

Function and platelet count in thrombocyte concentrate (TC) during the Storage

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Abstrak

Latar belakang: Evaluasi terhadap pemberian transfusi belum dilakukan secara optimal baik di hulu maupun di hilir. Tujuan penelitian ini untuk mengetahui pengaruh waktu penyimpanan terhadap perubahan pH, jumlah trombosit, dan fungsi agregasi yang terjadi pada trombosit pada beberapa hari penyimpanan.

Metode: Disain penelitian potong lintang terhadap sample kantong konsentrat trombosit yang telah lolos skrining infeksi penyakit menular melalui transfusi darah. Pengujian yang dilakukan ialah terhadap pH, jumlah trombosit dan fungsi agregasi terhadap sampel pada tiga waktu pengujian pada hari ke-0, ke tiga, dan ke lima penyimpanan.

Hasil: Pada 50 sampel kantong konsentrat trombosit didapatkan kenaikan pH pada hari ke tiga penyimpanan kantong trombosit yang disertai penurunan pada hari ke lima. Hal serupa ditemui pula pada jumlah trombosit. Sementara penurunan fungsi agregasi trombosit ditemukan lebih awal pada hari ke tiga penyimpanan dan didapatkan nilai rendah pada hampir semua sampel.

Kesimpulan: Ketiga parameter yaitu pH, jumlah trombosit, dan fungsi agregasi mengalami penurunan pada hari kelima. (*Health Science Journal of Indonesia;2015;1:48-51*).

Kata kunci: thrombocyte, concentrate, pH, agregasi, waktu penyimpanan.

Abstract

Background: Evaluation for platelet transfusion is not optimal for this moment even in upstream at the blood center or in downstream at the hospital. The purpose of this study was to determine the effect of storage time to changes in pH, platelet count and function that occurs on platelet aggregation during different time storage.

Methods: The study design was cross-sectional on selected bags of platelet concentrates that have passed the screening for infection transmitted through blood transfusions. The regular assessment in UTDD for PC has been done every month by random sampling with three parameters pH, platelets count and volume in the bag of blood. The testing for pH, platelet count, and aggregation functions for 50 samples with three different test time were conducted on day 0, third day, and fifth day storage.

Results: On 50 bags samples, pH, and number of platelets increased, but the platelet aggregation function decreased on the third day of storage. On the fifth day of storage the pH, number of platelets and the platelets aggregation function decreased and found the lowest number almost in all samples.

Conclusion: The three parameters: pH, platelet counts, and aggregation functions decreased on the fifth day of storage. (*Health Science Journal of Indonesia;2015;1:48-51*).

Keywords: platelets, pH, aggregation, platelet storage

Transfusion for medical treatment, the qualified blood and blood components must have sufficient blood cells which is still alive and keep it functioned optimally when it's being transfused.¹ One bag whole blood (WB) obtained from a donor consisted of several components such as packed red blood cell (PRC), Thrombocyte concentrate (TC), and the plasma.

The TC is a blood component needed by patients who suffer from bleeding to prevent and stop bleeding, due to thrombocytopenia.¹⁻³ In fact, clinicians in hospitals often complain about patients' non-optimal response after being platelet transfusions. According to Lubis study, it is found that about 33.3% patients with abnormalities of hemato-oncology at Cipto Mangunkusumo General Hospital had unsatisfied respond towards the platelet transfusions.⁴ Furthermore, Chunaeni's study noted that the state refractory towards the platelet transfusion is more prevalent in patients who get multiple platelet transfusions that are processed from pockets WB.⁵

The quality and quantity of platelets in TC are influenced by many things, among others, selection of donors, donor blood sampling, processing, storage, and distribution that require special treatment.⁶ During the storage, changes may occur in platelets blood bags as a result of platelets metabolism. According to Lo's study, it is observed that there are any changes in pH in the late storage significantly.⁷

Most of the standards use the amount of platelet and pH as a parameter to measure the TC quality.⁸ The American Association of Blood Banks (AABB) decides the value of $\text{pH} < 6.2$. The European Association of Blood Banks (EABB) decides a maximum limit of the pH be 7.4 at the end of TC storage.^{9,10} In addition, the expected number of platelets is 3.5×10^{10} per unit for about 90% of tested bags according to AABB standart.⁹

The function of platelets in hemostatic and thrombosis has been able to measure and it becomes gold standard examination and is widely used in determining platelet function; that is the aggregation function tests that use classical agonist is adenosine diphosphate (ADP), arachidonic acid, adrenaline, collagen or ristocetin.^{11,12}

This study aimed to identify the changes of the aggregation function, pH and amount of platelet during the storage which may affect the quality of the TC.

METHODS

This cross-sectional study was carried out at the Regional Blood Transfusion Unit (UTTD) Indonesia

Red Cross Jakarta and Clinical Pathology Department, Medical Faculty/Cipto Mangunkusumo General Hospital Jakarta. The study held on 28th March until 30th May 2013 towards selected 50 bags of platelet concentrates that have passed the screening of Infection Transmitted Through Blood Transfusions (IMLTD); that is Hepatitis B, Hepatitis C, Syphilis, and HIV.

The test was carried out on pH, amounts of platelet and aggregation functions for all 50 samples with three different test times; i.e day zero, third and fifth storage. Inclusion criteria were the blood met the specified requirement from Indonesia Red Cross¹³ initial blood taking volume (the main bag) 350 mL, using triple bags, fluent bloodstream while tapping, non-reactive of infection result. Meanwhile, exclusion criteria were blood from smoker donors, women with hormonal contraceptives, people who consume drugs a week before the donor, and were tapped more than once.

pH examination

The pH examination was carried out on day zero by using a pH meter by draining through the hose and collecting by the plastic tube about 5 mL volume, TC components that are left on the platelet agitator bag, they will be kept in an incubator with temperature of $22^\circ \pm 2^\circ \text{C}$ and taken back for examination on the third and fifth day after the storage.

Examination of the platelets amount

Samples were taken on day zero of the hose bag TC components and then inserted into the tube with sample number and time checks labels, then the platelet concentrate remaining in the bag is kept in platelet agitator incubator with temperature of $22^\circ \pm 2^\circ \text{C}$ to be taken back for the same examination on the third and fifth day after the storage.

Examination of aggregation functions

The TC was released into the bag about 5 mL tube that has been prepared by sticking the labels on the tube with the sample number and the retrieval dates. The plasma components obtained from the same donor were also taken the sample about 10 mL. The platelet aggregation examination was carried out on available samples by firstly testing the amount of platelet, if the results obtained $> 350000/\mu\text{L}$ then the sample diluted by Platelet Poor Plasma (PPP) of the same sample numbers and when the number of

platelets <15000/uL, the aggregation function tests could not be done due to the abnormal indication. Samples can be processed when the amount of platelet was about 150000-350000/uL. The next process was done by using aggregators such as ADP at a concentration of 1µM, 5µM, 10µM.

The comparing data analysis of platelets, pH, and platelet aggregation was conducted by paired T-test with normal distribution or by Wilcoxon test with the abnormal distribution. Pearson and Spearman correlation test were performed to assess the relationship between two numerical variables.

This study has passed the ethical clearance from Medical Faculty, University of Indonesia Number. 60/H2.F1/ETHICS/2013.

RESULTS

On 50 bags samples, we found increasing number of pH 7.432 about 0.257 on the third day and decreasing the number on the fifth day from the storage of 7.238 ± 0.353 pH. There is also an increasing number of platelets 618 about 256 x 109 / unit on the third day of storage, and decreasing on the fifth day of storage 599 about 271 x 109 / unit.

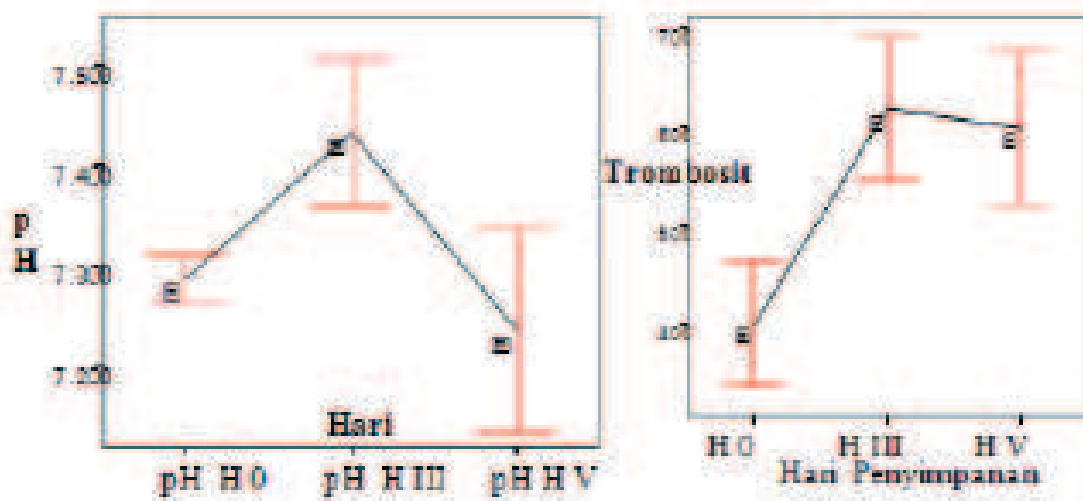


Figure 1: pH and thrombocyte count changes during the storage

The result of platelet aggregation examination showed the samples examined on day 0, there were 10 samples with normal limits aggregation by using ADP 10µM while 40 other samples were below the normal one or hypo-aggregation, where on the 3rd day all samples hypo-aggregation.

Table 1. Thrombocyte aggregation during the storage with ADP 10 µM in day 0 and day 3

Criteria	Day 0		Day 3	
	n	%	n	%
Normal	10	20%	0	0%
Hypoaggregation	40	80%	50	100%

DISCUSSION

In this study, we found that the number of pH and platelet increased on the third day after the storage. On the fifth day, both parameters started declining. On the other hand, the platelet aggregation did not

decreasing on the third day and getting down on the fifth day.

On the third day of storage, there was increasing number of pH rather than at the beginning or two hours after processing the components. The increasing in pH usually occurs due to the abundance of oxygen into the blood bag so that the pH was getting more alkali. Oxygen will be used for aerobic platelet metabolism. Platelets can also be metabolized using the anaerobe glucose. The results of platelet glycolysis are lactic acid that causes damage to the membranes of platelets (lysis), so the atmosphere is more acid by the end of the storage.^{14,15}

This increasing was analyzed as a consequence of bags of platelets that has greater permeability from the pores of bags, this situation brings into the exchange of O₂ and CO₂ in the TC bags which causes increasing number of CO₂ and decreasing of O₂, within the cell, there will be glycolysis process

that produces lactic acid ($C_3H_6O_3$) and starts to be imbalance of H_2CO_3/HO_3^- . The increasing number of lactic acid on platelet cells will cause damage to the platelet membrane and into the lysis of membrane and causes into a more alkali pH levels, it is becoming the explanation of the increasing number of pH on the third day of storage.^{14,15}

The number of platelet shows an increasing number based on the storage duration, it can happen since in *in vitro* platelet cells are shorter than the *in vivo* about five days. The cell death process is running through apoptotic mechanisms of cell division which begins with the platelets in the early phase, in counting the number of platelets by the calculation method named "flow cytometry" where the counting indicator was done by measuring 2 to 4 microns without assessing the condition of the platelet itself, and the next phase will be followed by decreasing number of the platelet cell lysis on the fifth day of storage.^{11,14} However, decreasing of pH on the fifth day of storage does not mean that the average 7238 ± 0.8 , this shows that the platelet did not activate and change the shapes.

On the platelet aggregation examination, on day 0 of storage or two hours after treatment, normal platelet cell aggregation shows only 20% or 10 of the 50 samples studied, it may possible because in most cases, the samples are examined more than 3 hours from the sample preparation. Then, the samples were diluted and continued by a process to homogenize the movement of platelet concentrate in the cuvette that can activate these cells. Another thing that cause this is the contamination of cell lysis of erythrocytes or leukocytes for both of the cell because inappropriate environment will cause the release of ADP and activate the trombosit cells.^{16,17}

In conclusion, the optimum time to use "Thrombocyte Concentrate" to the patients should be before the third day of the storage because there was decreasing of pH, number of platelet and platelet aggregation function on the fifth day of the storage.

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