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CORRELATION OF RED BLOOD CELLS STORAGE LESION F2α-ISOPROSTANES LEVELS WITH ERYTHROCYTE-DERIVED MICROPARTICLES

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Abstract

Background: PRC stored blood products undergo changes in morphology, biochemistry, and metabolic called storage lesion red blood cells (erythrocytes storage lesion). Morphological changes of erythrocyte membrane phospholipids and damage due to oxidative damage 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 allegedly one of the cause's 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 day 0, 7,14,21 and 28 at blood banks Hospital Dr. M. Djamil Padang. **Methods:** The cross-sectional study of 14 PRC units came from 14 heallthy donors at Blood Bank of Dr. M.Djamil Padang Hospital. The study lasted from the month of May 2016 to August 2017. The tests were conducted during 28 days of storage at 1-week intervals. Examination the level F2α-isoprostane with methods Enzyme-linked immunosorbant assay (ELISA) and examination of EMP levels (CD235a) with flowcytometry method. Bivariate statistical analysis of the correlation parameter storage lesion red blood cells (F2α-isoprostanes) with EMP (CD235a) using Pearson correlation test with a P value <0.05 indicates exhibited significantly. **Results:** Fourteen units of PRC obtained the largest donors were male (85.7%) with the highest blood type is O (42.9%). During storage there is an increased the amount of F2α-isoprostane and erythrocyte-derived microparticles (EMP) in the PRC product. There was no statistically significant correlation between the level F2α-isoprostane F2α and the amount of EMP in the during storage up to 28 days. **Conclusion:** There was no statistically significant correlation between the level-isoprostane F2α and the amount of EMP in the storage PRC.

Keywords: Red Blood Cells Storage Lesions, Erythrocyte-Derived Microparticles (EMP), F2α-Isoprostanes.

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 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) obtained with 200-250 mL separating plasma from one unit of Whole Blood (WB). Quality PRC stored for storage must be maintained while still a change in the morphology, biochemistry, and metabolic called storage lesion red blood cells (erythrocytes storage lesion). During storage the PRC, erythrocytes continuously oxidized by free radicals such as superoxide and hydrogen peroxide. Glutathione (GSH) as an important antioxidant in erythrocytes defense after storage PRC decreased 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 on the level of the membrane protein fractions and initiated 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 isomers formed in vivo, particularly through peroxidation of arachidonic acid induced free radicals after esterification into phospholipids. Isoprostane 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 damage 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 allegedly one of the causes bad outcomes in transfusi.12 This study aimed to determine the correlation red blood cells 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 at blood banks Hospital Dr. M. Djamil Padang.

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 inform consent were obtained from Dr. M. Djamil Central Hospital Blood Blank, Padang. The units were stored in a temperature controlled refrigerator at 2-6^{0}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 immunosorbant assay (ELISA), and examination of EMP levels (CD235a) with flowcytometry method. Statiscal analysis was performed using computer program. Bivariate statistical analysis of the correlation parameter storage lesion red blood cells (F2α-isoprostanes) with EMP (CD235a) using Pearson correlation test at any time of storage and continued with multivariate analysis with P <0.05 means significant.

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RESULTS AND DISCUSSION

Fourteen selected PRC units from donors with inform consent were studied weekly over 28 days starting at the age of 0 days old. Most of the donors were male (85.7%) with average age was 33 (9) years. Based on the number of donors by sex got more men than women are in accordance with the literature that women donors around 10% of all donors (Table 1). The average of hemoglobin level was 24.9 ± 1.4 g/dL and 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 in accordance with that recommended, which is in the hematocrit of 70-80%.

Table 1: Characteristics of donors

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

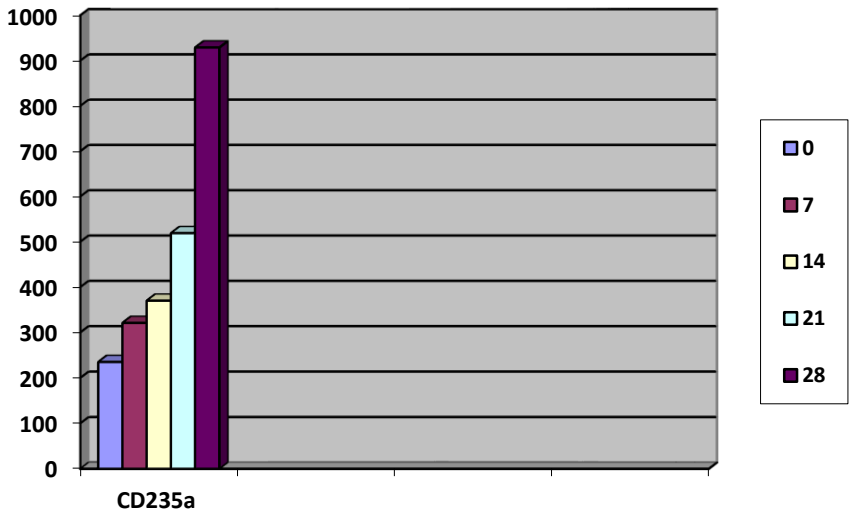

Figure 1: The differences between EMP (CD235a) mean level in packed red cell during the storage period

Figure 1 shows prolonged RBC storage results in elevated EMP (CD235a) levels during PRC storage period in the blood bank. In total 14 units we studied, 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 study.

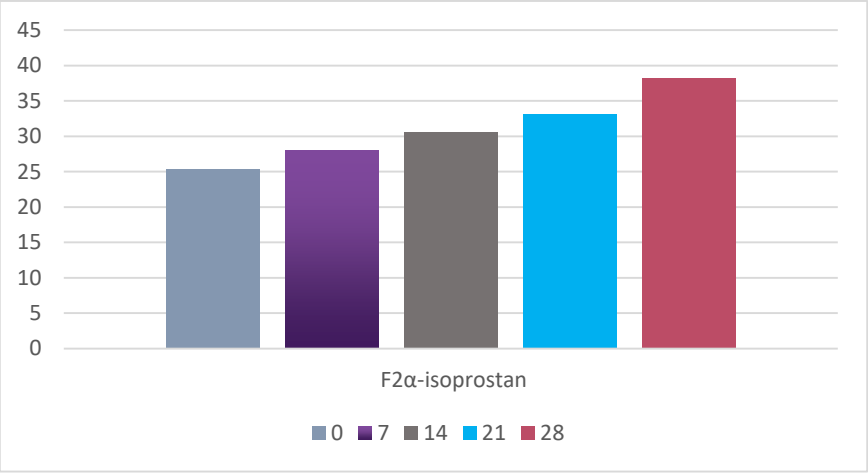

Figure 2: The differences between F2α-isoprostane mean level in packed red cell during the storage period

In this study shows prolonged RBC storage results in elevated F2α-isoprostane levels during 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 ± 3.9pg/mL in 0 days of storage and rose to 38.2 ± 8,5pg/mL at the end of study.

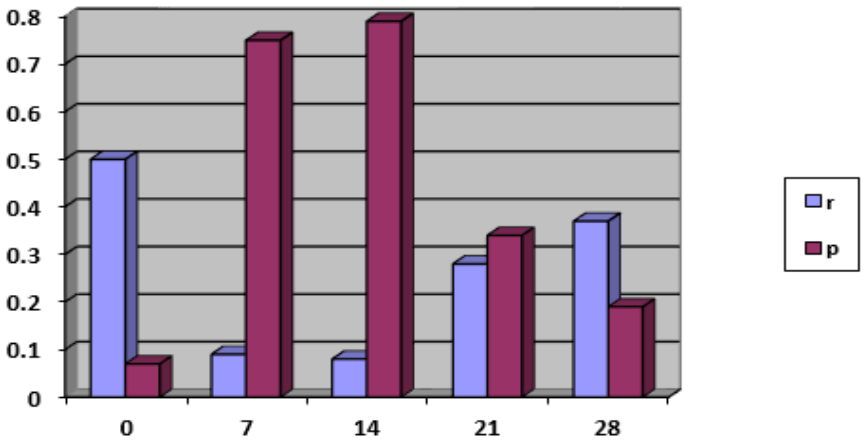

Figure 3: Correlation of F2α-isoprostane and EMP level in a packed red cell during the storage period.

On Figure 3 shows there was no statistically significantt correlation between the level-isoprostane F2α and the amount of EMP in the storage PRC. In this study, there was increasing erythrocyte-derived microparticles (EMP) level in PRC during the storage period (Figure 1). The EMP level rose significantly in linear with PRC storage duration ($p < 0.05$). In line with this study, Gao et al., (2013), also obtained that EMP level rose significantly in linear with PRC storage duration. EMP concentration increased 18-fold after 42 days of storage period in a temperature 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). Microparticles level 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). In a study by Beth et al. showed 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) In this study shows prolonged RBC storage results in elevated F2α-isoprostane levels during 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 linear with PRC storage duration, and indicating the status of oxidative stress increases with the duration of RBC storage. The mean of isoprostane level was 20pg/mL in 5 days of storage and rose to 40 pg/mL in 47 days of storage (Spinelli et al., 2014). In 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 that showed oxidative stress status and EMP levels (CD235a) rose significantly in lineat with PRC storage duration. Damage of Erythrocyte phospholipid membrane and hemolysis process that occurs during PRC storage will cause an increase in F2α-isoprostan and EMP (CD235a) levels in PRC products. However, in this study there was no statistically significant correlation between the level-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 in 42 days and 47 days of storage of the PRC product. (Czerska et al., 2015; Kor et al., 2009)

CONCLUSIONS AND SUGGESTIONS

In this study it was found that there was increasing erythrocyte-derived microparticles (EMP) level and F2α-isoprostane level in PRC during the storage period. The EMP level rose significantly in linear with PRC storage duration ($p < 0.05$). There was no statistically significant correlation between the level-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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