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The correlation of red cell membrane fragility with glucose and pH in PRC during storage



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ABSTRACT

Introduction: In packed red cells (PRCs), at least 70-80% of red blood cells (RBCs) are viable or circulating in the recipient's circulation 24 hours post-transfusion. Viability correlates with the osmotic fragility of the red cell membrane and the loss of ATP. PRC with citrate phosphate dextrose-adenine-1 (CPDA-1) as a preservative can be stored for up to 35 days, but the Indonesian Red Cross blood transfusion unit only permits up to 14 days. Based on reports that recipients of one-month-old blood developed icterus, the shelf life of red blood cells was established. This research aims to investigate the relationship between glucose and pH in PRC and the fragility of red cell membranes.

Methods: PRC were stored at $4 \pm 2^\circ\text{C}$ for 0, 7, 14, 21, 28, and 35 days in a cold room. Glucose, pH, and osmotic fragility tests were performed using samples from PRC. The data were analyzed statistically using SPSS.

Results: There were significant decreases in glucose, pH, and membrane fragility ($p < 0.05$). The decrease of glucose at each observed time is significant if compared with glucose at 0 days because the glucose was consumed for glycolysis. pH was also decreased from 7.56 ± 0.03 on day 0 to 6.81 ± 0.03 on day 35 because of lactate accumulation. The result of the osmotic fragility test shows that $23.45 \pm 2.63\%$ of RBC have hemolyzed on day 7 and $75.47 \pm 2.18\%$ of RBC have hemolyzed on day 14. The correlation between glucose and pH, glucose and membrane fragility, and pH and membrane fragility were strong and significant ($r > 0.8$ and $p < 0.05$). It showed that glycolysis proceeds in stored RBC, but because no glucose was added and pH decreased, the glycolysis diminished. As a consequence, the fragility of the red cell membrane increased.

Conclusion: There is a correlation between red cell membrane fragility and glucose and pH in PRC.

Keywords: glucose, membrane fragility, packed red cell, pH.

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INTRODUCTION

PRC with citrate phosphate dextrose adenine-1 (CPDA-1) as a preservative can be stored for 35 days based on the viability of human resources that are still good when transfused.^{1,2} In its implementation, Jakarta Blood Center applied a PRC storage period of only 14 days based on research conducted by Priyana et al. (1991) that there is a significant difference in ATP levels in the blood that has been stored for 2 weeks with fresh blood and also began to find significant differences in levels of 2.3 BPG in blood stored 3 weeks with fresh blood.³ The decreasing of ATP is associated with increased red cell membrane fragility and red cell membrane fragility correlates with red cell viability. Thus, when ATP decreases, red cell membrane fragility increases and red cell viability decreases.⁴ While in Jakarta, it is not yet known whether the decrease in ATP is accompanied by an increase in

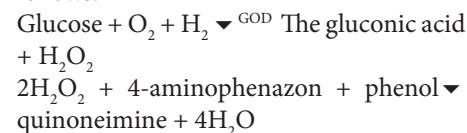
red cell membrane fragility, therefore we could measure the red cell viability.

The short storage time resulted in a poor blood supply management system at Jakarta Blood Center, especially during the fasting month and long holidays. Therefore, the researchers wanted to conduct red cell analysis during storage in PRC bags for 35 days by looking at changes in red cell membrane fragility associated with glucose and pH levels in PRC bags.

METHODS

Sixty PRC bags were taken randomly (random sampling) six times, namely shortly after completion of processing into PRC (day 0), storage days 7, 14, 21, 28, and 35 from the cold room ($2-6^\circ\text{C}$). The number of samples taken on each storage day is 10 bags. Each sample was checked for the glucose level, the pH level, and the percentage of hemolysis. The first step for glucose examination

was the deproteinization stage using TCA solution was then continued with glucose level examination using enzymatic methods.⁵ Glucose was determined by enzymatic oxidation in the presence of glucose oxidase (GOD).⁶ The hydrogen peroxide formed will react with phenol and 4-aminophenazone catalyzed by peroxidase (POD) to form quinoneimine which was purplish red.⁷ The reaction is as follows.



How to calculate:

$$\text{a. Glucose concentration (mg/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 100$$

b. Glucose level in red cell = Glucose concentration / Hemoglobin level (mg/gHb)

The pH level was checked using a pH meter.⁸ The Osmotic Fragility

Test (OFT) principle was placing the red cell in a normal saline solution with a concentration of 0.1% to 0.8%. The fragility of the red cell membrane would be seen based on its resistance in hypoosmotic solution. The normal values of osmotic fragility at 20°C and pH 7.4 can be seen in Table 1. If the fragility of the red cell membrane increased, the red cell would more easily undergo hemolysis, so that hemoglobin came out of the red cell and provided absorption at a wavelength of 540 nm. The calculation used tube 10 as the stamp and tube 1 as the standard 100% hemolysis.⁷

$$\% \text{ red cell fragile} = \frac{A_{\text{sample}} - A_{\text{stamp}}}{A_{\text{standard 100\%}} - \text{blanko}} \times 100$$

The data were processed using SPSS. The examination results data were tested for normality and homogeneity of variance. If the data obtained were normally distributed and the variance between groups was homogeneous, then an ANOVA statistical test was carried out with a meaning limit of $p = 0.05$, followed by an unpaired T-test. If the data obtained were not normally distributed or the variance between groups was not homogeneous, a non-parametric statistical test of Kruskal Wallis was performed. To see if there was a correlation between glucose levels with the percentage of red cell fragility, pH with the percentage of red cell fragility, and glucose levels with pH used linear regression statistical tests with a meaning limit of $p = 0.05$.

RESULTS

Data analysis showed there was a significant decrease in glucose levels in PRC bags during storage from 4.14 ± 0.63 mg/gHb at baseline to 0.13 ± 0.08 (Table 2 and Figure 1).

Data analysis also showed a significant decrease in pH in the PRC bag during storage from 7.56 ± 0.03 at the beginning of the observation to 6.81 ± 0.03 at the end of the observation (Table 3 and Figure 2).

Data analysis showed that there was a significant increase in red cell membrane fragility in PRC bags which was assessed from the percent (%) hemolysis that occurred in 0.54% NaCl solution. It

Table 1. Normal values of Osmotic Fragility at 20°C and pH 7.4

NaCl (%)	% fragility of red cell
0,4	50 – 90
0,45	5 – 45
0,5	0 – 5
0,54	0

Table 2. The glucose level in PRC bags for 35 days of storage at $4 \pm 2^\circ\text{C}$

Day	Glucose level (mg/gHb)	Range
0	4.14 ± 0.63	3.33-5.14 (n=10)
7	2.36 ± 0.26	1.88-2.81 (n=10)
14	0.90 ± 0.40	0.46-1.56 (n=10)
21	0.58 ± 0.19	0.24-0.89 (n=10)
28	0.30 ± 0.13	0.1-0.46 (n=10)
35	0.13 ± 0.08	0.03-0.25 (n=10)
Test	Anova 0.000	

Data are presented as the average value $\pm$ standard deviation (SD)

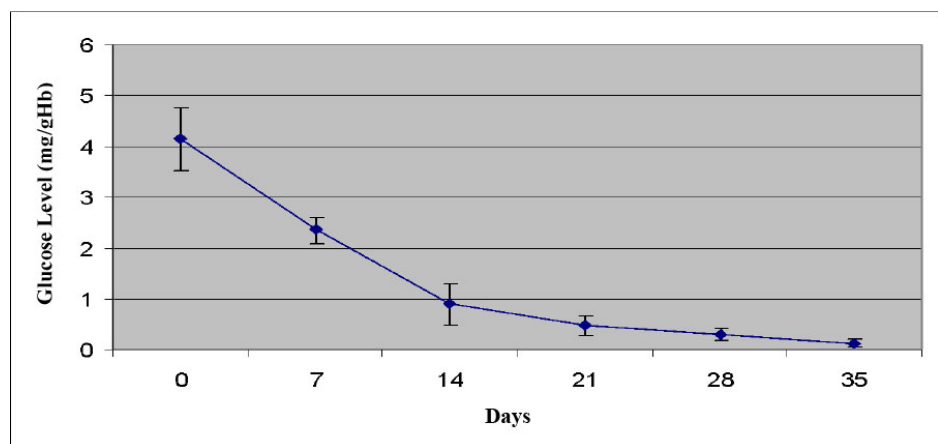


Figure 1. Decreased glucose levels (mg/gHb) in PRC bags at storage days 0, 7, 14, 21, 28, and 35.

Table 3. pH in PRC bags for 35 days storage at $4 \pm 2^\circ\text{C}$

Day	pH	Range
0	7.56 ± 0.03	7.53-7.64 (n=10)
7	7.09 ± 0.04	7.05-7.17 (n=10)
14	6.92 ± 0.09	6.8-7.09 (n=10)
21	6.88 ± 0.04	6.81-6.93 (n=10)
28	6.79 ± 0.04	6.72-6.84 (n=10)
35	6.81 ± 0.03	6.77-6.93 (n=10)
Test	Anova 0.000	

Data are presented as the average value of $\pm$ SD

appeared that at the beginning of the observation, there was already hemolysis $6.56 \pm 1.87\%$ to $75.47 \pm 2.18\%$ (Table 4 and Figure 3).

In this study, a strong and positive correlation ($r > 0.8$) was also obtained between glucose levels with pH (Table 5).

In table 6, it is showed that there was a strong ($r > 0.8$) and meaningful ($p < 0.05$) correlation between glucose levels and the percentage of red cell membrane fragility

and between pH and the percentage of red cell membrane fragility in blood bags during storage.

DISCUSSION

The decrease in glucose levels during storage shows the consumption of glucose by RBC. Glucose was the main nutrient for red cell energy metabolism through glycolysis. The source of glucose in the

PRC bag in this study only came from the preservative solution contained in the main bag. When the blood streamed down into the main bag, the preservative solution will mix with the blood. In the process,

the preservative solution will be separated from the red cell because the density of the preservative solution is smaller than the red cell. When plasma is separated from the red cell, the preservative solution

will be carried into the plasma bag so that the amount of preservative in the PRC bag became less than the amount of preservative contained in the plasma bag.⁹ Thus, the source of glucose became very limited to meet the need for red cells in PRC bags during storage. During storage, glucose levels would decrease so that glycolysis did not run optimally due to the unavailability of the main ingredient.

The pH decrease illustrated the accumulation of lactate in PRC during storage because lactate could not be converted into glucose. According to Guppy et al., at a temperature of 4°C, red cell glycolysis takes place more optimally at pH 7.67 than at pH 7.36.¹⁰ The decreasing of pH could inhibit enzymes such as glycolysis enzymes, methemoglobin reductase enzymes, and enzymes in hexose monophosphate (HMP) shunts so that red cell metabolism did not work optimally.

As a result of the decrease in ATP, there was a decrease in red cell deformability because ATP functions for the phosphorylation of spectrin and ion transport. The decreasing of ATP was associated with increased red cell membrane fragility. Damage to red cells during storage could also be caused by the presence of hydroxy radicals formed from the dismutation of superoxide ions in an acidic state. However, the damage to red cells due to free radical oxidation during storage could be reduced by giving antioxidants such as vitamin E, beta carotene, and vitamin C to donors before donation.¹¹

The alternate solution to extend the storage period was by adding an additive solution at the beginning of storage. With the presence of additives, red cells will get additional glucose for glycolysis so that energy remains available for the integrity of the red cell membrane. In addition to glucose, there is mannitol to maintain osmolarity in the PRC bag. Thus, the viability of red cells is better than without additives.

According to Harmening, it should be 75% of red cells transfused are still viable within 24 hours after transfusion. Viability correlates with red cell membrane fragility. In this study, it was found that on the 7th day, 23.45±2.63% of human resources had undergone hemolysis, which means

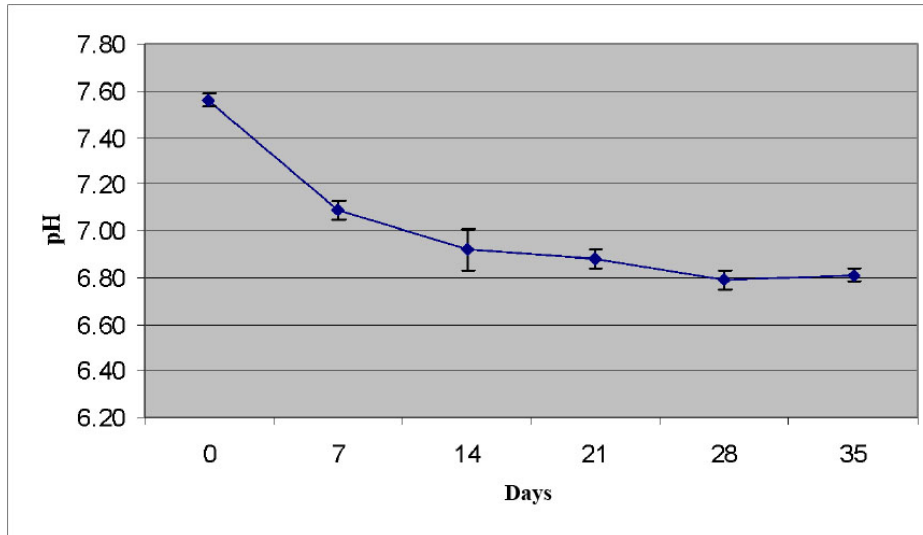


Figure 2. Decreased pH in PRC bags on storage days 0, 7, 14, 21, 28, and 35.

Table 4. The red cell membrane fragility in PRC bags for 35 days of storage at 4±2°C

Day	Red cell membrane fragility (% hemolysis)	Range
0	6.56 ± 1.87	4.43-8.93 (n=10)
7	23.45 ± 2.63	2.0-27.25 (n=10)
14	43.54 ± 3.85	39.16-49.74(n=10)
21	54.65 ± 3.25	51.07-58.84 (n=10)
28	70.11 ± 2.37	66.44-73.95 (n=10)
35	75.47 ± 2.18	70.77-78.8 (n=10)
Test	Anova 0.000	

Data are presented as the average value of ± SD

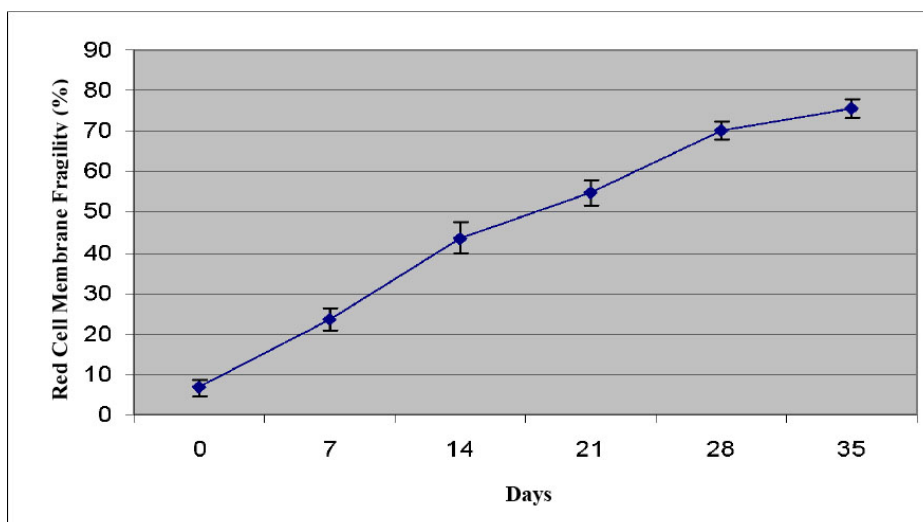


Figure 3. Percentage of red cell membrane fragility in PRC bags on storage days 0, 7, 14, 21, 28, and 35.

Table 5. The correlation between glucose levels and pH in PRC bags at storage (temperature $4 \pm 2^\circ\text{C}$) (n = 60)

Variable	Glucose levels
pH	r = 0.960 p = 0.000

Table 6. The correlation between pH, glucose levels, and red cell membrane fragility in PRC bags at storage (temperature $4 \pm 2^\circ\text{C}$) (n=60)

Variable	Percentage of red cell membrane fragility
Glucose levels	r = 0.932 p = 0.000
pH	r = 0.899 p = 0.000

76.55% of human resources were viable. Then on the 14th day, $54.65 \pm 3.25\%$ of human resources had undergone hemolysis, meaning that only 54.35% of human resources were viable. Therefore, whether the blood stored on the 14th day was still worth transfusing was still a question mark. From the research of Ho et al., it was not recommended to shorten the length of red cell storage.¹² However, it was not yet known with certainty the clinical impact of stored blood transfusions on patients due to various changes in the red cell above.

CONCLUSION

From the results of the study, it showed that there was a decrease in glucose levels, a decrease in pH, and an increase in red cell membrane fragility. This study also showed a relationship between a decrease in glucose levels and a decrease in pH, a relationship between a decrease in glucose levels and an increase in red cell membrane fragility, and a relationship between a decrease in pH and an increase in red cell membrane fragility.

CONFLICT OF INTEREST

There are no conflicts of interest in this study, according to all the authors.

ETHICAL CONSIDERATIONS

The research publication ethical guidelines for this study were in accordance with ICMJE and COPE protocols.

AUTHOR CONTRIBUTIONS

Equal contributions to this study were made by each author.

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