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Correlation of Red Blood Cells Storage Lesion F2α-Isoprostanes Levels with Erythrocyte-Derived Microparticles

Zelly Dia Rofinda[1*], Dian Pertiwi[1], Dwitya Elvira[2]

[1]Clinical Pathology Department, Faculty of Medicine, Universitas Andalas / Dr. M. Djamil General Hospital, Padang, Indonesia

[2]Internal Medicine Department, Faculty of Medicine, Universitas Andalas / Dr. M. Djamil General Hospital, Padang, Indonesia

***Corresponding Author:**

Zelly Dia Rofinda,

Clinical Pathology Department, Faculty of Medicine, Universitas Andalas / Dr. M. Djamil General Hospital, Padang, Indonesia

Email ID: zellydiarofinda@med.unand.ac.id

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ABSTRACT

Background: PRC stored blood products change morphology, biochemistry, and metabolism, called storage lesion red blood cells (erythrocyte storage lesion). Morphological changes of erythrocyte membrane phospholipids and damage due to oxidative stress and hemolysis allegedly produce microparticles known as erythrocyte-derived microparticles (EMP) and F2α-isoprostanes. F2α-isoprostanes are the indicators used to assess lipid peroxidation and cellular oxidative stress. Increased EMP-isoprostanes F2α in the PRC product is allegedly one of the causes of bad outcomes in transfusions.

Aim: to know the correlation between red blood cells storage lesions (F2α-isoprostane) with erythrocyte-derived microparticles (CD235a) in the PRC product based storage time, days 0, 7,14,21, and 28 at the blood banks Hospital Dr. M. Djamil Padang. Methods: The cross-sectional study of 14 PRC units came from 14 healthy donors at the Blood Bank of Dr. M.Djamil Padang Hospital. The study lasted from May 2016 to August 2017. The tests were conducted during 28 days of storage at 1-week intervals. Examination of the level F2α-isoprostane with the methods Enzyme-linked immunosorbent assay (ELISA) and examination of EMP levels (CD235a) with the flow cytometry method. Bivariate statistical analysis of the correlation parameter storage lesion red blood cells (F2α-isoprostanes) with EMP (CD235a) using the Pearson correlation test with a P value <0.05 indicates exhibited significant correlation. Results: Fourteen units of PRC obtained the largest donors were male (85.7%), with the highest blood type being O (42.9%). During storage, there is an increased amount of F2α-isoprostane and erythrocyte-derived microparticles (EMP) in the PRC product. There was no statistically significant correlation between the level of F2α-isoprostane F2α and the amount of EMP during storage up to 28 days.

Conclusion: There was no statistically significant correlation between the level of isoprostane F2α and the amount of EMP in the storage PRC

Keywords: *red blood cells storage lesions, erythrocyte-derived microparticles (EMP), F2α-isoprostanes*

1. INTRODUCTION

Blood transfusion is a medical treatment that is most often given, with about 14 million units transfused PRC in the United States in 2011 and average 40% of critically ill patients received at least one unit in the intensive care unit (ICU) (Spinelli et al., 2014; Widiarsih & Resa, 2022). Average monthly demand for blood at the Blood Bank Dr M. Djamil Padang is as much as 1,047 bags, consisting of whole blood as much as 3.9% and 96.1% PRC (Isti et al., 2018). Packed red cells (PRC) are obtained with 200-250 mL separating plasma from one unit of Whole Blood (WB). Quality PRC stored for storage must be maintained while still maintaining a change in the morphology, biochemistry, and metabolism, called storage lesion red blood cells (erythrocyte storage lesion). During storage, the PRC erythrocytes are continuously oxidized by free radicals such as superoxide and hydrogen peroxide. Glutathione (GSH) is an important antioxidant in erythrocytes' defense after storage. PRC decreased for more than 14 days, and the consequence is an increase in oxidative damage and decreased nitric oxide (NO). (Flatt et al., 2014)

Damage to the erythrocyte membrane phospholipids is very likely to be a factor that causes the loss of red cell deformability and its ability to survive in vitro (Kor et al., 2009). Reactive oxygen species (ROS) attack the level of the membrane protein fractions and initiate lipid peroxidation reactions that cause damage to the integrity of the membrane and death eritrosit (D'Alessandro, 2013; Stafforini et al., 2006). Erythrocyte membrane lipid peroxidation will generate isoprostane. Isoprostane (IsoPs) is a prostaglandin isomer formed in vivo, particularly through peroxidation of arachidonic acid, induced by free radicals after esterification into phospholipids. Isoprostane is released into plasma by phospholipase activity. Isoprostan measurement is a comprehensive method for assessing cellular oxidative stress status. (Spinelli et al., 2014). The use of isoprostane as an oxidative stress marker has several advantages over other oxidative stress markers, namely, isoprostane is chemically stable, specific as a peroxidation product, formed in vivo, and isoprostane is present in the amount detected in tissues and biological fluids, and isoprostane is not affected by lipid content in food. (Czerska et al., 2015)

Erythrocytes in the bag, PRC degradation, and loss of efficiency during storage. Morphological changes of erythrocyte membrane phospholipids and damage due to oxidative stress and hemolysis allegedly produce microparticles known as erythrocyte-derived microparticles (EMP). EMPs are considered a sign of RBC storage lesions, which can cause several effects in patients transfused with stored blood for more than 21 days, when the stability of the RBC membrane is lost and hemolysis occurs in the storage bag. (Rubin et al., 2012). EMP role in the innate immune system messenger paracrine and as proinflammatory mediators that induce or propagate an inflammatory signal (Straat et al., 2016). Increased EMP in the PRC product is allegedly one of the causes of bad outcomes in transfusion.12 This study aimed to determine the correlation between red blood cell storage lesions (F2α-isoprostanes) with erythrocyte-derived microparticles (CD235a) in the PRC of products based on the time-to-day storage 0, 7,14,21, and 28 days at the blood banks Hospital Dr. M. Djamil Padang.

2. METHODS

This research was an analytic study with a cross-sectional design, conducted from May 2016 to August 2017. The study protocol was approved by the Ethical Review Board of the Andalas University Faculty of Medicine. Fourteen selected PRC units from donors with informed consent were obtained from Dr. M. Djamil Central Hospital Blood Bank, Padang. The units were stored in a temperature-controlled refrigerator at 2- 60 °C and sampled weekly over 28 days starting at the age of 0 days old. Plasma separation days 0, 7, 14, 21, and 28, then the plasma was divided into 3 aliquots were stored at -20ºC. Examination of F2α-isoprostanes levels with Enzyme-linked immunosorbent assay (ELISA), and examination of EMP levels (CD235a) with the flowcytometry method. Statistical analysis was performed using a computer program. Bivariate statistical analysis of the correlation parameter storage lesion red blood cells (F2α-isoprostanes) with EMP (CD235a) using the Pearson correlation test at any time of storage, and continued with multivariate analysis, with P <0.05 meaning significant.

3. RESULTS AND DISCUSSION

Fourteen selected PRC units from donors with informed consent were studied weekly over 28 days, starting at the age of 0 days old. Most of the donors were male (85.7%) with an average age was 33 (9) years. Based on the number of donors by sex, there are more men than women is in accordance with the literature that women donors around 10% of all donors (Table 1). The average hemoglobin level was 24.9 ± 1.4 g/dL, and the hematocrit level was 77.1 ± 4.8%, and the highest blood type was O (42.9%) (Table 2). The hematocrit value of the PRC unit in this study is below that recommended, which is in the range of 70-80%.

Table 1. Characteristics of donors

No.	Characteristics	Mean ± SD	N	%
1	Age	33 ± 9 years		
2	Gender:			
	- Male		12	85.7
	- Woman		2	14.3
3	Hemoglobin (Hb)	14.7 ± 0.9 g/dL		
4	Blood group:			
	- A		3	21.4
	- B		4	28.6
	- O		6	42.9
	- AB		1	7.1

Table 2. Characteristics of product Packed Red Cells (PRC)

No.	Characteristics	Mean ± SD
1	Hb	24.9 ± 1.4 g/dL
2	Haematocrit	77.1 ± 4.8%

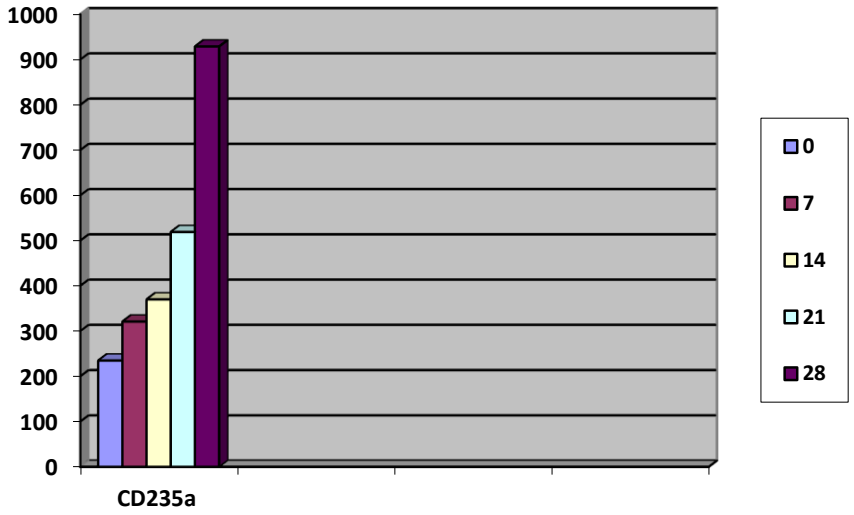

Figure 1. The differences between EMP (CD235a) mean level in packed red cells during the storage period

Figure 1 shows that prolonged RBC storage results in elevated EMP (CD235a) levels during the PRC storage period in the blood bank. In total, 14 units we studied, and the EMP (CD235a) level was increased with time. The mean of EMP level was 228 ± 125/uL in 0 days of storage and rose to 956 ± 644 mL at the end of the study.

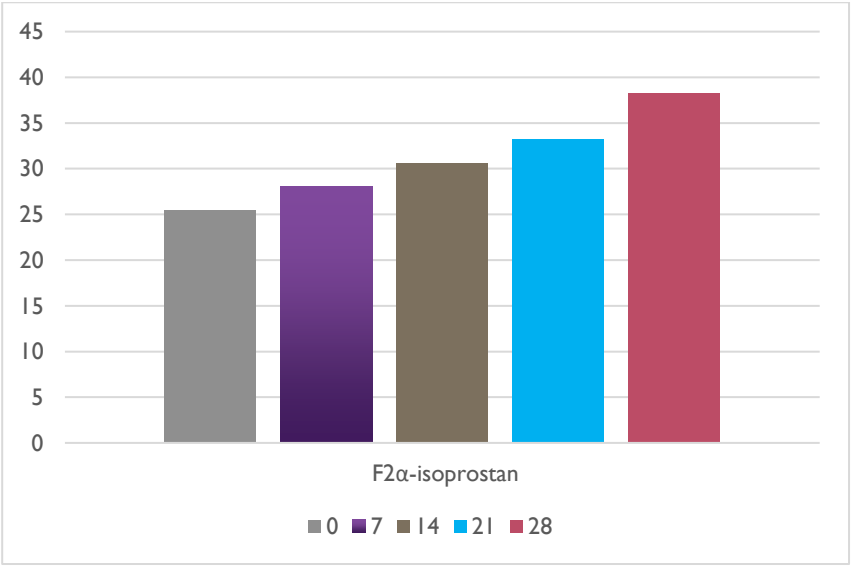

Figure 2. The differences between F2α-isoprostane mean level in packed red cells during the storage period

This study shows that prolonged RBC storage results in elevated F2α-isoprostane levels during the PRC storage period in the blood bank. In total, 14 units we studied, F2α-isoprostane level was increased with time. The mean of F2α-isoprostane level was 25.4 ± 3.9 pg/mL in 0 days of storage and rose to 38.2 ± 8,5 pg/mL at the end of the study.

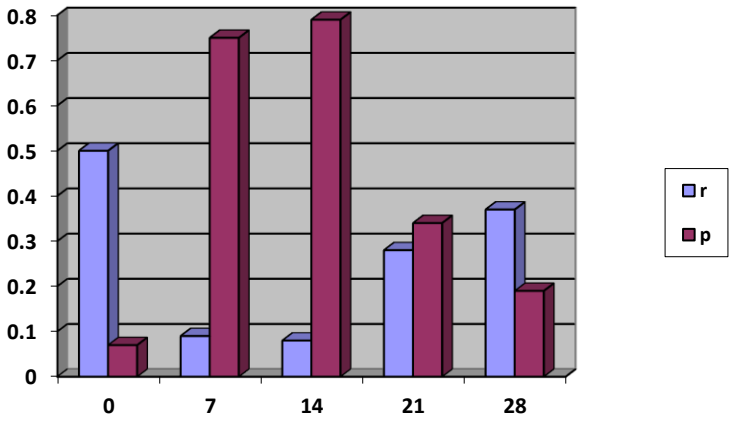

Figure 3. Correlation of F2α-isoprostane and EMP level in a packed red cell during the storage period.

Figure 3 shows that there was no statistically significant correlation between the level of isoprostane F2α and the amount of EMP in the storage PRC.

In this study, there was an increasing erythrocyte-derived microparticle (EMP) level in PRC during the storage period (Figure 1). The EMP level rose significantly in a linear relationship with PRC storage duration ($p < 0.05$). In line with this study, Gao et al., (2013)Also, observed that the EMP level rose significantly in a linear relationship with PRC storage duration. EMP concentration increased 18-fold after 42 42-day storage period at a temperature of 4°C. The mean of EMP level was 3389 ± 218/µL in 0 days of storage and rose to 61,586 ± 2237/µL in 42 days of storage. Another study also documented (Aleshnick et al and Rubin et al). microparticle levels isolated from red blood cells varied. (Aleshnick et al., 2016)increasing during storage duration (Rubin et al., 2008, 2013). Microparticles in stored PRC units experienced a gradual increase in size and protein content. (Bosman et al., 2010; Kriebardis et al., 2008). A study by Beth et al. showed a statistically significant linear increase in red blood cell lysis along with the duration of storage, from 0.024 ± 0.22% in 7 days of storage, and rose to 1.33 ± 0.47% in 42 days of storage ($P = 0.002$). (Bouchard et al., 2018)

This study shows that prolonged RBC storage results in elevated F2α-isoprostane levels during the PRC storage period in the blood bank. In total, 14 units we studied, F2α-isoprostane level was increased with time (Figure 2). This relates to the erythrocyte membrane damage that occurs during the storage process due to the presence of free radicals. In line with this study, Spinelli et al 2014) also obtained that isoprostane level rose significantly in a linear manner with PRC storage duration, indicating that the status of oxidative stress increases with the duration of RBC storage. The mean of the isoprostane level was 20 pg/mL in 5 days of storage and rose to 40 pg/mL in 47 days of storage. (Spinelli et al., 2014). A study conducted by Silliman et al showed that the precursors for F2α-isoprostan accumulated in plasma RBC products in storage for 42 days on nonleukoreduced and leukoreduced RBCs (Silliman et al., 2011). Isoprostane F2α (8-isoprostane) is a specific product of the nonenzymatic peroxidation of arachidonic acid and is shown to have adverse biological activity, and as such, has been used as an indicator of lipid peroxidation and oxidative stress. In the study of Karon et al, 2012, there was a statistically significant increase of 8-isoprostane levels in the supernatant of the PRC product during the storage period. The mean of isoprostane level was 136 ± 105 pg / mL in days 0, 198 ± 89 pg/mL in days 7, 246 ± 86 pg/mL in days 14, 351 ± 138 pg/mL in days 21, and rose to 376 ± 104 pg/mL in 42 days of storage. (Kor et al., 2009). Increasing the amount of isoprostane in PRC products transfused for critically ill patients or individuals with chronic inflammatory conditions can be a mechanism that contributes to adverse transfusion outcomes.(Czerska et al., 2015)

In this study, an increase in F2α-isoprostane levels, which showed oxidative stress status and EMP levels (CD235a), rose significantly in line with PRC storage duration. Damage to the Erythrocyte phospholipid membrane and the hemolysis process that occurs during PRC storage will cause an increase in F2α-isoprostane and EMP (CD235a) levels in PRC products. However, in this study, there was no statistically significant correlation between the level of isoprostane F2α and the amount of EMP in the storage PRC (Figure 3). This might be related to the duration of storage duration and the number of study samples. Based on Siliman and Spinelli's research, the increase of F2α-isoprostane level in the PRC product was significant up to 42 days and 47 days of storage of the PRC product. (Czerska et al., 2015; Kor et al., 2009)

4. CONCLUSIONS AND SUGGESTIONS

In this study, it was found that there was an increasing erythrocyte-derived microparticles (EMP) level and F2α-isoprostane level in PRC during the storage period. The EMP level rose significantly in a linear relationship with PRC storage duration

(p <0.05). There was no statistically significant correlation between the level of isoprostane F2α and EMP level in the storage PRC.

In this study, there was no statistically significant correlation between F2α-isoprostane levels and EMP levels, further research needs to be done by increasing the number of samples and the length of days of storage of PRC products and it is necessary to conduct a study of outcomes in patients given blood transusions based on storage time to determine the effect of the presence of red blood cells storage lesions on the PRC

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