PHARMACOLOGY, MEDICAL REPORTS, ORTHOPEDIC, AND ILLNESS DETAILS
(COMORBID)
VOLUME 1 ISSUE 2 (2022)

COMPARISON OF ERYTHROCYTES IN 3 ML, 2 ML, & 1 ML OF BLOOD SAMPLES WITH K2EDTA ANTICOAGULANTS AFTER A 4-HOUR DELAY IN THE DR. H. ABDUL MOELOEK BANDAR LAMPUNG HOSPITAL

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Abstract

The volume of blood samples, especially the number of erythrocytes in laboratory examinations, has an important role in determining the results of the diagnosis, in this case pre-analytic errors often occur so that a definite diagnosis will be difficult to establish. Therefore, research on the comparison between the results of the examination of the number of erythrocytes in the volume of blood samples of 3 mL, 2 mL, and 1 mL after being delayed for 4 hours with K_2EDTA anticoagulant helps to determine a definite diagnosis from laboratory tests, one of which is the count of the number of erythrocytes. This research aims to identify the comparison of erythrocytes in the volume of blood sample 3 mL, 2 mL, and 1 mL with K_2EDTA anticoagulant after postponed for four hours. The study uses primary data with hematology analyzer in Dr. H. Abdul Moeloek Bandar Lampung Hospital. This type of research is a quantitative, using observational analytic design with a cross sectional view and with consecutive method, through hematology analysis using a Alayzer Mindray BC-3600 hematology. The research has 50 respondents. The results of erythrocytes comparison between blood volume 3 mL, 2 mL and 1 mL with K_2EDTA anticoagulant after 4 hours have different results but statistically the results have the same with 3 ml, that show the lowest result. Therefore, it can be concluded that there are no significant differences between the examination of the erythrocytes with the volume of blood sample 3 mL, 2 mL, and 1 mL in the vacutainer K_2EDTA tube after delayed for 4 hours.

Keywords: *Hematology Examination, Blood Volume, K_2EDTA*

1. INTRODUCTION

The utilization of supporting examinations in the health sector is preferable when determining a diagnosis in this field. Typically, laboratory examination is used to support the findings of the clinical investigation (Permenkes, 2010). A hematological examination is the most common laboratory examination. A count of the number of erythrocytes is one of various types of hematological exams that can be performed. The amount of erythrocytes in a blood sample has an important role in diagnosing disease (Ardina & Rosalinda, 2018). The erythrocyte quantity is essential to confirm the diagnosis and to use anticoagulants to

prevent clotting (Handayani, 2017) because the erythrocytes that have been taken from the vein will experience freezing when left at room temperature if not using anticoagulants as clotting delays, erythrocytes will experience damage/destroyed. The most effective anticoagulant is K_2EDTA recommended by *International Council for Standardization in Haematology* (ICSH) and North Celebes Creative Lab (NCCL) (Danastri, 2020). When performing an examination, it is expected that K_2EDTA must match the blood volume to get reliable results (Gandasoebrata, 2013).

Laboratory examination are classified as pre-analytic, analytical, and post-analytic. The laboratory testing level was discovered to be identical to the test error, which obtain at a rate of 62% pre-analytic, 25% analytic, and 14% post-analytic (Mengko, 2013). Failure to perform hematological examinations to count the quantity of erythrocytes in the field was caused by a variety of factors, including power outages, damaged tools, and tool calibration (Dameuli, 2018). However, there are many cases of blood morphological damage as a result of extended mixing with anticoagulants at the time of evaluation and storage at room temperature in several circumstances (Sriwati, 2018).

However, according to the International Council for Standardization in Haematology (ICSH) in 2002, suggesting that anticoagulants contained in blood samples will experience cell morphology changes after 30 minutes of collection, therefore ICSH recommends that the examination should be carried out for a maximum of 4 hours (Vives-Corrons et al., 2014).

Research on delayed blood tests and anticoagulants has been carried out by several researchers including the study conducted by Daniel et al. (2020) which turned out that from a complete blood test using the K_2EDTA anticoagulant there was no significant difference after being delayed for 2 to 6 hours with results in the amount of erythrocyte of 4,7 when the sample was examined using K_2EDTA, while getting a score of 4,6 on the K_3EDTA examination, hence it can be concluded that there is no significant difference (Daniel, 2020). However, different result was obtained by Cinthia (2018) with an examination of 3 ml of blood volume on examination of differences in blood morphology with K_3EDTA anticoagulant which was immediately examined and delayed for 3 hours, and obtained the results that there were differences in the shape of erythrocytes so that it also affected the number of erythrocytes, with a p-value of 0,025 ($p<0,05$) (Cinthia, 2018). Consequently, the author wants to performed research comparing the number of erythrocytes in 3 ml, 2 ml, and 1 ml blood samples given with K2EDTA anticoagulant after a 4-hour delay at the UTD (Blood Transfusion Unit) of Dr. H. Abdul Moeloek Bandar Lampung Hospital.

2. RESEARCH METHOD

The type of research used is analytic observation with a Cross Sectional approach. This research was conducted at UTD (Blood Transfusion Unit) of Dr. H. Abdul Moeloek Bandar Lampung Hospital. The sample of this study were voluntary blood donors. The research subjects selected were those who met the inclusion and exclusion criteria with the non-probability sampling technique (not random), using the convenience technique. From 53 people, 55 people were selected as the sample. Based on gender, there were 25 men and 25 women. In this study, the anticoagulant used was the K_2EDTA vacutainer tube with a standard volume of 3 mL. The examination method used in this study is the automatic

method using the Hematology Alayzer Mindray BC-3600 tool. After the anticoagulant has been mixed with the blood volume, it will be left for four hours before being tested.

The data were analyzed by computer using the IBM SPSS Statistics version 26 program which was then tested for normality. The normality test used was the Shapiro-Wilk test and the data obtained were not normally distributed, then analyzed using the Kruskal-Wallis test. Because the Kruskal-Wallis test was significant and had the same variance, a Bonferroni Post Hoc analysis was carried out to find out the significant differences between groups.

3. RESULT AND DISCUSSION

3.1. Result Research

The results of the study on the number of erythrocytes showed that 3 mL of K_2EDTA blood had an average of 5,10x10^6/µL, the number of erythrocytes found in 2 mL of K_2EDTA blood has a mean of 5,11x10^6/µL, while the number of erythrocytes found in the blood K_2EDTA 1 mL has a mean of 5,12x10^6/µL. The results of the erythrocyte examination are presented in table 1 as follows.

Table 1 Comparison of the results of the examination of the number of erythrocytes in blood samples

Volume of Sample	Mean of Erythrocytes amount (10^6/µL)	Lowest Value of Erythrocytes (10^6/µL)	Highest Value of Erythrocytes (10^6/µL)
3 mL	5.10	3.93	6.80
2 mL	5.11	3.96	6.46
1 mL	5.12	3.98	6.47

Table 2 Comparison of the results of the examination of the number of erythrocytes in blood samples by gender

Gender	Volume of Sample	Mean of Erythrocytes amount (10^6/µL)	95% Confidence Interval	
			Lowest Value of Erythrocytes (10^6/µL)	Highest Value of Erythrocytes (10^6/µL)
Man	3 mL	5.44	4.65	7.42
	2 mL	5.44	4.68	7.54
	1 mL	5.45	4.61	7.52
Woman	3 mL	4.76	4.05	5.60
	2 mL	4.78	4.27	5.56
	1 mL	4.80	4.27	5.64

Table 3 Comparison of erythrocyte number examination results in blood samples based on age

Age Range	Volume of Sample	Mean of Erythrocytes amount (10^6/µL)	95% Confidence Interval	
			Lowest Value of Erythrocytes (10^6/µL)	Highest Value of Erythrocytes (10^6/µL)
17-25	3 mL	5,21	4,42	7,42
	2 mL	5,21	4,52	7,54
	1 mL	5,22	4,49	7,52
26-35	3 mL	5,26	4,12	6,16
	2 mL	5,29	4,27	6,16
	1 mL	5,32	4,27	6,27
36-45	3 mL	4,84	4,05	5,98
	2 mL	4,86	4,32	5,95
	1 mL	4,88	4,36	6,00

Furthermore, Kruskal-Wallis Test was performed to see the average difference in blood samples of 3 ml, 2 ml and 1 ml with K2EDTA anticoagulants after 4 hours delay, the result can be seen in the following table.

Table 4 Kruskal-Wallis Test

Volume of Blood	n	Mean (10^6/µL)	Standard Deviation	(Min-Max)	*P-Value*
3 mL	50	5,10	0,611	4,92-5,27	0,995
2 mL	50	5,11	0,602	4,94-5,28	
1 mL	50	5,12	0,608	4,95-5,29	

3.2. Discussion

Based on table 1, it is known that of the 50 respondents studied, the number of donor erythrocytes showed that of the three different sample volumes, the lowest examination results were found at 3 mL volume, which was 5,10 x 10^6/µL. Meanwhile, the highest was at 1 ml blood sample volume, which was 5,12 x 10^6/µL.

Furthermore, based on table 2, it can be seen that the frequency distribution of respondents based on gender is 25 men with a presentation of (50%) and 25 women with a presentation of (50%), it is known that the results of data analysis related to gender are known that men and women have the same number.

Moreover, based on table 3, it is known that the frequency distribution of donor respondents based on age there are patients aged 17-25 years as many as 28 respondents with a presentation of (56%), while respondents aged 26-35 years as many as 6 respondents with a presentation of (12%), and respondents who aged 36-45 years as many as 16 respondents

with a presentation of (32%). The results reveal that the concentration of erythrocytes declines as one becomes older and older.

Lastly, based on table 4, the results of the Kruskal-Wallis Analysis Test on the results of count the number of erythrocytes p = 0,995 it can be concluded that p > 0,05 because the significant value is greater than the significance level used, so Ho is accepted and Ha is rejected, it can be concluded that there is no significant comparison from the results of the examination of the count of the number of erythrocytes in the sample. blood volumes of 3 mL, 2 mL, and 1 mL with the K_2EDTA vacutainer tube after a delay of 4 hours with a standard blood volume of 3 mL. The results of this study are in line with research conducted by Daniel et al. (2020) it turns out that from a complete blood test using the K_2EDTA anticoagulant there is a non-significant difference after being delayed for 2 to 6 hours with results in the erythrocyte count of 4,7 when the sample is examined using K_2EDTA while getting a value of 4,6 on K_3EDTA examination, and it can be concluded that there is no significant difference Daniel et al. (2020). In contrast, Cinthia (2018) with an examination of 3 ml of blood volume on examination of differences in blood morphology with K_3EDTA anticoagulant which was immediately examined and delayed for 3 hours, and got the results that there are differences in the shape of erythrocytes so that it also affects the number of erythrocytes, with a p-value of 0,025 (p <0,05) (Cinthia, 2018).

4. CONCLUSION

Based on this research, it can be concluded that there is no significant difference between the examination of the number of erythrocytes and the blood sample volume of 3 mL, 2 mL, & 1 mL in the K_2EDTA vacutainer tube after being delayed by 4 hours in blood samples of healthy people.

Suggestion

The findings of this study revealed that there was no significant difference between the examination of the number of erythrocytes and the volume of blood samples of 3 mL, 2 mL, and 1 mL with anticoagulant K_2EDTA after a delay of 4 hours; therefore, it is recommended that if there is a delay in the examination, it is expected to be no longer than 4 hours; and that further research is also expected to examine the influence of insufficient blood volume in the vacutaine K_2EDTA tube to the sick. Aside from that, it is able to perform an edge blood smear examination on a blood sample in which the erythrocytes have clumped together, as well as inserting blood into the K_2EDTA vacutainer tube using a vacuette needle.

REFERENCES

Ardina, R., & Rosalinda, S. (2018). Morfologi eosinofil pada apusan darah tepi menggunakan pewarnaan Giemsa, Wright, dan kombinasi Wright-Giemsa. *Jurnal Surya Medika (JSM)*, *3*(2), 5–12.

Cinthia, A. (2018). *Perbedaan Morfologi Eritrosit Pada Spesimen Darah K3edta Yang Segera Diperiksa Dan Ditunda Selama 3 Jam*. Universitas Muhammadiyah Semarang.

Dameuli, S. (2018). *Perbedaan Kadar Hemoglobin Menggunakan Hb Meter, Spektrofotometer Dan Hematology Analyzer Pada Sampel Segera Diperiksa Dan*

Ditunda 20 Jam. Universitas Muhammadiyah Semarang.

Danastri, L. N. E. (2020). *Pengaruh Perbedaan Volume Darah Pada Tabung Vakum K2edta Terhadap Hitung Jumlah Leukosit*. Sekolah Tinggi Ilmu Kesehatan Nasional.

Daniel, V. R., Eem, H., Nina, M., & Adang, D. (2020). *Pengaruh Waktu Simpan Darah dengan Antikoagulan Dipotassium dan Tripotassium Ethylenediaminetetraacetic Acid Terhadap Jumlah Leukosit*. Politeknik Kesehatan Kemenkes Bandung.

Handayani, E. M. (2017). *Pengaruh Penundaan Pemeriksaan Spesimen Darah Pada Suhu Ruang, Ruang AC dan Lemari Es Terhadap Jumlah Trombosit*. Universitas Muhammadiyah Semarang.

Mengko, R. (2013). Instrumen Laboratorium Klinik. *Institut Teknologi Bandung*.

Permenkes. (2010). *Peraturan Menteri Kesehatan Republik Indonesia Nomor 1191/Menkes/Per/Viii/2010 Tentang Penyaluran Alat Kesehatan*. Pusat Komunikasi Publik Departemen Kesehatan.

Sriwati, C. (2018). *Perbedaan Kadar Hemoglobin Pada Sampel Yang Segera Diperiksa Dan Di Tunda 3 Jam*. Universitas Muhammadiyah Semarang.

Vives-Corrons, J.-L., Briggs, C., Simon-Lopez, R., Albarede, S., de la Salle, B., Flegar-Meatrii, Z., Nazor, A., Guyard, A., Lipsic, T., & Nagai, Y. (2014). Effect of EDTA-anticoagulated whole blood storage on cell morphology examination. A need for standardization. *International Journal of Laboratory Hematology*, *36*(2), 222–226.