The Effect of Long Storage of Whole Blood Components on the Level of 2,3 Diphosphoglycerate and Lactic Acid in the Blood Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia

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Abstract

The purpose of this study was to analyze changes in levels of 2,3 DPG and lactic acid on WB storage time. This research is an observational analytical with time series design was conducted at the Clinical Pathology Installation and Blood Bank Dr. Soetomo General Hospital Surabaya in the period July - September 2020. Levels of 2,3 DPG and lactic acid were measured in 16 bags of Whole Blood components on the day 1, day 5, day 10, day 20 and day 30. Statistical analysis was performed using the Friedman test. The results were statistically significant if $p < 0.05$. The Friedman statistical test showed that there were significant differences in levels of 2.3 DPG ($p < 0.001$) and levels of lactic acid ($p < 0.001$) during storage. The results showed that the level of 2,3 DPG in WB which was stored decreased according to the duration of storage. The yield of lactic acid on stored WB increased with the duration of storage. Therefore, saving WB is recommended to be given within <6 days to reduce the risk of acidosis. Further research is needed for other parameters that can affect the storage process.

Keywords: *2,3 diphosphoglycerate, lactic acid, whole blood*

Introduction

Blood transfusion is an important part of modern health care. Blood transfusion is a series of processes for transferring donor blood to the recipient's blood circulation as a treatment effort. The main purpose of giving blood to a patient is to rapidly improve tissue oxygenation. Whole Blood component transfusion is used for resuscitation of patients with massive bleeding, hemorrhagic shock, and patients who need blood quickly.[1]

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The ideal WB transfusion is actually using fresh blood, which is blood that has just been taken from a donor until 6 hours after collection. The advantage of using fresh blood is that the clotting factors are still complete, including labile factors (V and VIII), the function of erythrocytes and platelets is still relatively good. Fresh blood is difficult to obtain because it takes more than 4 hours to check blood groups, cross reactions and transportation and the risk of disease transmission is relatively high.[2]

Fresh blood (fresh whole blood) is difficult to get, therefore WB transfusion can use stored blood. Storage of WB is recommended at refrigerator temperature (2-6°C) with storage time depending on the anticoagulant used. The advantage of using stored blood is that it is easily available at any time,

but the disadvantage is that clotting factors, especially factors V and VIII, have been used up and the ability of erythrocyte oxygen transport decreases. This is caused by a progressive decrease in levels of 2,3 Diphosphoglycerate (2,3 DPG).[3]

The decrease in the level of 2,3 DPG causes the release of oxygen in the tissue is low, resulting in tissue hypoxia. Blood with a low 2,3 DPG does not increase tissue oxygenation even though the hemoglobin level is raised. Blood with a low 2,3 DPG is not appropriate for patients requiring rapid oxygenation or resuscitation.[3] The level of 2,3 DPG is important to pay attention to in blood transfusions, because blood stored quickly can lose 2,3 DPG so that its ability to deliver oxygen will decrease.[4]

Whole blood (WB) during storage at the blood bank will experience a series of chemical changes that affect the viability and function of transporting oxygen from the lungs to the tissues. This change is known as a storage lesion. It is estimated that 1-5% of erythrocytes will be damaged during the time of donor collection and every day the erythrocyte viability will continue to decrease as a result of decreased levels of Adenosine Triphosphate (ATP). If the ATP level decreases, there is a loss of membrane lipids, the membrane becomes stiff and its shape changes from disc to spherical (without a central palor and small size), then this can cause potassium to leave and sodium to enter the cell. This lysis of red blood cell (RBC) causes a decrease in the RBC count and an increase in hemoglobin and iron.[5]

Erythrocytes do not have a nucleus and mitochondria so that energy is generated for oxidative metabolism through the breakdown of glucose. The breakdown of glucose into lactate or pyruvate is generally referred to as glycolysis. The process of glycolysis causes glucose to be consumed, resulting in a decrease in glucose levels. Glycolysis that occurs also results in the production of lactic acid by producing a low pH. Lactate, as an end product of red blood cell metabolism, will increase during storage. Transfusions of stored blood with increased lactate concentration by a mean concentration of 8 mmol/L during the first week of storage will increase metabolic acidosis.[6]

This research was conducted on the blood component of WB and measured the levels of 2,3 DPG and lactic acid at the storage period of the 1st, 5th, 10th, 20th, and 30th day. The selection of 2,3 DPG and lactic acid was based because they were the most influencing parameters for oxygenation to the tissues. The choice of measurement time for the day 1, day 5, day 10, day 20, and day 30 of measurements is based on the literature which states that the WB components are stored for 35 days at most to avoid the increased risk of contamination by bacteria.

Method

This research is an observational analytical study with a time series design which was conducted at the Clinical Pathology Installation and Blood Bank Dr. Soetomo General Hospital, Surabaya, Indonesia. The samples were 16 units of WB components stored at refrigerator temperature (2-6°C) on storage day 1, day 5, day 10, day 20 and day 30. Samples were taken by consecutive sampling technique from July-November 2020.

WB which is stored on the 1st day at 2-6°C temperature is accommodated in a plain tube, as much as 3 ml by clamping the hose in the WB bag first then cutting the ends and accommodating in a tube as much as 3 ml. The hose connecting the WB bag is closed again using a heat sealer. A plain tube containing 3 ml of WB was centrifuged at 3000 rpm for 15 minutes. Plasma was removed and put into an aliquot. The aliquots were then stored in a refrigerator at -200C until 16 samples were collected. Measurement of 2,3 DPG was carried out by the competitive ELISA method based on the insert kit from Bioassay[7] and measurement of lactic acid was carried out by the enzymatic method based on the insert kit from Randox.[8] The remaining WB blood components are again stored at 2-6°C. The same

method used for the first day sampling was carried out for the fifth day, tenth day, twentieth and thirtieth day of sampling. Data analysis was performed using the SPSS statistical program. The statistical test used is the Friedman test. The result of the test is statistically significant if the p value is <0.05.

Results and Discussion

The results of the normal distribution test using the Shapiro-Wilk test showed that the chlorhexidine gluconate group data was normally distributed (p >0.05), while the n-propanol and hydrogen peroxide groups were not normally distributed (p <0.05) (Table 1).

Table 1. Characteristics of the Research Sample.

Group Blood	Whole Blood (Bag)	Amount (%)
A +	6	37.5
B +	5	31.25
O +	5	31.25
AB +	0	0
Total	16	100

The sample of this study used 16 bags of Whole Blood (WB) components and the most WB bags were obtained with group A+ as many as 6 bags (37.5%) and no AB+ blood group was obtained as shown in Table 1.

Table 2 shows the results of the examination of 2.3 DPG on days 1, day 5, day 10, day 20 and day 30 of WB storage in the blood bank. There was a difference in the mean level of 2.3 DPG which was statistically significant at storage on day 1, day 5, day 10, day 20 and day 30 (p <0.001), namely the highest on day 1 (32.2 ± 11.2) compared to day 5 (30.0 ± 8.9), day 10 (28.4 ± 7.6), day 20 (27.1 ± 6.9) and day 30 (26.2 ± 7.2).

Table 2. Comparison of 2.3 DPG Levels (n = 16).

Parameter	Range	Median	Mean	SD	P value
2,3 DPG (µmol/ml) Day 1 Day 5	 23.5 - 58.7 22.4 - 56.8	 28.5 27.0	 32.2 30.0	 11.2 8.9	
Day 10	21.6 - 52.9	26.6	28.4	7.6	<0.001
Day 20 Day 30	21.3 - 51.7 19.5 - 51.4	26.2 25.2	27.1 26.2	6.9 7,2	

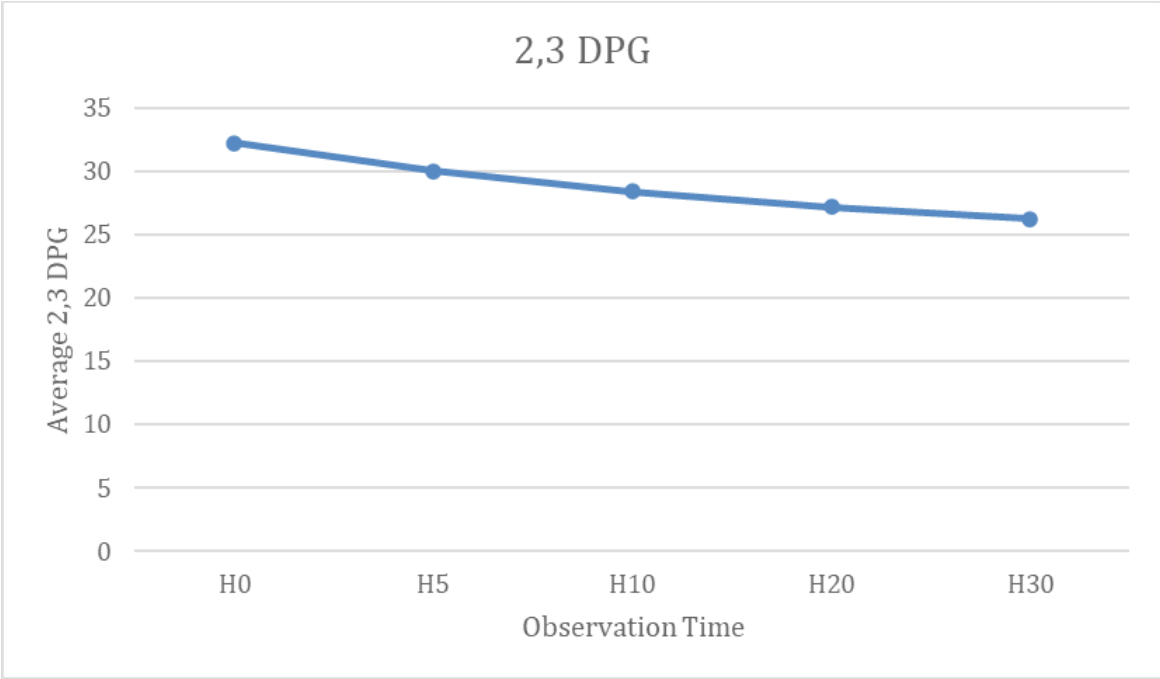

Figure 1. Line diagram of the average 2.3 DPG levels between observation times.

The results also showed that the levels of 2.3 DPG on day 5 were significantly lower than on day 1 ($p < 0.001$) and levels of 2.3 DPG on day 30 were significantly lower than on day 1, day 5, day 10 and day 20 ($p < 0.001$, respectively) (Figure 1).

Decreased levels of 2,3 DPG during the storage period, WB can be caused by the glycolysis process of red blood cells against WB components which are said to be the main cause of Red Blood Cell (RBC) Storage Lesions. During the storage period in the refrigerator, the temperature of 2-6°C red blood cell metabolism continues and glycolysis will also continue.[9,10]

Blood stored in the preservative Citrate Phosphate Dextrose Adenine (CPDA) and stored at a temperature between 2-6°C will undergo biochemical structural and functional changes that affect the viability and function of red blood cells due to differences in the atmosphere compared to invivo. These changes are called storage lesions, namely decreased concentrations of Adenosine Triphosphate (ATP) and 2,3 Diphosphoglycerate (2,3 DPG), decreased blood pH, increased potassium and lactate concentrations, changes in erythrocyte shape, loss of erythrocyte vitality, and hemolysis. 2,3 DPG is important for Hemoglobin (Hb) affinity for oxygen and for oxygen delivery to tissues. 2,3 DPG binds to deoxyhemoglobin and stabilizes it and facilitates the transport of oxygen from the lungs to the tissues by oxyhemoglobin. A decrease in 2,3 DPG leads to an increase in oxygen affinity for hemoglobin and therefore less oxygen delivery to the tissues.[11]

A reduction in 2,3 DPG levels in blood transfusion therapy should be considered because blood stored quickly can lose 2,3 DPG. However, the in vivo regeneration of 2,3 DPG after transfusion of depleted blood is a rapid process. The rate of in vivo regeneration depends on the quality and quantity of blood transfused as well as the state of the recipient.[12] In a study conducted by Juel reported that after 42 days of storage at 4^0C, the red blood cell concentration of 2,3 DPG decreased only by 92% from its original level.[14]

Table 3 shows the results of lactic acid tests on days 1, day 5, day 10, day 20 and day 30 of WB storage in the blood bank. There was a statistically significant difference in the mean levels of lactic acid

at storage on days 1, day 5, day 10, day 20 and day 30 ($p < 0.001$), namely the lowest on day 1 (4.7 ± 0.6) compared to day 5 (8.4 ± 1.2), day 10 (12.7 ± 2.0), day 20 (19.1 ± 2.2) and day 30 (22.2 ± 1.8).

Table 3. Comparison of Lactic Acid Levels (n=16).

Parameter	Range	Median	Mean	SD	P value
Lactic acid (mmol/ml)					
Day 1	3.7-5.9	4,7	4,7	0.6	<0.001
Day 5	6.8-11.2	8,4	8.4	1.2	
Day 10	9.9-16.6	12.4	12.7	2.0	
Day 20	16.9-23.1	18.4	19.1	2.2	
Day 30	19.3-25.2	22.0	22.2	1.8	

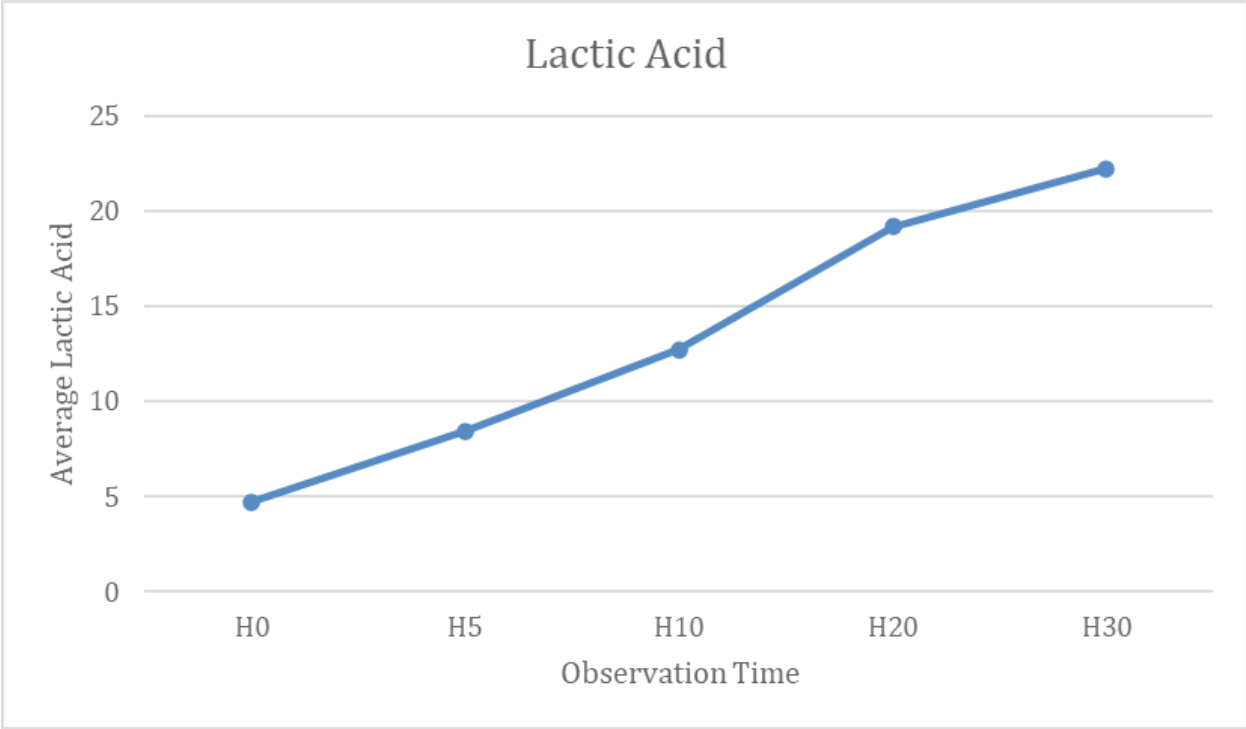

Figure 2. Line diagram of mean lactic acid levels between observations time.

The results also showed that the levels of lactic acid on day 5 were significantly higher than on day 1 ($p < 0.001$) and the levels of lactic acid on day 30 were significantly higher than on day 1, day 5, day 10 and day 20 ($p < 0.001$, respectively) (Figure 2).

The increase in lactic acid levels occurs during storage in the blood bank at a temperature of 2-6^{0}C due to glycolysis for ATP production and is associated with changes in pH. Blood stored for 35 days showed a higher lactic acid content than fresh blood.[15]

Glycolysis (breakdown of glucose) in normal red blood cell metabolism is the only source of energy for red blood cells. Red blood cells do not have mitochondria and depend entirely on glycolysis for energy needs. Red blood cells produce energy through the anaerobic glycolysis pathway, one glucose

molecule through anaerobic glycolysis produces ATP and pyruvic acid molecules, then pyruvic acid will be converted into lactate as the end product of red blood cell metabolism. Lactate as the end product of red blood cell metabolism will increase during storage.[16]

Increased levels of lactic acid in stored Whole Blood (WB) can affect the condition of the recipient's body or worsen the condition of the recipient's body experiencing acid base balance disorders.[15] Transfusion of stored blood with an increased concentration of lactic acid and an average concentration of lactic acid 8.0 mmol/l during the first week of storage, will increase metabolic acidosis.[13] The normal level of lactic acid in the blood is 0.6-1.5 mmol/l or 0-20 mg/dl and the level of lactic acid that can be accepted by the body is around >2,0-5 mmol/l or >18.02-45.05 mg/dl.[17,18]

Conclusion

In summary, acid 2,3 DPG in stored WB decreased and lactic acid in stored WB increased with the duration of storage. It is recommended that WB transfusion be given to save WB blood <6 days so that the risk of acidosis is lower. Also recommended further research is needed for other parameters that can affect the storage process.

Conflict of Interest: The author declare that they have no conflict of interest.

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