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Comparison of K2 and K3 EDTA Anticoagulant on Complete Blood Count and Erythrocyte Sedimentation Rate

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ABSTRACT

The use of anticoagulants is one of the important pre-analytic factors in hematological tests. Both dipotassium (K2) and tripotassium (K3) Ethylene Diamine Tetraacetic Acid (EDTA) are widely used anticoagulants. International Council Standardization of Hematology (ICSH) and several researchers recommend the use of K2 EDTA due to its less hyperosmolar effect on blood cells compared to K3 EDTA. This study aimed to compare the results of Complete Blood Count (CBC) and Erythrocyte Sedimentation Rate (ESR) using anticoagulant K2 EDTA and K3 EDTA. This study was an analytic observational study with a cross-sectional design conducted from April to December 2018. The subject of the study were 103 healthy adults selected by consecutive sampling. Blood samples were collected in both anticoagulant tubes with a volume of 3 mL each. Samples were tested twice, in the first 0 hours and the next 6 hours using Sysmex XN 1000 and Alifax Roller 20 LC. Kolmogorov-Smirnov test, paired T-test and Wilcoxon rank test were used for statistical analysis. The agreement test between both anticoagulants was carried out using the Bland Altman plot for parameters with a significant difference. There was a significant difference between both anticoagulants for the parameters of hemoglobin, hematocrit MCV, MCHC, RDW, PDW, MPV, PLC-R, and erythrocyte sedimentation rate in both the first and second tests. The agreement test using the Bland Altman plot showed that the difference in these parameters was within the Limit of Agreement (LOA) range of 95%. This study showed that there were differences in some parameters of complete blood count and erythrocyte sedimentation rate between the two anticoagulants (K2 K3 EDTA), but these differences were within the LOA range.

Keywords: Complete blood count, erythrocyte sedimentation rate, K2 EDTA, K3 EDTA

INTRODUCTION

Ethylene Diamine Tetraacetic Acid (EDTA) is an anticoagulant commonly used for routine hematological test.[1] Ethylene diamine tetraacetic acid works by inhibiting platelet aggregation and binding free calcium to prevent the formation of blood clots. Three types of EDTA salts are widely used for the hematological test, such as disodium (Na2), dipotassium (K2), and tripotassium (K3) EDTA. Disodium and dipotassium are usually used in dry form; while tripotassium EDTA is usually used in liquid form to enhance anticoagulant activity.[2] All types of EDTA anticoagulant are hyperosmolar, causing fluid to move from intracellular to extracellular, which can cause shrinkage of erythrocytes. These effects are known to influence some parameters, including decreasing the value of Packed Cell Volume (PCV) and Mean Corpuscular Volume (MCV). These effects are mainly reported in the use of K3 EDTA; therefore, some researchers recommend the use of K2 Ethylene Diamine Tetraacetic Acid (K2 EDTA) compared to K3 Ethylene Diamine Tetraacetic Acid (K3 EDTA).[3] The International Council Standardization of Hematology (ICSH) also recommends the use of K2 EDTA as the anticoagulant of choice for blood cell count.[4] Dipotassium Ethylene Diamine Tetraacetic Acid (K2 EDTA) is widely used in the United States and the United Kingdom, whereas Tripotassium Ethylene Diamine Tetraacetic Acid (K3 EDTA) is used in Europe and Japan.[3,5,6]

The variation in the use of the EDTA underlies the researchers to compare the measurement results of the hematological test, such as complete blood count and erythrocyte sedimentation rate using the K2 EDTA and K3 EDTA. Ethylene Diamine Tetraacetic Acid (K2 EDTA) was used as a measurement reference in this study and the results of the measurement using K3 EDTA were compared with those using K2 EDTA.

METHODS

This research was an analytic study with a cross-sectional design. Sample collection was carried out by consecutive sampling during June-August

2018. The research sample was taken from healthy adult patients >18 years old who underwent a medical check-up or blood transfusion test. The anticoagulant tube used was BD vacutainer with K2 EDTA and K3 EDTA. A total of 3 mL of venous blood samples were collected in each EDTA tube. The blood test was carried out twice, immediately after collection (R1;0 hours) and 6 hours after collection (R2;6 hours) at room temperature.

Complete blood count was carried out using Sysmex XN 1000 and erythrocyte sedimentation rate test was carried out using Roller Alifax 20 LC device. The complete blood parameters analyzed in this study were 17 parameters in total, consisting of hemoglobin (Hb), hematocrit (HCT), erythrocytes (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width (RDW), leukocytes (WBC), neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), platelets (PLT), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), and Platelet Large Cell Ratio (P-LCR).

This research has been approved by the Ethics Committee of Dr. Soetomo General Academic Hospital with Number 0302/KEPK/V/2018. SPSS version 17.0 was used in this study for the statistical analysis. The comparative test in this study was carried out using the Wilcoxon sign rank test for data with abnormal distribution and the paired T-test for data with normal distribution. The Bland Altman plot test was used to analyze the agreement between both measurements (for data with significance difference). The percentage of bias between both tubes was calculated based on the following formula: % mean differences: [(mean K3 EDTA-mean K3 EDTA/mean K2 EDTA) X100%]. The results of the % mean differences of all the parameters were compared with the % desirable bias that has been determined based on biological variation, except for PDW and LED because the % desirable bias for these two parameters has not been determined. The desirable bias for LED used in this study was ±10%, according to Alifax.[7,6]

RESULTS AND DISCUSSIONS

There was a total of 103 samples obtained in this study, consisting of 74 samples from blood donors and 29 samples from medical check-up. Research subjects in this study consisted of 55 males and 48 females an age range of 18-58 years as shown in Table 1.

Comparison of statistical analysis results between

Table 1. Characteristics of research subjects

Profile	Results
Gender–n (%)	
Male	55 (53.4%)
Female	48 (46.6%)
Age–years	
Range	18–58
Median	36
Mean±SD	37.48±10.77

Table 2. Complete blood count and erythrocyte sedimentation rate test results using K2 EDTA and K3 EDTA

	Mean % Differences (p-value)						
Parameter	K2 EDTA R1	K2 EDTA R2	K3 EDTA R1	K3 EDTA R2	K2K3 EDTA R1	K2K3 EDTA R2	Desirable Bias %
Hemoglobin (Hb)	13.6 (10.9-17.0)	13.7 (10.8-17.8)	13.6 (10.4-16.6)	13.6 (10.3-16.6)	0.0 (0.001)	0.7 (0.191)	1.8
Hematocrit (HCT)	41.7±3.6	42.8±4.0	41.2±3.7	42.2±4.0	-1.2 (0.000)	1.4 (0.000)	1.7
Erythrocyte							
RBC [a]	4.9 (3.7-37.4)	4.9 (3.6-6.7)	4.9 (3.5-6.7)	4.9 (3.4 6.7)	0.0 (0.066)	0.0 (0.069)	1.7
MCV [a]	86.9 (65.0-96.5)	87.4 (68.4-98.4)	86.6 (57.0-97.4)	87.1 (66.3-100.0)	0.3 (0.000)	-0.3 (0.000)	1.2
MCH [a]	28.5 (19.6-31.8)	28.5 (19.7-32.0)	28.5 (19.2-36.7)	28.4 (19.3-31.5)	0.0 (0.041)	-0.3 (0.002)	1.4
MCHC [b]	32.8±1.1	32.4±1.1	33.2±1.1	32.6±1.1	0.6 (0.000)	0.6 (0.000)	0.8
RDW-CV	12.9 (11.6-18.6)	13.1 (11.7-18.6)	12.9 (11.5-18.8)	13.1 (11.6-112.7)	0.0 (0.216)	0.0 (0.000)	1.7
Leukocyte							
WBC% [a]	6.8 (4.0-11.4)	6.6 (4.2-11.3)	6.7 (4.1-11.6)	6.7 (0.9-11.3)	-1.5 (0.053)	1.5 (0.095)	5.6
Eo% [b]	3.0±1.7	3.1±1.7	3.1±1.7	3.1±1.7	3.3 (0.271)	0.0 (0.518)	19.8
Baso% [a]	0.6 (0.1–1.6)	0.6 (0.1–1.7)	0.6 (0.2–1.5)	0.6 (0.1–1.9)	0.0 (0.105)	0.0 (0.693)	15.4
Neut% [b]	58.3±7.2	58.3±7.1	58.2±7.2	58.0±6.7	-0.2 (0.725)	-0.5 (0.968)	9.1
Lymph% [a]	30.0 (12.0-46.5)	30.5 (11.2-46.9)	30.6 (12.9-46.5)	30.4 (17.6-46.8)	2.0 (0.140)	-0.3 (0.425)	7.4
Mono% [b]	6.9±1.7	6.6±1.6	6.9±1.7	6.8±1.7	0.0 (0.763	3.0 (0.443)	13.2
Platelet							
PLT [b]	296.8±62.2	300.7±62.1	294.6±61.6	300.4±61.6	-0.7 (0.183)	-0.09 (0.063)	5.9
PDW [b]	11.2±1.4	11.1±1.3	11.4±1.4	11.4±1.4	1.8 (0.001)	2.7 (0.014)	NA
MPV [b]	10.03±0.65	9.98±0.65	10.10±0.67	10.14±0.68	0.7 (0.007)	1.6 (0.028)	2.3
P-LCR [b]	25.0±5.3	24.5±5.3	25.4±5.5	25.8±5.6	1.6 (0.087)	5.3 (0.008)	NA
LED [a]	24 (2-80)	23 (2-82)	22 (2-75)	22 (2-75)	-8.3 (0.019)	4.3 (0.012)	10%

NA: Not Available, a: data with normal distribution, b: data with abnormal distribution
p-value: Wilcoxon ranked- paired T-test $p < 0.05$: statistically significant

the two anticoagulant tubes is shown in Table 2. From the results of the study, statistical differences were found between K2 EDTA and K3 EDTA on the first test (R1;0 hours) for Hb, HCT, MCV, MCH, MCHC, PDW, MPV and LED parameters. Statistical differences were also obtained on the second test (R2;6 hours) for hematocrit, MCV, MCHC, RDW, PDW, MPV, P-LCR and LED parameters. The difference in a number of these parameters was within the desirable bias range based on the previously mentioned formula, because the % mean differences did not exceed the upper limit of the % desirable bias. However, there was no significant difference in leukocyte parameters ($p > 0.05$) between K2 EDTA and K3 EDTA, either in the first test (R1;0 hours) or in the second test (R2;6 hours).

The recommended anticoagulants for a complete blood count are K2 EDTA and K3 EDTA.[8] Several studies have shown the anticoagulant effect of K2 EDTA and K3 EDTA on hematological test, especially the erythrocyte "shrinkage" in K3 EDTA due to its higher hyperosmolar properties and the dilution effect of K3 EDTA in liquid form; therefore, many researchers recommend the use of K2 EDTA

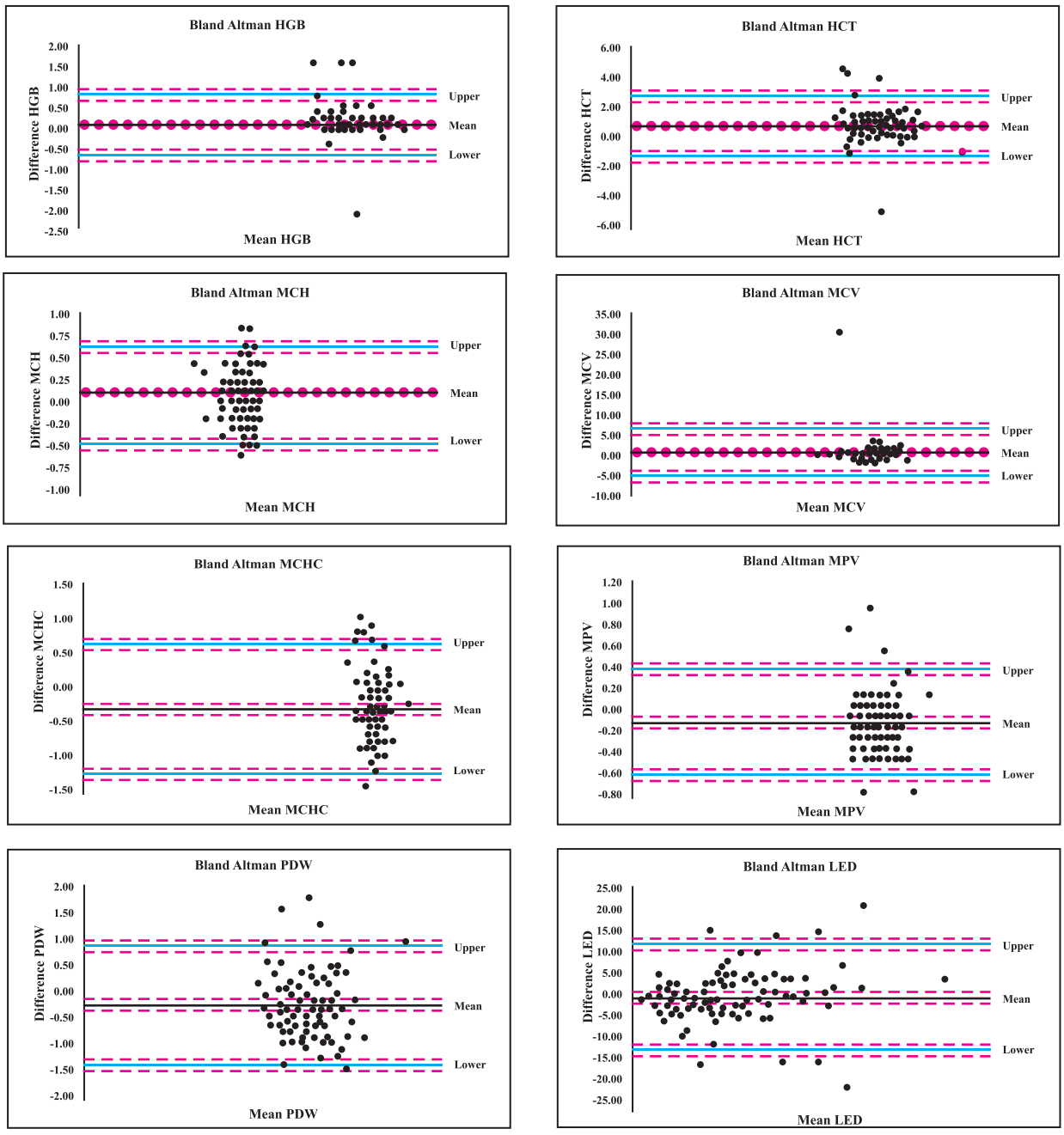

Figure 1. Bland Altman plot curve to analyze the agreement between the use of K2 EDTA and K3 EDTA for parameters showing the significant difference with LOA 95%

compared to K3 EDTA.[3,6] The ICSH also recommends the use of K2 EDTA as the anticoagulant of choice for hematological tests.[9]

This study showed that there was a significant difference in Hb between K2 EDTA and K3 EDTA tubes on the first test (R1;0 hours). This was different from a study by Mehmood *et al*., which showed no statistical difference for Hb parameters between K2 EDTA and K3 EDTA.[5] A significant difference was also found in the HCT parameter between K2 EDTA and K3 EDTA both in the first and second test (R1;0 hours, R2;6 hours). Hematocrit was lower in K3 EDTA when compared to K2 EDTA in both the first and second test (R1;0 hours, R2;6 hours). This might be caused by the shrinkage effect of erythrocytes due to stronger hyperosmolar effect of K3 EDTA compared to K2 EDTA.[3,5] EDTA causes cell shrinkage due to hypertonicity of plasma caused by increased ion concentration. The results of this study were different compared to the research by Ahn *et al*., which found a higher increase of hematocrit in K3 EDTA compared to K2 EDTA.[6]

The statistical tests for MPV, PDW showed a significant difference between K2 EDTA and K3 EDTA both in the first and second tests (R1;0 hours, R2;6 hours). Changes in MVP depend on the time of contact with the EDTA anticoagulant. The mechanism that plays a role is a change in membrane permeability through the Cyclic AMP (cAMP) cycle. Platelets undergo a change in shape from discoid to spherical, causing an increase in platelet volume when platelets pass through an automatic device with the principle of impedance. In a hemocytometer with the principle of measurement using volume and refractive index, various MPV sizes will be obtained.[9]

The statistical tests for MCV showed a significant difference between K2 EDTA and K3 EDTA tubes in both the first and second test (R1;0 hours, R2;6 hours). It is known that K2 EDTA can affect MCV results, but lower MCV values are more often found in K3 EDTA due to its stronger hyperosmolar effect.[10] Gossen *et al*. showed that MCV was not affected by the concentration of K3 EDTA even though the concentration was increased 10 times. However, the use of K2 EDTA at high concentrations could slightly increase MCV. The difference in MCV between K2 EDTA and K3 EDTA usually occurs under conditions of low blood pH. For example, in conditions of sepsis or severe infection, which tend to lower the pH, significant differences in MCV can be found.[3,11] A significant difference was also found in the Erythrocyte Sedimentation Rate (ESR) parameter between K2 EDTA and K3 EDTA both on the first (R1;0 hours) and the second test (R2;6 hours).

Statistical analysis in this study showed significant differences in the erythrocyte sedimentation rate and some complete blood parameters (Hb, HCT, MCV, MCH, MCHC, PDW, MPV, and ESR) both on the first (R1;0 hours) and on the second tests (R2;6 hours), but the difference was within the desirable bias range for each parameter. The agreement test using the Bland Altman plot was carried out for several parameters with a significant difference. It was found that the difference between both anticoagulants was mostly within the LOA range of 95% (Figure 1). This result was in accordance with a study by Ahn *et al*. and Mehmood *et al*.[5,6]

The different results obtained from this study might be due to environmental factors, the use of different equipment, and the use of different test methods. In addition, this study merely distinguished the results of complete blood tests and erythrocyte sedimentation rates using both anticoagulants.

CONCLUSIONS AND SUGGESTIONS

This study showed significant differences ($p < 0.05$) in Hb, HCT, MCV, MCH, MCHC, MPV, PDW, and ESR between both anticoagulants. The agreement test using the Bland Altman plot showed that the differences in the parameters of Hb, HCT, MCV, MCH, MCHC, MPV, PDW and LED between both anticoagulants were within the Limit of Agreement (LOA) range of 95%. Further research was needed to determine a better anticoagulant between K2 EDTA and K3 EDTA.

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