Analysis of Whole Blood Quality: Number of Erythrocytes, Leukocytes, Platelets, and pH Value during 28-day Storage

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Abstract: Whole blood contains all the elements of blood such as all blood cells, plasma, and clotting factors. It is used in the treatment of massive bleeding. However whole blood has an expiration time limit. The quality of blood decreases gradually due to storage time and causes blood cell lysis, so it directly affects blood cell counts and pH. The aim of this study was to determine the quality of whole blood during 28day storage. Whole Blood from blood bags containing CPDA-1 was used as a sample. The number of erythrocytes, leukocytes, and platelets was measured by Hematology Analyzer. The results of the study showed that there was a decrease in the number of erythrocytes from 5,02 x 10^6cell/µL into 4,92x10^6cell/µL. The number of leukocytes decreased from 6,31x10^3cell/µL to 3,17 x 10^3cell/µL. Platelet count also decreased from 195x10^3cell /µL to 81x10^3cell/µL. The pH value decreased from 7.2 on to 6.9. This study concluded that there was a decrease in the number of erythrocytes, leukocytes, platelets, and pH. The number of erythrocytes and pH was still normal, while the number of leukocytes and platelets was below the normal range.

1 INTRODUCTION

Blood transfusion is a therapy to save someone's life using blood and its components (Booth and Allard, 2017). The main principle of blood transfusion is to safely and effectively replace blood (Seghatchian et al., 2011). This way, transfusion of various blood components is required according to the indications in order to reduce the risks of transfusion (Eldin and Teruya, 2012).

Whole blood is one of the blood components that can be transfused when there is massive bleeding (Avery and Avery, 2010). Whole blood contains all the cellular components which include erythrocytes, leukocytes, and platelets contained in plasma (Hall et al, 2015). Whole blood can be stored for 21 days at the temperature of 1-6°C in anticoagulant citrate phosphate dextrose (CPD), and can be stored for 35 days at 1-6°C in anticoagulant citrate phosphate dextrose adenine (CPDA-1) (Kurup et al., 2003; AABB, 2017). 450 ml of whole blood contains a 63 ml CPDA-1 anticoagulant solution; the amount of hemoglobin is at least 45 grams per bag; the number of leukocytes is <1 x 10^6 per bag and it is free from bacterial contamination (*Kementerian Kesehatan Republik Indonesia,* 2015 and World Health Organization, 2001).

An indication of administering whole blood to replace red blood cells is when there is acute blood loss with hypovolemia (WHO, 2001). Unfortunately during storage, there are changes in the structure and function of erythrocytes in whole blood which can reduce the function and viability of cells after transfusion (Kucukakin et al., 2011). Besides, the storage period which is too long can also decrease pH, DPG content, platelets and coagulation factors in plasma (Hughes et al., 2007). Thus, it is crucial to conduct a study of the quality of whole blood by finding out the number of erythrocytes, leukocytes, platelets, and pH during the storage period of 28 days.

2 MATERIALS AND METHOD

Materials. Hematology analyzer Sysmex XS-80i, blood bank (temperature 2-6°C), blood collection tubes were purchased from BD vacutainer.

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Sample. One whole blood in CPDA-1 blood bag was used as a sample.

Measurement of the number of erythrocytes, leukocytes, and platelets. The blood bag was homogenized evenly by gently shaking the bag for two minutes. The blood bag tube was cut so blood could come out and 3mL of it was put into 28 sample tubes (red tubes). Prior to measurement, the sample was left at room temperature for 1 minute and then homogenized. The number of erythrocytes, leukocytes, and platelets in the sample tubes was counted using a hematology analyzer, every day for 28 days.

Measurement of pH. The blood samples in the sample tubes were homogenized, then a pH electrode was put into the samples until the maximum line. The results obtained on the pH meter were then read.

3 RESULTS AND DISCUSSION

Based on the results of this study, the pH and the number of erythrocytes are as shown in Figure 1.

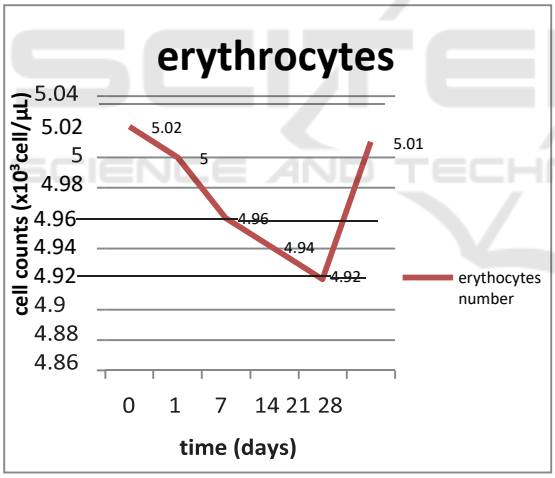

Figure 1: Number of erythrocytes on whole blood during 28-day storage.

Based on the results shown in Figure 1, the number of erythrocytes declined gradually starting from the baseline to the 21st day. On the baseline, the number of erythrocytes was 5.02x10^{6}cell/µL, which declined on the 1st day to 5x10^{6}cells/µL. On the 21st day, the number of erythrocytes decreased to 4.96x10^{6}cells/µL on the 7th day, to 4.94x10^{6}cells/µL. However, there was an increase in the number of erythrocytes on the 28th day, i.e. 5.01x10^{6}cell/µL.

A decrease in the number of erythrocytes from the baseline to the 21st day is due to "storage lesion", namely biochemical and biomechanical changes in erythrocytes and storage media during storage (Vani et al., 2015). This may change the structure, function, and viability of erythrocytes during the storage period (Kucukakin et al., 2011). In addition, a factor that causes changes in the viability of erythrocytes during storage is glycolysis which continues to take place, resulting in accumulation of metabolic waste which leads to acidosis or a decrease in pH. Limited carbon source in the blood bag can also slow down the rate of glycolysis and the synthesis of energy in the form of ATP, thus causing abnormal cell shape. Unfortunately, the Hematology Analyzer could not detect the presence of abnormal erythrocytes (Zandecki et al., 2007), thus further observation of cell shapes is necessary.

An increase in the number of erythrocytes on the 28th day was believed to be due to uneven homogenization during testing which caused cell deposits on the bottom of the tubes. In fact, despite a gradual decrease in the number of erythrocytes, it is still within the normal range, i.e. 4.5 – 6.5 x 10^6 cell/µL (Riswanto, 2013).

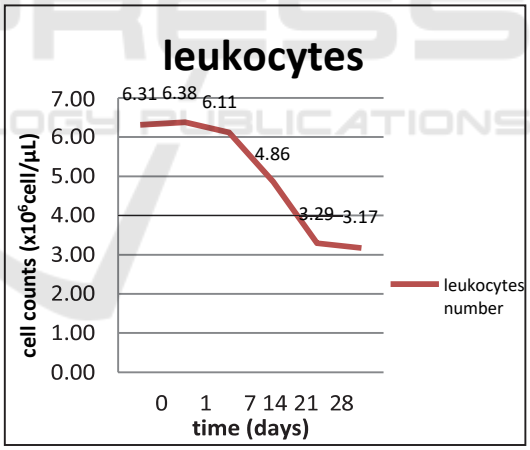

Figure 2: Number of leukocytes on whole blood during 28-day storage.

Figure 2 presents the number of leukocytes in whole blood during the 28-day storage. It can be seen that there was a gradual decrease in the number of leukocytes from the baseline to the 28th day. On the baseline, the number of leukocytes was6.31x10^3/µL, which decreased to 6.38x10^3/µL on the 1st day. Finally, on the 28th day, the number of leukocytes decreased to 3.17x10^3/µL.

The decrease in the number of leukocytes could occur in whole blood during storage. This is related

to the functions of leukocytes, i.e. recognizing and eliminating various foreign antigens such as foreign proteins, viruses, and bacteria that enter the body (Roenhorst et al., 1988). Thus, when there is no antigen or viral infection in the whole blood bag during storage, the number of leukocytes will decrease.

Based on Regulation of *Kementerian Kesehatan Republik Indonesia No.91 tahun 2015*, the number of leukocytes in each bag of leukodepleted whole blood (whole blood of which the number of leukocytes has been reduced) is <1 x 106 per bag. This way, it can be said that the number of leukocytes in the whole blood in this study exceeded the normal range stipulated in this regulation. This is because the whole blood used was not leukodepleted whole blood so the number of leukocytes had not been reduced using a filter.

According to Ampofo et al. (2002) and Seidl et al. (1987), whole blood can be safely transfused if screening for infectious diseases has been performed and it is free from allogeneic leukocytes. Allogeneic leukocytes can cause a reaction in donor blood. The decrease in the number of leukocytes in whole blood is very important for blood recipients because it can prevent the effects of leukocyte-mediated adverse reactions such as alloimmunization, transfusion-mediated cytomegalovirus (CMV) infections, and non-hemolytic transfusion reactions (Dellinger and Anaya, 2004; Vamvakas, 2006).

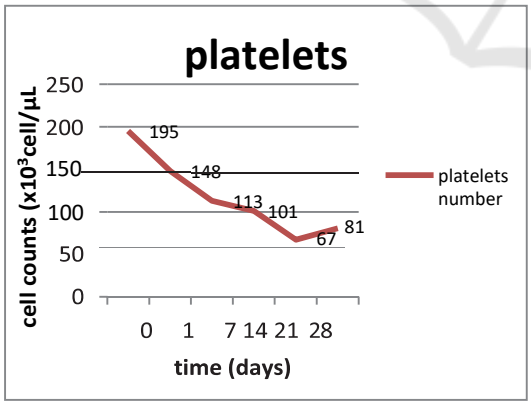

Figure 3: number of platelets on whole blood during 28- day storage.

Platelets are cells that maintain vascular integrity and play a role in hemostasis, i.e. in the process of coagulation and prevention or stopping bleeding. Platelets are produced from megakaryocytes in the bone marrow and its life span is around 8-10 days.

Based on the results of this study, the platelet count in whole blood during the 28-day storage decreased gradually from the baseline, i.e. 195x10^3/µL, to 67x10^3/µL on the 21st day although there was a slight increase to 81x10^3/µL on the 28th day (Fig3). The normal platelet count in the blood is 150,000-400,000/mm3. Thus it can be said that the platelet count started to decrease on the baseline from the normal range.

In general, a decrease in platelet count in whole blood during storage is believed to be influenced by hypoxia and anaerobic glycolysis due to oxygen depletion. This makes the atmosphere in the blood bag acidic, causing platelets to lose viability. In addition, platelets should be stored at room temperature because storage below 15^{o}C may change the structure of the platelet membrane which also affects viability (Getz, 2019; Zucker and Borreli, 1954).

Thus at present, regarding the use of platelets for transfusion in blood clotting disorders, it is recommended to use platelet concentrate components stored in plasma. This is to reduce the risk of platelets losing their viability after transfusion due to the storage period.

However, previous studies showed that storing platelets in whole blood by agitation up to 15 days at a temperature of 4°C can maintain the functions of platelets and can be used in traumatic wound healing during surgery (Slichter et al., 2019).

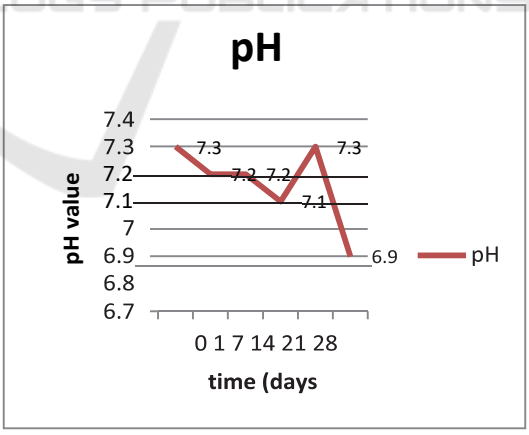

Figure 4: number of platelets on whole blood during 28- day storage.

The level of pH is a measure of the balance between acidity and alkalinity in the body. The normal pH of the blood is neutral to alkaline, i.e. 7.35 to 7.45. However, when the blood is outside the body, i.e. in a blood bag, there are many factors that cause changes in blood pH. This is what

underlies the measurement of blood pH as an important part of the efforts to control the quality of blood products. That is, at the end of storage, the pH level of the blood in the blood bag may not be ≤ 6.4. In this study, the blood bag sample used anticoagulant Citrate Phosphate Dextrose Adenine (CPDA-1) of which the ratio between blood volume and anticoagulant volume was x: x. During the 28 day storage, the blood pH was measured on days 0, 1, 7, 14, 21 and 28 days. According to the measurement done 6 hours after tapping (day 0), the blood pH was
7.30. On the 1st day, the pH level was observed to decrease by 0.1 to 7.20 and this remained stable until the 7th day. However, there was another pH decline on the 14th day by 0.2 to 7.10. On the last storage day, i.e. the 28th day, there was a quite significant decrease in the pH, i.e. 6.90. Some of the factors that might have an important effect on lowering the pH level during storage are:

1. The use of anticoagulant CPDA-1 which contains an acid compound
2. Respiration by blood cells produces carbon dioxide. In the body, carbon dioxide is processed by the lungs, but the blood bag does not have such function, causing respiratory acidosis which disrupts blood buffer retention, thus lowering pH level.
3. Cellular metabolism which continues to take place during storage, decreasing the ATP level and glucose in the blood. One of the products of cellular metabolism is lactic acid. Lactic acid accumulation could also lower the pH level.

4 CONCLUSIONS

Based on the results of this study, in general, the pH value and the number of erythrocytes, leukocytes, and platelets decrease during the 28-day storage. In fact, the number of erythrocytes and pH values are still within the normal range, while the number of leukocytes and platelets is below the normal range. Therefore, it is important to process blood into particular blood components to guarantee and provide the quality of the components needed.

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