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THE DIFFERENCE LEVEL OF MYELOPEROXIDASE IN PLATELET CONCENTRATE BASED ON PREPARATION METHOD AND STORAGE DURATION

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ABSTRACT

The Platelet Concentrate (PC) preparation process through its storage affects the platelets contained inside. The contaminating leukocytes in PC is important factors implicated in storage lesion on PC during storage. Leukodepletion is a method to reduce leukocytes contaminantion. Myeloperoxidase (MPO) is an enzyme produced by polymorphonuclear cells that have the potential to change the structure and function of platelets when there is an interaction between them during storage. The aim of this study was assessing the difference in myeloperoxidase level of PC based on its preparation method (leukodepleted and nonleukodepleted) and time storage. A cross-sectional observational study was conducted at the Blood Transfusion Services Unit, Dr. Sardjito Hospital, Yogyakarta from April to December 2014. Platelet concentrate products were grouped based on storage time (≤ and >72 hours) and preparation method (leukodepleted and nonleukodepleted), MPO was then measured. Mean difference in each group was analyzed using ANOVA test and Post hoc test with a statistical significance level of $p < 0.05$. There were 64 eligible subjects, consisting of 29 leukodepleted PCs and 35 nonleukodepleted PCs, based on their storage time, 31 PCs had ≤72 hours storage time and the other 33 PCs > 72 hours. There were significantly lower median MPO level in ≤ 72 hours TCs than > 72 hours in nonleukodepleted PC group (13.23±6.47 ng/mL vs. 15.58±7.82 ng/mL; p=0.017). In PC group with more than 72 hours storage time, median MPO level in nonleukodepleted was significantly higher than leukodepleted PC (15.58±7.82 ng/mL vs. 11.11±3.97 ng/mL; p=0.001). Myeloperoxidase level was lower in nonleukodepleted PC group with ≤ 72 hours than > 72 hours storage time. Furthermore, the MPO level was higher in leukodepleted PC than nonleukodepleted PC in > 72 hours storage time.

Key words: Platelet concentrate, time storage, leukodepleted, myeloperoxidase, storage lesion

INTRODUCTION

The World Health Organization (WHO) reported that at least 9 million patients from 90 countries worldwide need blood donation every year.[1] In the United States, around seven thousand doses of Platelet Concentrate (PC) are transfused into patients daily, and the number is increasing every year.[2] This trend also occurs in Indonesia, especially in the blood transfusion services unit of the Dr. Sardjito Hospital. More than 3,000 bags of blood are transfused every month and especially PC product. There is increasing demand from 29.52% in 2011 to 36.92% in 2017.[3] Platelet concentrate transfusion is a therapeutic procedure for patients with thrombocytopenia; however, it may increase the risk of disease transmissions caused by viruses and bacteria. This procedure may create a risk of fever and anaphylactic reactions. The accumulation of cytokines and chemokines from contamination of leukocytes during storage of cellular blood products were major factor, including PC.[4]

Storage lesions that occur during PC storage are presented with accumulation of active substances due to the disintegration of contaminating leukocytes from residual leukocytes. It is as a result of imperfections in separating blood components with PC, such as the expression of P-selectin and platelet-derived soluble mediators histamine, surface Cluster of Differentiation/CD-40 ligand (sCD40L), chemokine ligand/CCL-5, platelet factor 4 (PF4), transforming growth factor-β (TGF-β), interleukin-6 (IL-6) and IL-27.[5] Previous studies suggested that platelet function is starting to significantly decline after 72 hours of storage, particularly in its aggregation function.[6] Myeloperoxidase (MPO) enzyme is one of the mediators from the intracellular granules of neutrophils and monocytes that are

active during the storage of blood components. This enzyme may serve as a signal by binding themselves to the cell membrane, modulating human platelet aggregation via actin cytoskeleton reorganization and Store-Operated Calcium Entry (SOCE).[7]

Activated platelets will reduce effectiveness in improving the hemostasis function. During the storage process, in addition to the change of extracellular environmental conditions, the platelets themselves will also automatically change so that it may affect its function. Increased number of active platelets will be different from what happened in vivo. Activated platelets will be destroyed by the spleen and liver as soon as possible. However, during in-vitro conditions, activated platelets during storage will occur and disrupt other platelets activity by releasing mediators and direct interaction with other cells.[8]

At present, there are several leukodepletion methods developed in many blood banks using filters on blood products before storage that aim to reduce the number of leukocytes in the product. The decline in leukocyte counts is expected to reduce the levels of cytokines released by leukocytes during blood products storage, thus reducing the incidence of post-transfusion reaction.[9] Platelet concentrate storage time has become an essential factor in PC transfusion procedure because of platelet storage lesion. Myeloperoxidase is secreted by contaminating leukocytes in PC during storage. Myeloperoxidase induce platelet activation and in decrease efficacy of platelet transfusion. Leukodepletion is a method that may reduce the contaminating leukocyte count in blood products. Differences in the levels of myeloperoxidase in PC products with and without leukodepletion with certain storage time has not been proven yet.

METHODS

A cross-sectional study was conducted to compare MPO levels between PC with and without leukodepletion and between storage time ≤72 hours and >72 hours. The consecutive sampling method was used, based on PC request timing from the Pediatrics Hematology-Oncology Wards to Blood Transfusion Service Unit of the Dr. Sardjito Hospital. Samples were all PC products with or without leukodepletion to be transfused in pediatric patients in the Estella 1 and 2 Wards of the Dr. Sardjito Hospital from April to December 2014. Data collection was performed on pooled PC unit volume, estimated from the weight of PC, platelet and leucocyte counts per unit PC was measured using a hematology analyzer Sysmex XN-1000; and MPO levels were measured using ELISA with R&D Systems Human Myeloperoxidase on PC products when there was a request from the ward for transfusion.

The inclusion criteria for this study were all PC products with or without leukodepletion to be transfused in pediatric patients from the Estella 1 and 2 Wards of the Dr. Sardjito Hospital. Exclusion criteria included PC that underwent hemolysis during processing and PC products stored for more than five days.

Characteristics of PC (PC volume, platelet count per PC unit, and leukocyte count per PC unit) was presented according to the group, which was based on the method of PC preparation (leukodepleted and nonleukodepleted) and storage time (≤72 hours and > 72 hours). Numerical data are presented in mean and standard deviation. The mean difference between groups was analyzed using ANOVA and Kruskal-Wallis test according to the normality of data distribution. The variations in MPO levels in each group were analyzed in the same way and continued with a post hoc test to analyze the significance of differences between groups. Mean difference was significant if the p-value was <0.05. This study was ethically approved by the Ethics Committee of the Faculty of Medicine, Gadjah Mada University, number KE/FK/426/EC.

RESULT AND DISCUSSION

A total of 64 PC samples were collected, consisting of 29 leukodepleted PC and 35 nonleukodepleted PC. Characteristics of PC can be seen in Table 1, based on group preparation method and storage time.

The difference in mean pooled PC volume in all groups was not statistically significant (p> 0.05). Post hoc analysis found no significant differences in mean volume between the test groups. In nonleukodepleted PC group, median leukocyte count was higher than leukodepleted PC in both subgroups of storage time.

In this study, there were no differences between mean platelet count in all groups of PC (p> 0.05). A post hoc analysis found significantly lower mean platelet count in leukodepleted PC than nonleukodepleted PC in ≤ 72 storage time groups. Also, mean platelet count in leukodepleted PC with storage time ≤ 72 hours were significantly higher than nonleukodepleted PC with storage time > 72 hours.

Table 1. Characteristics of PC

Parameter	Leukodepleted		Nonleukodepleted		p
	Storage time ≤72 hours (n = 17)	Storage time >72 hours (n = 12)	Storage time ≤72 hours (n = 14)	Storage time >72 hours (n = 21)	
Pooled PC volume (mL)	348. 7 ± 149. 63	353. 1 ± 135,63	321. 9 ± 144. 85	329. 9 ± 125. 66	0.26 *
Leukocyte count per TC unit ($x10^6$)	0.01 ± 0.002 a,c	0.01 ± 0.003 b,d	0.03 ± 0.01 a,b	0.045 ± 0.01 c,d	0.001 **
Platelet count per TC unit ($x10^{10}$)	1.61 ± 0.47 e,f	1.65 ± 0.77	1.78 ± 0.84 e	1.84 ± 0.98 f	0.889 *

* = mean±SD was analyzed with ANOVA test
** = median±IQR was analyzed Kruskal-Wallis test
Post hoc Tukey analysis
a, b, c, d, e, f = same character showing mean/median difference with p< 0.05

Table 2. Differences of MPO levels based on production method and storage time

Parameter	Leukodepleted		Nonleukodepleted		P
	storage time ≤72 hours	storage time >72 hours	storage time ≤72 hours	storage time >72 hours	
MPO level (ng/mL)	10.69±3.16[a]	11.11±3.97[b]	13.23±6.47[c]	15.58±7.82[a,b,c]	<0.01*

* = median±IQR was analyzed with Kruskal-Wallis test
Post hoc Tukey analysis
a, b, c = same character showed mean/median difference with p< 0.05

Table 2 shows that, overall, there were differences in MPO levels between four groups tested, i.e., based on PC production method (leukodepleted and nonleukodepleted) and storage time (≤ 72 hours and > 72 hours) with p<0.01.

Furthermore, the post hoc analysis found that the mean difference was statistically significant in which median MPO level in storage time ≤ 72 hours was lower than >72 hours in nonleukodepleted PC, meanwhile in leukodepleted PC group, the difference in MPO levels was not significant (10.69±3.16 ng/mL vs. 11.11±3.97 ng/mL; p=0.106).

In the PC group with storage time >72 hours, median MPO level in nonleukodepleted PC was significantly higher than leukodepleted PC (11.11±3.97 ng/mL vs. 15.58±7.82 ng/mL; p=0.001) and no significant difference in MPO level was found between PC groups with storage time ≤ 72 hours (10.69±3.16 ng/mL vs. 13.23±6.47 ng/mL; p=0.106). The strongest difference in median MPO was found between leukodepleted group with storage time ≤ 72 hours and nonleukodepleted PC with storage time >72 hours (10.69±3.16 ng/mL vs. 15.58±7.82 ng/mL; p <0.01).

In this study, contaminating leukocyte count in nonleukodepleted PC products was still within the reference range, less than 108-109 cells. A significant difference between the two PC groups based on production method suggested that leukodepleted method with leukofilter was quite effective to decrease contaminating leukocytes. According to the American Association of Blood Banks (AABB), in leukodepleted PC there should be residual leukocytes less than 0,83x106 per PC unit.[10]

Platelet count in leukodepleted PC was lower than nonleukodepleted PC. This result may be explained that during the production process, a small fraction of leukocytes can be carried in the PC bag. These residual leukocytes may have a negative effect during storage. Leukocytes may interact with activated platelets during storage. The longer PC is stored, the more platelets become activated.[11] However, this study found that the PC with storage time ≤ 72 hours had a platelet count less than PC with storage time > 72, although the difference was not significant. This finding was probably related to lower mean donor platelet count in leukodepleted PC.

Comparing leukocyte count and MPO level, it was found that nonleukodepleted PC had significantly higher leukocyte count than leukodepleted PC, as well as significantly higher MPO levels. This condition indicated that leukocyte count influenced MPO level in PC. Leukodepletion method not only can reduce contaminating leukocyte count, but also reduce MPO level. However, between PC with storage time ≤ 72 hours and >72 hours, MPO levels were not significantly different but contaminating leukocyte count was significantly different. This condition indicated that the leukocyte count did not affect the association between MPO production and PC storage time. Myeloperoxidase accumulation was due to the accumulation of leukocytes which will release MPO in PC rather than its storage time.

A previous study found significantly elevated MPO levels among donor plasma with different time storage. Myeloperoxidase levels in PC from days 0, 5, and 7 were significantly increased as well. Granulocytes disintegrated during storage of blood components. Some granules containing active substances including MPO, was accumulated extracellularly, associated with storage time. Increased MPO during early storage was 10-25 times higher after 35 days of storage.[12]

Myeloperoxidase accumulation in PC product was shown to increase platelet activation in-vitro before the PC was transfused to patients. Platelets activated during the storage process will release pro-inflammatory cytokines, and there was evidence that platelet aggregation and MPO concentration in blood plasma increased concomitantly with the progression of a cardiovascular disease, revealing the interrelations between platelets and neutrophils in inflammation and thrombosis. The MPO itself could also exert effects that were independent of its catalytic activity and affected various processes involved in cell signaling and cell-cell interactions. Also, MPO modulated inflammatory responses when activated platelet and MPO-rich PC were transfused, it would harm the patients and increased the morbidity and mortality of their disease.[7,13]

Decreased efficacy of activated platelet-rich PC may have adverse impacts on patients. Correction of thrombocytopenia may not be reached, requiring repeated PC transfusions. This condition is found in 18-34% of patients with hematologic-oncology disorders who had repeated PC transfusions.[14] Platelet activation, indirectly indicated by MPO levels during the storage, is closely related to storage time and production process (leukodepletion). By indirectly reducing MPO level through leukodepletion and shortening storage time, it is expected that the efficacy of PC transfusion is maintained properly and reduce the risk of post-transfusion morbidity and mortality.

This study had some limitations. MPO levels were not measured in the early production of PC thus, this study was not able to prove that elevated MPO levels were associated with storage time. The use of myeloperoxidase marker in this study was an indirect marker of platelet activation. Thus, direct marker on platelet activation such as p-selectin/CD62P was required as a comparison. Also, donor characteristics data were not analyzed, showing possible bias in this study.

CONCLUSION AND SUGGESTION

With storage time > 72 hours, MPO levels in leukodepleted PC was significantly lower than in nonleukodepleted whereas nonleukodepleted PC group. Myeloperoxidase levels were significantly higher in PC with storage time > 72 hours than storage time ≤ 72 hours.

Further research is needed with the cohort research design approach so that the measurement of serial MPO levels from the beginning of PC production (1st day) to transfusion could assess the relationship between MPO levels and PC storage time.

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