassium increased with PRC storage time, changes in PRC plasma potassium levels were found on the 4th day of storage and over time

increased by 77.0 mmol/L on the 14th day of storage. . This is the same as a study conducted by Tuti Asryani where there was an increase in potassium levels in the blood stored for more than 3 days.

According to research in 2019, it was also found that there was an increase in plasma potassium concentration in blood preparations stored in storage before being given, followed by an increase in the patient's plasma potassium level after the blood preparation was transfused and when it was transfused, there was no change in clinical signs and symptoms experienced by the patient. . In this study, it was also stated that the average potassium level in stored blood preparations increased by 0.5-1.0 mmol/L every day (Antwi-Baffour, 2019). In the Rizos study, 2017 in Greece, it was found that there was an increase in plasma potassium levels in patients transfused with blood using stored blood for more than 4 days, but no side effects were found in the study sample (Rizos, 2017). During storage, blood cells undergo metabolic changes, so in vitro storage must be considered in an effort to reduce changes that occur in blood cells during storage, because the atmosphere is very different from in vivo. Changes that occur during storage are decreased levels of Adenosine 5'-triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG), decreased blood pH, increased levels of potassium and lactate, changes in erythrocyte cell shape, loss of erythrocyte viability. and hemolysis (Lelubre C, 2013).

Biochemical changes that occur in PRC due to storage of erythrocytes, one of which is an increase in potassium levels in PRC plasma. The activity of the Na⁺/K⁺ATPase pump is strongly influenced by temperature. The pump becomes inactivated at 4°C causing potassium to leave the cell and sodium into the cell. During blood storage, potassium leaks into the plasma due to failure of the Na⁺/K⁺ATPase pump, this process occurs slowly and continuously. Potassium levels in plasma PRC may increase

by 0.5-1 mmol/L per day. Potassium leaks progressively from erythrocytes after three days of storage and extracellular levels may increase to 50 mmol/L. The peak plasma potassium level of PRC after two weeks of storage can reach 90 mmol/L (Madina UU, 2014). Fergusson's study reported that transfusion of PRC with longer shelf-life resulted in significantly worse clinical outcomes or improvements. This is due to the continued metabolism of blood cells during blood storage.

V. CONCLUSION

The duration of storage of blood in stored blood was >3 days, there was an increase in potassium levels after transfusion, while for potassium levels <3 days, there was no increase in potassium levels after transfusion. Potassium levels before packed red cell (PRC) transfusion were mean in patients with blood storage duration < 3 days 3.79 ± 0.54 and > 3 days 4.01 ± 0.79 within normal limits. Potassium levels after being given a packed red cell (PRC) transfusion, the average potassium level of patients with blood storage < 3 days was 4.25 ± 0.55 and > 7.75 ± 0.95 . In the long storage of blood > 3 days after transfusion, there is an increase in potassium levels. It is hoped that in further research, it is hoped that the length of storage of blood can be divided according to time and it is hoped that the blood transfusion procedure uses blood with a storage period of < 3 days.

REFERENCES

- [1.] Koch CG, Li L, Sessler D, Figueroa P, Hoeltge G, Mihaljevic T, et al. Duration of Red Cell Storage and Complications After Cardiac Surgery. *New England Journal Medicine*. 2008; 358:1229-39.
- [2.] Lelubre C, Louis V. Relationship Between Red Cell Storage Duration and Outcomes. Adults Receiving Red Cell Transfusion: A Systematic Review. *Crit Care*. 2013;17(2):1-18.
- [3.] Leo MG, Anneke B. Effects of storage of Red Cells. *Transfusion Medicine and Chemotherapy*, 2008;35(5):359-67.
- [4.] Madina UU, Abdullah M. Blood Transfusion Strategy in Gastrointestinal Tract Bleeding : Liberal or Restrictive. Department of Internal Medicine Faculty of Medicine University of Indonesia. 2014 Volume 15, Number 3.
- [5.] Maharani, Rheva. 2017. Apa Yang Dimaksud Dengan Uji Silang Serasi Darah (Crossmatch) Pada Darah Donor. Diakses pada tanggal 05/01/2021 di situs <https://www.dictio.id>
- [6.] Mangku G, Senapathi TGA. *Buku Ajar Ilmu Anestesia dan Reanimasi*. Indeks. Jakarta Barat. 2010. hal 302-315.
- [7.] Marino PL. *The ICU Book*. 3rd edition. New York: Lippincott Williams & Wilkins, 2007.
- [8.] Martí-Carvajal AJ, Simancas-Racines D, Peña-González BS. 2015. Prolonged storage of packed red blood cells for blood transfusion (Review). *The Cochrane Collaboration*
- [9.] Maxwell MJ, Wilson MJ. Complication of Blood Transfusion. *British Journal of Anaesthesia*. 2006;6(6):225-229
- [10.] Mc Cullough J. Preparation, Storage, and Characteristic of Blood Components and Plasma Derivatives. Dalam: *Transfusion Medicine*. Edisi ke-3. New Delhi: Blackwell Publishing Ltd; 2012. hlm. 68-77.
- [11.] Miller RD. *Miller's Anesthesia*. 8th edition. Philadelphia: Elsevier Saunders; 2015.
- [12.] Mohamed Saleh R, Zefarina Z, Che Mat N. F. et al. (2018) Transfusion medicine and molecular genetic methods. *Int J Prev Med*; 9:45.
- [13.] Morgan GE, Mikhail MS. *Clinical Anesthesiology*. 5th Edition. United States: Lange; 2013.
- [14.] Mukherjee S, Marwaha N, Prasad R, Sharma R, Thakral B. Serial Assessment of Biochemical Parameters of Red Cell Preparations to Evaluate Safety for Neonatal Transfusions. *Indian J Med Res*. 2010; (132):715-20.
- [15.] Monica Verma, Kiran Dahiya , Deepika Malik , 2015. Effect of Blood Storage on Complete Biochemistry. Verma et al., *J Blood Disord Transfus* 2015, 6:6 DOI: 10.4172/2155-9864.1000329
- [16.] Normund S et al. The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patient- First result. *Acta Chirurgica Laviensis*. 2009; 9 (1): 24–7
- [17.] Opoku-Okrah C, Acquah BKS, Dogbe EE. Changes In Potassium and Sodium Concentration In Stored Blood. *Pan African Medical Journal*. 2015;20(1):1-6.
- [18.] Raza S, Baig M, Chang C, Dabas R, Akhtar M, Khan A, et al. A Prospective Study On Red Blood Cell Transfusion Related Hyperkalemia In Critically Ill Patients. Original Article *J Clin Med Res*, 2015;7(6):417-21.
- [19.] Rizos CV, Milionis HJ, Elisaf MS. Severe Hyperkalemia Following Blood Transfusions: Is There a Link?. *World Journal of Nephrology*. 2017; 6(1)53-56
- [20.] Sacher R, Mc Person R. Ilmu Kedokteran Transfusi. Dalam *Tinjauan Klinis Hasil Pemeriksaan Laboratorium*. Edisi ke-11. Jakarta: EGC; 2002. hlm. 235-40.
- [21.] Scott GM, LeGrys VA, Hood JL. Electrolytes and Blood Gas. In: *Tietz Fundamental of Clinical Chemistry*. 6th Ed., Philadelphia, Elsevier, 2008; 431–4
- [22.] Setyati, S. 2010. *Transfusi Darah yang Rasional*. Pelita Insani : Semarang
- [23.] Sharma S, Sharma P, Tyler LN. Transfusion of Blood and Blood Products: Indications and Complications. *Am Fam Physician*. 2011;83(6):719-724
- [24.] Smith H, Farrow S, Ackerman J, Stubbs J, Sprung J. Cardiac Arrest Associated with Hyperkalemia Red Blood Cell Transfusion. *Anesthesi Analg*. 2008;106(4):1062-9.
- [25.] Sutjiyanto A, Nurulita A, Mangarengi F. Kadar Kalium Di Packed Red Cell Simpanan. *Indonesian Journal of Clinical Pathology and Medical Laboratory*. 2014;

- 22(2):147-9. 23. Karon B, Buskirk C, Jaben E, Hoyer J, Thomas D. Temporal Sequence of Major Biochemical Events During Blood Bank Storage of Packed Red Blood Cells. *Blood Transfusion*. 2012; 10:453-61.
- [26.] Uvizl R, Klementa B, Adamus, Neise J. Biochemical Changes in The Patients Plasma After Red Blood Cell Transfusion. *Signa Vitae*. 2011;6(2):64-71.
- [27.] van de Watering L, Brand A. Effects Of Storage Of Red Cells. Review Article *Transfusion Medicine and Hemotherapy*. 2008;100(1):359-67.
- [28.] Watering LMG. Alternatives to Blood Transfusion in Transfusion Medicine. *Research Gate*. 2008 Nov. doi: 10.1111/j.1778- 428X.2008.00114.x
- [29.] Yaddanapudi S, Yaddanapudi LN. Indications For Blood And Blood Product Transfusion. *Indian J Anaesth*. 2014 Sep-Oct, 58(5): 538-542
- [30.] Yap CH, Lau L, Khrisnaswamy M, Gaskell M, Yii M. Age of Transfusid Red Cells and Early Outcomes After Cardiac Surgery. *Ann Thorac Surg*. 2008; 86:554-9.