ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 2: 501-505
P-ISSN.2089-1180, E-ISSN: 2302-2914

Correlation between hemolysis index and storage period to potassium levels of Packed Red Cell in Sanglah General Hospital, Bali, Indonesia

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Received: 2022-03-09
Accepted: 2022-05-10
Published: 2022-05-20

ABSTRACT

Background: The quality of a blood product is determined by collecting, processing, storing, and transporting it. When blood is stored, there will be mechanical and biochemical changes. Mechanical changes consist of morphological changes and hemolysis, while one of the biochemical changes is increased plasma potassium levels. This study aimed to determine the correlation between the hemolysis index and the storage period to potassium levels of the packed red cell (PRC).
Methods: This study was a cross-sectional study at Sanglah Hospital. The sample was taken from the tube in the PRC blood bag and then a complete blood count and potassium analysis were performed. Data were analyzed using SPSS version 25 for Windows.
Results: From 70 subjects, the median hemolysis index was 0.15% (0-1.69%), the mean storage period was 12.06±4.95 days, and the mean potassium level was 12.06±3.92 mmol/L. The Spearman test results showed a positive weak significant correlation between the hemolysis index and potassium levels (r=0.360; p=0.002). The Pearson test results also showed a positive weak significant correlation between the parameter of the storage period and potassium levels (r=0.357; p=0.002).
Conclusion: There was a positive weak significant correlation between the hemolysis index and the storage period to potassium levels of the PRC.

Keywords: Hemolysis Index, Potassium, PRC, Storage Period.
Cite This Article: Wardhana, C.A., Lestari, A.A.W., Wande, I.N., Herawati, S., Mahartini, N.N. 2022. Correlation between hemolysis index and storage period to potassium levels of Packed Red Cell in Sanglah General Hospital, Bali, Indonesia. *Bali Medical Journal* 11(2): 501-505. DOI: 10.15562/bmj.v11i2.3604

INTRODUCTION

The quality of a blood bag is determined by the process of making, storing, and transporting it. There will be mechanical and biochemical changes when the blood is stored. Mechanical changes are in the form of morphological changes and hemolysis, while one of the biochemical changes is an increase in plasma potassium levels.[1,2]

When the blood bag is stored, there will be a constant release of intracellular to extracellular potassium due to the failure of the sodium-potassium pump system. Plasma potassium levels are estimated to increase by 0.5–1.0 mmol/L per day when stored in the refrigerator. The estimated total extracellular potassium in blood stored for 35 days is between 7–25 mmol/L.[2]

Apart from the storage duration of blood cells, other factors are thought to correlate with increased potassium levels in the blood bag, such as the hemolysis index.[3] The hemolysis index is the ratio between the hemoglobin level of plasma and the hemoglobin of blood in the bag, which is determined by calculating the percent of hemolysis. Hemolysis percentage was calculated using hemoglobin in plasma, hematocrit, and total hemoglobin in Packed Red Cells (PRC). Percent hemolysis = plasma hemoglobin x (100 - hematocrit) / total hemoglobin.[3]

Hemolysis is an important marker in evaluating the quality of blood storage. Regulation of the Minister of Health of the Republic of Indonesia Number 91 of 2015 concerning blood service transfusion standards recommends that the acceptable hemolysis index in the red blood cell component is <0.8%.[4] Sanglah Hospital Denpasar is one of the Bali government hospitals with a blood bank. Until now, there is no data about the quality of PRC storage in the blood bank.

Hyperkalemia is a potentially life-threatening condition characterized by increased potassium concentrations in the blood and is a fairly rare complication of blood transfusion.[6] Until now, there has been no international agreement regarding the definition of hyperkalemia. The European Resuscitation Council states hyperkalemia is a condition with a plasma potassium concentration of >5.5 mmol/L and severe hyperkalemia if the plasma potassium concentration is >6.5 mmol/L. Hyperkalemia is known to be associated with poor outcomes and risk of death in patients.[5,6]

A study conducted on a population of critically ill patients at the Intensive Care Unit (ICU) stated that out of 125 patients who received a transfusion of 160 bags of PRC blood, 4% of patients developed hyperkalemia with plasma potassium concentrations >5.5 mmol/L after transfusion. PRC.[5] In addition, there have been published case reports that report the risk of fatal complications such as heart rhythm disturbances, cardiac arrest to death in pediatric patients or infants due to hyperkalemia after PRC transfusion.[7]

The pathogenesis of hyperkalemia due to PRC transfusion is complex and remains unclear. Various factors can affect, among others, the volume of blood transfused, the rate of transfusion, the condition of peripheral perfusion in the patient, and the storage of lesions in transfused blood.[2] Storage lesions are loss of viability and cell function associated with biochemical and cell morphology changes during in vitro blood storage.[8]

Based on those mentioned above, this study aimed to determine the correlation between the hemolysis index and the storage period to potassium levels of the Packed Red Cell (PRC), so that it is expected to know the quality of blood storage and prevent transfusion complications.

METHODS

This study was an analytic observational study with a cross sectional approach to determine the correlation between storage time and hemolysis index on potassium levels in PRC blood bags at the Sanglah Hospital Blood Bank. This research was conducted in September 2019-December 2019 at the Sanglah Hospital Blood Bank. The population in this study was the PRC blood bag in the Sanglah Hospital Blood Bank which was selected by consecutive sampling technique. The blood sample was taken from the tube of the PRC blood bag. The whole blood from the blood bag tube was used to measure total hemoglobin and hematocrit using the Advia 2120i instrument, then the whole blood was centrifuged to obtain the blood plasma. The blood plasma was used to measure the plasma hemoglobin using the Advia 2120i and potassium using the GEM Premier 3000 instrument. The inclusion criteria were all PRC blood bags that would be taken by the room nurse to be transfused into the patient and the storage temperature of the blood bags was 2-6°C. Exclusion criteria were blood bag age >42 days since blood collection.

The storage period is the number of days from blood collection to sample analysis. Hemolysis index is the ratio between plasma hemoglobin levels and blood hemoglobin in the bag obtained from examination using the cyanide-free hemoglobin method, measured spectrophotometrically at a wavelength of 550 nm in g/dL units using the Advia 2120i instrument. Potassium is the value of potassium from a sample taken from a blood bag tube and measured by the direct ion-selective electrode method, which compares the voltage difference between the sample electrode and the reference electrode with units of mmol/L using the GEM Premier 3000 instrument.

All data were analyzed using SPSS software (IBM Corporation, Armonk, NY, USA) version 25 for Windows. The Kolmogorov-Smirnov normality test is used on data with a numerical measuring scale to determine whether the data is normally distributed with a p-value >0.05. Univariate analysis was used for demographic data to obtain a characteristic distribution of study participants. Bivariate analysis was used to determine the correlation between storage period and hemolysis index to potassium levels in PRC blood bags using the Pearson correlation test and the Spearman correlation test. A p-value of less than 0.05 is considered statistically significant.

RESULTS

In this study, 70 bags of PRC blood met the inclusion and exclusion criteria. This study indicated that most of the PRC blood bags used had blood type O Rhesus positive (65.7%) and followed by blood type B Rhesus positive (21.4%). The normality test showed that the parameters of storage period and potassium were normally distributed, while the hemolysis index parameter was obtained with an abnormal distribution (Table 1). The median value for the hemolysis index was 0.15%, with a minimum value of 0% and a maximum value of 1.69%. In the parameter of storage time, the mean value was 12.06 ± 4.95 days, while the mean value of potassium content was 12.60 ± 3.92 mmol/L. This study also found 9 samples (12.9%) who had a hemolysis index ≥0.8%. Data on the basic characteristics of research blood bags can be seen in Table 1.

The correlation was tested using the Pearson correlation test for normally distributed parameters, while the parameters with an abnormal distribution used the Spearman correlation test. Figure 1 illustrates the graph of the results of the Spearman correlation test, which shows a significant weak positive correlation between the hemolysis index and potassium levels (r = 0.360; P = 0.002) with an R^2 of 16.5%. The results of the Pearson correlation test between the parameters of storage time to potassium levels also showed a significant weak

Table 1. General characteristics of blood bags.

Variable	Frequency (n=70)	Median (min-max)/ Mean±SD
Blood type, n (%)		
A Rhesus positive	4 (5.7)	
B Rhesus positive	15 (21.4)	
AB Rhesus positive	5 (7.2)	
O Rhesus positive	46 (65.7)	
Hemolysis index (%), n (%)		0.15 (0.00-1.69)
<0.8%	61 (87.1)	
≥0.8%	9 (12.9)	
Plasma hemoglobin (g/dL)		0.1 (0.00-0.6)
Total hemoglobin of PRC (g/dL)		13.3 (7.00-25.00)
Hematocrit of PRC (%)		38.1 (20.50-70.50)
Storage period (days)		12.06 ± 4.95
Potassium (mmol/L)		12.60 ± 3.92

SD = standard deviation

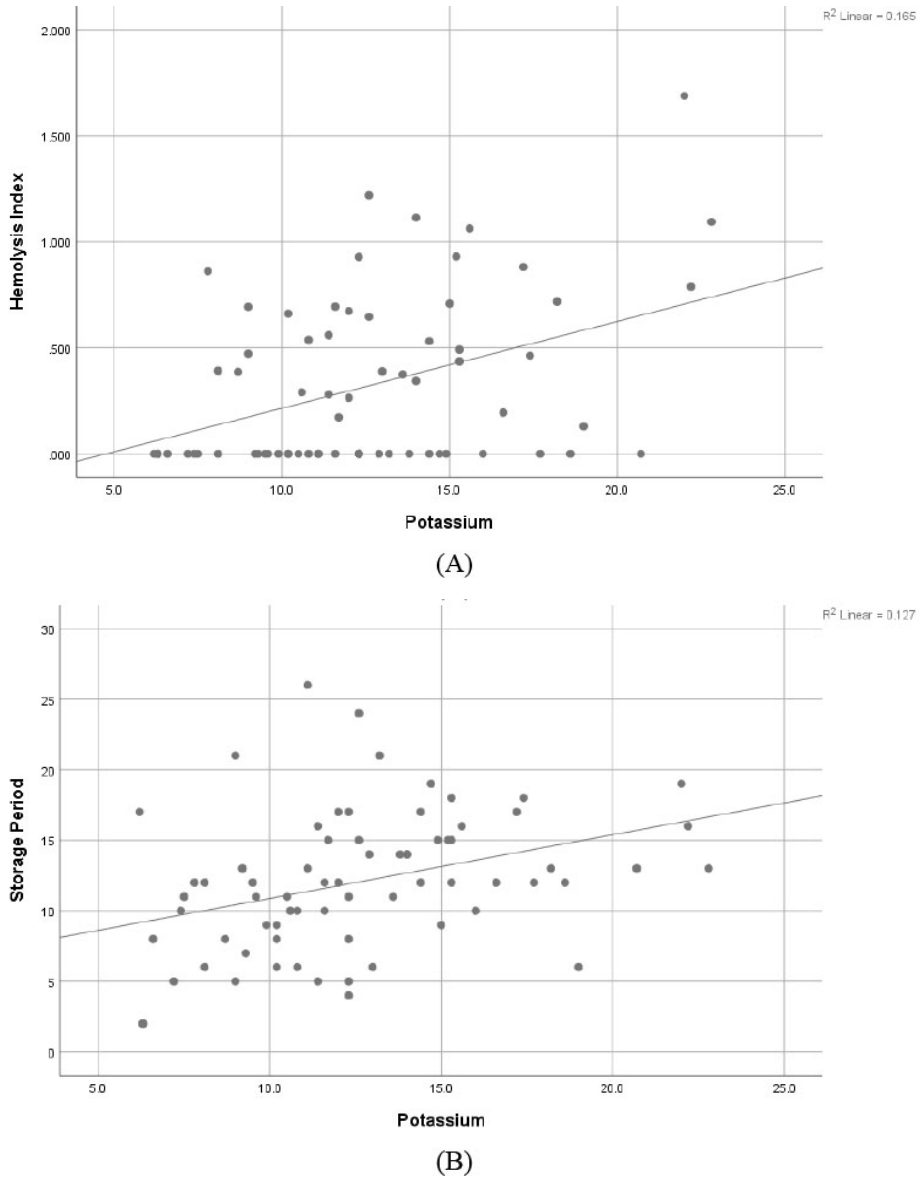

Figure 1. Scatter plot of the correlation between hemolysis index to (A) potassium levels and (B) storage period to potassium levels.

positive correlation (r = 0.357; P = 0.002) with an R^2 of 12.7%.

DISCUSSION

Blood type O is the most common blood type in Southeast Asia and then followed by blood type B.[9] A study in China that collected data from blood donors also found the most common blood type was O Rhesus positive and only 0.55% Rhesus negative from the total study population.[10] This study indicated that most of the PRC blood bags used had blood type O Rhesus positive (65.7%) and followed by blood type B Rhesus positive (21.4%).

Plasma potassium levels in the blood bag will constantly increase with the length of time the blood bag is stored until the transfusion is carried out. Adenosine triphosphate (ATP) is needed to maintain erythrocyte viability, phospho rylate glucose, and sodium-potassium pump. When the blood is mixed with anticoagulants and stored in the refrigerator, there will be a decrease in ATP levels and stimulate erythrocytes to release potassium out of cells due to failure of the sodium-potassium pump. In addition, there is also a change in erythrocyte morphology from biconcave discs to echinocytes, spherocytes, more rigid, and finally, erythrocytes lose their ability to survive while in circulation. This increase in potassium can also occur due to erythrocyte hemolysis which causes the efflux of potassium from cells to plasma.[2,11]

A significant weak positive correlation was found between the hemolysis index and potassium levels in the PRC blood bag (r=0.360; P=0.002). Hemolysis is an important parameter for evaluating the quality of RBC storage. Hemolysis is characterized by hemoglobin red and indicates erythrocyte lysis, which has a higher potassium concentration relative to plasma. The hemolysis index is the ratio between the hemoglobin plasma level and the hemoglobin blood in the bag, which is determined by calculating the percent hemolysis. Hemolysis percentage was calculated using hemoglobin in plasma, hematocrit, and total hemoglobin in PRC. Percent hemolysis = plasma hemoglobin x (100 - hematocrit)/total hemoglobin.[3]

The weak positive correlation between the hemolysis index and the potassium level is in accordance with the hemolysis theory that the occurrence of hemolysis in erythrocytes causes an increase in potassium levels in the blood bag due to the transfer of potassium contained in erythrocytes to the extracellular. The normal ratio between intracellular and extracellular potassium is 40:1. Therefore a change in the ratio of just 2.5% due to hemolysis can cause significant changes in plasma potassium levels.[12]

This study divided the hemolysis index group using a cut-off of <0.8% and ≥0.8%, according to the cut-off of the hemolysis index permitted by the Regulation of the Indonesian Minister of Health and the European Directorate for the Quality of Medicines & Healthcare (EDQM).[11-13] The study results found that 9 samples (12.9%) had a hemolysis index ≥0.8%. In Indonesia, based on the Regulation of the Indonesian Minister of Health number 91 of 2015 concerning blood service transfusion standards, the hemolysis index that is still allowed at the end of the storage period of the PRC bag is <0.8%. The regulation also requires the blood transfusion unit to conduct a monthly sampling of at least 4 PRC bags with quality control (QC) that can be accepted by 75% of all samples.[4]

Based on this regulation, it is known that

the quality of blood storage at the Sanglah Hospital Blood Bank still complies with the Regulation of the Minister of Health with the number of PRC blood bags whose hemolysis index <0.8% is 87.1% and does not exceed the permissible limit.

A significant weak positive correlation was also found between the parameters of storage period to potassium levels (r = 0.357; P = 0.002). When blood is stored in the refrigerator, there will be biochemical and morphological changes in erythrocytes with an increase in storage period. Biochemical changes that occur include a decrease in glucose, a decrease in ATP, a reduction in 2,3-diphosphoglycerate (DPG), an increase in pH, a disruption in the sodium-potassium pump, an increase in intracellular calcium, and a decrease in nitric oxide. Erythrocyte morphology also undergoes irreversible changes, the formation of echinocytes, spherocytes, and microvesicles.[8] The presence of leukocytes in packed red cell components also plays an important role in hemolysis during storage. This is due to various chemicals and enzymes, especially proteases, from the leukocytes.[14]

A change in potassium and LDH levels in the PRC blood bag from 0 days to 42 days was observed in the research. That research also reported an increase in potassium and LDH levels linearly with the storage period. This study also stated that the volume of erythrocytes was maintained by the sodium-potassium pump, which was dependent on the level of ATP in the erythrocyte membrane. The decrease in ATP levels is related to the continuous efflux of potassium.[11]

The results of this study are also in line with other studies that observed biochemical changes in the PRC blood bag over 6 weeks. Another study reported a significant difference (p <0.001) between the levels of measured parameters such as plasma potassium, plasma hemoglobin, and hemolytic index every week.[3] In this study, it was reported that there were significant weekly increases in the parameters of plasma potassium, plasma hemoglobin, hemolytic index, hematocrit, hemolysis, mean corpuscular volume (MCV), and plasma lactate. Other parameters such as sodium and glucose are reported to experience a significant decrease in levels every week.[3]

Another study found a positive correlation between storage age (particularly after 20 days of storage) of transfused PRC with increased markers of hemolysis and the inflammatory chemokine monocyte chemoattractant protein (MCP)-1 in very low birth weight infants. The study suggested special attention to PRC stored for more than 20 days when transfused into the neonate.[15]

The limitation of this study is that the samples analyzed came from a blood bag tube, which may not be representative or not reflect the biochemical changes that occur in the blood bag as a whole. This study only analyzed potassium levels in blood bags. A further study assessing potassium levels before and after transfusion in groups of patients at risk for hyperkalemia is needed to determine the effect of hemolysis on blood components transfusion.

CONCLUSION

There was a weak and significant positive correlation between the hemolysis index and the storage period of potassium in the PRC blood bag. Periodic evaluation of storage lesions in blood bags is needed to determine the quality of blood storage in the hospital blood bank and avoid transfusion complications.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICAL CLEARANCE

This study had been approved by the Research Ethics Commission of Sanglah Hospital, Denpasar (Ethical Clearance No. 2447/UN14.2.2.VII.14/LT/2020).

FUNDING

The author funded this research. The authors declare no conflict of interest related to the material presented in this article.

AUTHOR CONTRIBUTION

All authors were equally involved in making concepts and planning the research, data collection, calculating the experimental data, performing analysis, and critically revising the manuscript.

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