International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

The Effect of Donor Blood Storage Time to the Counts of Erythrocyte and Thrombocyte

Nurlia Naim[a*], Muhammad Nasir[b]

[a]*Department of Health Analyst, Health Polytechnic, Ministry of Health Makassar*

[a]*Email: nurlianaim2018@gmail.com*

Abstract

Blood transfusion is a process of transferring blood from donator to recipient that serves to improve the condition of anemia by using good quality blood and the amount needed. It is done by giving erythrocytes or blood component as needed. Until now, the effect of storage time on erythrocyte and thrombocyte stability is not known clearly. This research aims to determine the effect of storage time on blood and how large the effect of donor blood storage time to erythrocytes and thrombocyte counts. The research is quasy experiment with time series design modification or Time Series Design and Non Randomized Pretest-Postest Control Group. The sample of this research is blood donor (whole blood) blood group A, B, and O. Data analysis was used One Way Anova test (α = 0,05). In the amount of erythrocytes obtained F. Count (0.14) <F. table (5.14), which means there is no effect of donor blood storage time on the amount of erythrocytes. For platelets in the F. Count (426,2)> F. table (5.14), which means there is an influence between the number of platelets in the blood before being stored with the number of platelets stored for 1 week and 2 weeks. Advice for Indonesian Red Cross institutions to provide a tool for hematological examination as one of the preliminary tests of donor blood before donating to recipients.

Keywords: Transfusion ; Storage ; Erythrocytes ; Thrombocyte.

---

* Corresponding author.

1. Introduction

Blood transfusion is a process of transferring blood from donator to recipient that serves to improve the condition of anemia by using good quality blood and the amount needed. It is done by giving erythrocytes or blood component as needed.

Blood and its various components can also be transfused separately as needed. Blood composed of various components, such as erythrocytes (red blood cells), thrombocyte concentrate, cryoprecipitate, and fresh frozen plasma. The transfused blood component as required will reduce the likelihood of transfusion reaction, circulatory overload and transmission of infection occurring in comparison with complete blood transfusion [1].

Before blood is transfused to the recipient it is first stored in the refrigerator, which is expected to maintain the function of components in the blood. However, storage temperature greatly affects the quality of blood. The temperature for donor blood storage ranges from 2-6^{0}C, at this temperature the process of glycolysis in the blood can be inhibited. The cold is expected to maintain the function of components in the blood.

After blood is stored for 2 weeks in ACD, although almost all red blood cells live normally after transfusion, approximately 10% die within 24 hours. After 4 weeks storage in ACD, survival after transfusion decreased and as many as 25% of red blood cells were destroyed. The longer the blood is stored the more red blood cells are destroyed and the small number of red blood cells that can survive [2].

As previous research, showed that erythrocytes are a major part of blood cells, each milliliter of blood contains about 5 billion erythrocytes, which is clinically frequent reported in the count of red blood cells as 5 million per mm^3. Erythrocytes like biconcave discs, which are disc-shaped flat cells that on both sides in the center are concave, like a donut with flat center and not tubular, 8 μm diameter, 2 μm outer and 1 μm center [3, 4]. It is known that platelets derived from megakaryocyte cytoplasmic fragmentation in bone marrow. The size is small (2 - 4 μm), without nucleus [5]. Platelets have a function associated with hemostasis (the process of cessation of blood flowing from a wound) [6].

Erythrocytes and platelets (thrombocyte) have susceptibility to environmental influences, such as temperature at the time of storage where temperatures suitable for donor blood storage are in the range of 2-6^{0}C. They are also very susceptible to storage containers, where the donor blood storage container itself is equipped with special anticoagulants such as *Acid Citrates Dextrose* (ACD), after blood is stored for 2 weeks in ACD approximately 10% of blood cells will be destroyed in 24 hours. At storage time, platelets will group themselves in a tight group with a diameter of 15 pm so it will form aggregates or clots that will reduce the amount in the count per mm^3 [7, 8].

Until now, the effect of storage time on erythrocyte and thrombocyte stability is not known clearly. As described above, the authors want to do a research on "The Effect of Donor Blood Storage Time to Erythrocyte and Thrombocyte Counts." This research aims to determine the effect of storage time on blood and how large the effect of donor blood storage time to erythrocytes and thrombocyte counts.

2. Method of Research

The research is a simple experimental research with the modification of *time series design* to determine the effect of donor blood storage time to erythrocytes and thrombocyte count [9, 10, 11]. It was conducted at the Laboratory of Regional Hospital Salewangang starting from 27 June – 11 June 2016. The populations of research were all whole blood in the Indonesian Red Cross of Makassar. The sample of research is blood donor (Whole Blood) includes A**am**, B, and O groups. Total sample used are 3 samples. Sampling was done with *non-randomized pre-test* and *post-test control group* techniques. Data analysis used is One-Way ANOVA test (α = 0,05). The data of research is presented in table and narration

3. Result

As result of research was conducted in Regional Hospital Salewangang - Maros for 3 samples whole blood includes A, B, O with automatic method obtained results as follows:

Table 1: Examination result of erythrocytes on sample whole blood at the Regional Hospital Salewangang

No.	Sample Code	Examination Result of Erythrocyte		
		Pre-Storage	1 Week Storage	2 Weeks Storage
1.	Sample A	4.590.000/mm^3	4.040.000/mm^3	4.240.000/mm^3
2.	Sample B	4.600.000/mm^3	4.310.000/mm^3	4.310.000/mm^3
3.	Sample O	4.720.000/mm^3	4.150.000/mm^3	4.200.000/mm^3

Source: Primary data, 2016

Table 2: Examination result of thrombocyte on sample whole blood at the Regional Hospital Salewangang

No	Sample Code	Examination Result of Thrombocyte		
		Pre-Storage	1 Week Storage	2 Weeks Storage
1.	Sample A	365.000/mm^3	85.000/mm^3	55.000/mm^3
2.	Sample B	171.000/mm^3	57.000/mm^3	33.000/mm^3
3.	Sample O	275.000/mm^3	72.000/mm^3	42.000/mm^3

Source: Primary data, 2016

Table 1 showed the examination result of erythrocytes and Table 2 showed the examination result of thrombocyte. Both examination results are raw data or data that have not been assessed with statistical formula, for that then data from table 1 and 2 will be processed into the statistical formula One-Way ANOVA test.

The following is an assessment of research results in the statistgical formula One-Way ANOVA:

Table 3: Result of One-Way ANOVA test for examination of erythrocyte count α = 5%

Observation	Sample Code			Σ
	A	B	O	
ERYTHROCYTE	4,59	4,60	4,72	
	4,04	4,31	4,15	
	4,24	4,31	4,20	
ΣXi	12,87	13,22	13,07	39,16
Average	4,29	4,41	4,36	
n_i	3	3	3	9

Source: Primary data, 2016

$$FK = \frac{39,16^2}{9} = 170,39$$

$$JKT = 4,59^2 + 4,04^2 + 4,24^2 + 4,60^2 + 4,31^2 + 4,31^2 + 4,72^2 + 4,15^2 + 4,20^2 - 170,39 = 0.43$$

$$JKP = \frac{12,7^2}{3} + \frac{13,22^2}{3} + \frac{13,07^2}{3} - 170,39 = 0,02$$

$$JKS = 0,43 - 0,02 = 0,41$$

Table 4: Result of ANOVA test

Variation Source	Db	JK	KT	F_{count}	F_{table}
Treatment	2	0,02	0,01		
Residual	6	0,41	0,07	0,14	5,14
Total	8	0,43	0,08		

Source: Primary data, 2016

F_{count} (0.14) < F_{table} (5.14) then H_0 is accepted and H_a is rejected, it means there is no significant influence between donor blood storage time to erythrocytes count.

Table 5: Result of One-Way ANOVA Test for examination of thrombocyte count α = 5%

Observation	Sample Code			Σ
	A	B	O	
THROMBOCYTE	365	171	275	
	275	57	72	
	55	33	42	
ΣXi	505	261	389	1155
Average	168,3	87	129,6	
n_i	3	3	3	9

Source: Primary data, 2016

$$FK = \frac{1155^2}{9} = 148{,}225$$

$$JKT = 365^2 + 275^2 + 55^2 + 171^2 + 57^2 + 33^2 + 275^3 + 72^2 + 42^2 - 148{,}225 = 111.402$$

$$JKP = \frac{505^2}{3} + \frac{261^2}{3} + \frac{389^2}{3} - 170{,}39 = 9.930{,}6$$

$$JKS = 111.402 - 9.930{,}6 = 101.471{,}4$$

Table 6: Result of ANOVA test

Variation Source	Db	JK	KT	F_{count}	F_{table}
Treatment	2	9.930,6	4.965,3		
Residual	6	101.471,4	16.911,9	426,2	5,14
Total	8	111.402	21.877,2		

Source: Primary data, 2016

F_{count} (426,2) > F_{table} (5.14) then H_0 is rejected and H_a is accepted. It means there is a significant influence of donor blood storage time to thrombocyte count.

4. Discussions

In this research, the examination in a laboratory is conducted regularly by using Hematology Analyzer OL-3800 tool on blood donor samples that have been drawn before, but only taken 2 parameters examination as an

examination result in accordance with the title that has been received i.e the examination of erythrocytes and thrombocyte counts by automatic method.

The sample in this research is a sample of donor blood (whole blood) taken from the Indonesian Red Cross Unit, where the sample obtained is fresh blood that has not been stored in the refrigerator. Before examination, the firstly prepares tool to be used is to ensure that the automatic device will be used in good condition without any problems and ensure the reagents are available, samples to be examined are available in EDTA tube, then homogenized and perform routine blood tests (calculate erythrocytes and thrombocyte counts) whose results were taken as control or pre-tests in this research. Then, the sample is stored neatly in a special refrigerator with a temperature of 2-80C for 1 week.

The next process is to perform routine blood examination on samples that have been stored in the refrigerator for 1 week. Preparation is similar to the first namely to ensure the tool will be used in good condition, it is just not used samples that are on EDTA tube again. This time the sample is taken directly from the stored blood bag, before the temperature of sample should be adjusted to the room temperature first while homogenized so that the components in the blood are well together. After sample is homogeneous and in accordance with the room temperature, then perform samples collection into the tube by removing blood from a small tube that is in the blood bag that first disposed of approximately 1 ml before collected, then perform routine blood examination using an automatic method. And then, sample is wrapped neatly and stored again in the refrigerator for 1 week.

After 2 weeks storage, the routine blood examination is performed again with similar material and tools as previous. The results obtained are recorded and attached to the results of the research.

On Hematology Analyzer OL-3800 tool, RBC/PLT is counted and measured using impedance method. This method is based on measurements of electrical resistance changes as generated by particles, which in this case are blood cells, suspended in a conductive diluent when passing through a known dimensional aperture. Electrodes submerged in fluid on both sides of aperture to make electric paths. Since each particle passes through the aperture trough, a temporary change in the resistance between the electrodes is generated. This change produces measurable electrical pulses. The number of pulses generated shows the number of particles that pass through the gap. The amplitude of each pulse is proportional to the internal reference voltage channel, which only receives pulses of certain amplitude. If pulse generated above RBC/PLT is at a low limit, it is calculated as RBC/PLT [12, 13].

Based on the research, considering the method used is automatic method that means it give confidence in the generated result by automatic tool that used to conduct examination such as routine blood examination (erythrocytes and thrombocyte counts). It is likely creates error or mistakes either a fault of analyst or device itself. Possible errors are errors during the preparation of tools and materials that are part of pre-analytics, for example the sample to be used as examination material is not homogenized first before it is inserted into the sample container. Another mistake that may occur is error device for example reading error due to poor control.

To minimize errors that may occur can be done by controlling the device and ensure diluent or reagents are used

is still in good condition, and also the capability or skill of analyst itself [14].

The results of research on donor blood sample (whole blood) before the storage (pre-test) for sample A, the count of erythrocytes is 4.590.000/mm3 and thrombocyte is 365.000/mm3, sample B; the count of erythrocytes is 4,600,000/mm3 and thrombocyte is 171.000/mm3, sample O; the count of erythrocyte is 4.720.000/mm3 and thrombocyte is 275.000/mm3. After 1 week storage for sample A is 4.040.000/mm3 for erythrocyte and 85.000/mm3 for thrombocyte, sample B is 4.110.000/mm3 for erythrocyte and 57.000/mm3 for thrombocyte, sample O is 4.150.000/mm3 for erythrocyte and 72.000/mm3 for thrombocyte. And after 2 weeks storage, for sample A is 4.240.000/mm3 for erythrocyte and 55.000/mm3 for thrombocyte, sample B is 4.310.000/mm3 for erythrocyte and 33.000/mm3 for thrombocyte, sample O is 4.200.000/mm3 for erythrocyte and 42.000/mm3 for thrombocyte.

From the research results it can be seen that there is no significant change from the results of erythrocytes examination based on storage time which means there is no significant effect on the erythrocytes count in storage, whereas on the results of thrombocyte examination, there are significant differences which means there is influence of time storage against thrombocyte count.

Based on the results obtained there is no significant effect between the storage time to the count of erythrocytes, even in 2 week storage there is an increase in the count. This can be due to the long life span of erythrocyte, which is 120 days while the shelf-life as performed in this study is only 14 days. In addition, the increase in 2 weeks storage can be due to the erythrocyte concentrate as result of partial loss of plasma fluid due to cold temperature of the refrigerator with low pressure can cause water evaporation in the cell.

Meanwhile, on the examination of thrombocyte count, there is decrease of average count in 1 and 2 weeks storage significantly. This is due to the short life span of thrombocyte, which is only 9-10 days. Also, it can be due to the nature of thrombocyte aggregation that is easily attached to the media (blood bag), and will cluster. It may also be due to an intrinsic or extrinsic path of blood clotting that causes damage to thrombocyte cells. Another possibility, the ability of thrombocyte to conduct thrombocyte satellites by surround leukocyte, it can only be seen through the blood smear beneath the objective lens of the microscope.

5. Conclusion

As results of research on 3 sample of donor blood (whole blood) includes A, B, O groups can be concluded on the examination of erythrocytes count that there is no effect of donor blood storage time to the count of erythrocytes, while for thrombocyte there is effect of donor blood storage time to the count of thrombocyte count. It is recommended to the Indonesian Red Cross institution to conduct a hematological examination as one of screening test of donor blood before transfused to the patient or recipient.

References

[1] Bermawi, H. Transfusi Darah dan Komponen Darah.In: M. S. Kosim, et al.eds. Buku Ajar Neonatologi. 1ed.s.l: Ikatan Dokter Anak Indonesia, 2010. p. 285.

[2] Erviana I. Pengaruh Waktu Penyimpanan Darah Donor Terhadap Jumlah Leukosit Metode Konvensional.Makassar: Analis Kesehatan. 2011.

[3] Vaught JB, Henderson MK, Compton CC. Biospecimens and biorepositories: from afterthought to science. Cancer Epidemiol Biomarkers Prev. 2012;21:253–255. [PMC free article] [PubMed]

[4] Campbell LD, Betsou F, Leiolani Garcia D, Giri JG, Pitt KE, Pugh RS, Sexton KC, Skubitz AP, Somiari SB. Development of the ISBER Best Practices for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research. Biopreservation and Biobanking. 2012;10:232–233.[PubMed].

[5] National Cancer Institute Best Practices for Biospecimen Resources. [Accessed October 21, 2013];http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf.

[6] Departemen Kesehatan Republik Indonesia,. Hematologi.Jakarta: Pusdiknes Depkes RI. 1989

[7] Dumont LJ, AuBuchon JP. Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials. Transfusion. 2008 Jun;48(6):1053–60. [PubMed].

[8] Dumont LJ, AuBuchon JP, Gulliksson H, et al. In vitro pH effects on in vivo recovery and survival of platelets: an analysis by the BEST Collaborative. Transfusion. 2006;46(8):1300–5. [PubMed].

[9] Gandasoebrata R. Penuntun Laboratorium Klinik. Jakarta: Dian Rakyat.1969.

[10] Hoffbrand A.V, Pettit J.E, Moss P.A.H. Kapita Selekta Hematologi. Jakarta: EGC. 2005.

[11] Latief. Transfusi Darah. In: R. Hassan & H. Alatas , eds.Buku Kuliah 1 Ilmu Kesihatan Anak. Jakarta: Bagian Ilmu Kesehatan Anak Fakultas Kedokteran. Universitas Indonesia, 2007.p. 483.

[12] Chandra, S. Transfusi Darah dan Infus Cairan. In: T.Rachimhadhi, G.H.Wiknjosastro & A. B. [13] Saifuddin, eds.Ilmu Kebidanan. Jakarta: Yayasan Bina Pustaka Sarwono Prawirohardjo, 201.p.419.

[13] Sherwood L. Fisiologi Manusia dari Sel ke Sistem, Edisi 2. Jakarta;EGC. 2001.