RESEARCH PAPER INTERNATIONAL JOURNAL OF PURE MEDICAL RESEARCH

Physiology

KEYWORDS: EDTA; Cell blood count; platelets; Mean Cell Volume; Hematocrit

EVOLUTION OF THE CELL BLOOD COUNT (CBC) PARAMETERS IN THE CONSTANT K2.EDTA CONCENTRATION WITH DIFFERENT BLOOD VOLUMES: A RANDOMIZED STUDY

Volume-3, Issue-3, March - 2018

Azam Mosleh	Department of Physiology &Pharmacology, School of Medicine, Qom University of Medical Sciences, Qom, Iran
Akram Moslehi	Clinical Science Laboratory, 22 Bahman Polyclinic, Qom Social Security Management, Qom, Iran
Reza Moosavi	Clinical Science Laboratory, 22 Bahman Polyclinic, Qom Social Security Management, Qom, Iran
Tahere Alijani	Clinical Science Laboratory, 22 Bahman Polyclinic, Qom Social Security Management, Qom, Iran
MaryamTabibi	PhD Candidate of Microbiology, Cellular and Molecular Research Center, Qom University of Med Sciences, Qom, Iran. .* Corresponding Author ph.d.tabibi @gmail.com

Article History
Received: 28.12.2017
Accepted: 03.02.2018
Published: 10.03.2018

ABSTRACT:

Background: All of we know that the phlebotomy is a very hard practice in some conditions. So, it's important to get the best results with the lowest blood sample value. K2.EDTA is usually used as a routine anticoagulant to measure cell blood count (CBC) and has been shown that its chelating function can decrease platelets activation. The aim of this study is to determine minimum volume of blood which has no significant effect on CBC indexes.

Methods: 0.5, 1 and 2 ml blood samples of 70 healthy donors were collected in K2.EDTA tubes and then CBC (RBC , WBC , PLT counts, MCV, MCH, MCHC, MPV, HGB and HCT) parameters were measured by Sysmex KX-21N automated cell counter. K+ concentration was measured in plasma and lysed RBCs by Flame photometer.

Results: The statistical analyses of the results revealed that MCV and HCT increased in 0.5ml tubes but others parameters have no significant differences. Also, K+ concentration increased in plasma and lysed RBCs in 0.5ml compared to 1ml tubes.

Conclusion: This study showed that low blood sample amount in K2.EDTA tubes increased HCT and MCV indexes, however it didn't influence on other CBC parameters. It seems that these be the result of high potassium concentration in external environment.

Introduction:

Mostly, blood sampling is an unpleasant and difficult condition for patient and lab staffs, especially in newborns, children, elders and the confined patients in ICU and CCU departments. In these cases, laboratory tests are usually plenty and the blood sample is low and it is necessary that one sample can be distributed in several tubes. So, the blood volume is reduced in every tube.

One of the most common tests in laboratory is cell blood count (CBC). Current anticoagulant in routine CBC tubes is EDTA (Ethylene DiamineTetraacetic Acid) salt in three forms: K2.EDTA, K3.EDTA, and Na2.EDTA. In 2010, the Clinical and Laboratory Standards Institute (CLSI) recommended K2.EDTA and K3.EDTA for venous blood sampling. K2.EDTA is usually used in Middle East and Iran and K3.EDTA is used in USA and European countries [1].

As much, EDTA values should be proportionate to blood volumes. DIN ISO 6710 stated that the concentration range for EDTA is 1.2 to 2mg/ml blood (4.1 - 6.8 mmol/L blood) based on anhydrous EDTA [2,3], most of Manufacturer companies recommend 2 to 3 ml blood for every disposable CBC (Count Blood Cell) tubes that is optimal to measure CBC parameters. This ratio is optimal for measuring CBC parameters after keeping for 4h in room temperature and 24h in refrigerator [4].Previous studies have shown that EDTA decrease platelets and provides pseudothrombocytopenia [5].

So, if EDTA value to blood volume ratio be low, sample will be clot and the outcomes are false definitively. But, if EDTA value to blood volume ratio be high what will happen? Therefore, our aim of this study is to determine minimal volumes of blood samples which have no significant effect on CBC parameters.

Materials and methods:

CBC tubes (Labtron) were plastic, no vacuum and containing dried K2.EDTA anticoagulant. K2.EDTA concentration was 3.5mg. The best quantity of blood volume was 2mL for this EDTA concentration (according to producer company recommendation).

Seventy healthy donors, 20-45 years old were selected randomly. They were studied according to the ethical committee of Qom University of Medical Sciences (QUMS). After phlebotomy, the donor's blood was distributed in volumes of 0.5, 1 and 2mL into CBC tubes.2mL blood volume considered as control group versus 0.5 and 1mL blood volumes in every tube. Then, samples were analyzed by automated hematology analyzer (KX21N sysmex, Japan). The assessments were included 9 parameters in hematology: RBC (Red Blood Cell), WBC(White Blood Cell) and PLT (Platelet) counts as well as MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), MCHC (Mean RBC Hemoglobin Concentration), MPV (Mean Platelet Volume), Hb (Hemoglobin) and HCT (Hematocrit). For Hb measurement, the Non-cyanide hemoglobin method was used. Analysis principle of HCT was the measurement of RBC pulse height ratio (%) of whole blood RBC volume in whole blood. The rest of the parameters were calculated by the following methods:

MCH per RBC(pg) = Hb(g/dl)×10/ RBC×(106 µl)
MCHC(g/dl) = Hb(g/dl)×100/ Hct(%)
MPV(fl) = PCT(Platelet-crit or Platelet volume ratio)(%)×1000/ PLT×(103 µl)
MCV(fl) in whole blood = Hct(%) ×10/ RBC×(106 µl)
Also, plasma K+ concentration and K+ concentration in lysed RBCs were measured by the Flame photometer (IL 943,UK) in the blood

samples.

Statistical Analysis:

Data were expressed as mean ± SD. Statistical analysis was performed by one way analysis of variances (ANOVA) and Tukey's post hoc test using in STAT5 program. P < 0.05 considered to be statistically significant.

Results:

Results analysis showed that WBC, RBC, PLT counts and Hb, MCH, MCHC, MPV values among 1ml and 2ml groups were no significant differences compared with control group (P>0.05) (Table 1).

Hematocrit analysis revealed a significant increase in the 0.5mL blood group compared with the 1ml and the 2ml groups(41.9±0.35vs39.9±0.34 and 39.6±0.34, respectively)(Fig. 1A). MCV value also, markedly increased in the 0.5ml group compared with the 2mL group (89.1±0.66vs87±0.64 and 86.6±0.63 respectively) (P<0.05) (Fig. 1B).

Results of plasma K+ concentration showed that K+ concentration significantly increased in the 0.5mLblood group compared with the 1ml group (50.19±6.72vs 19.8±1.88mmol/L, respectively) (Fig. 2A). Measurement of K+ concentration of lysed RBCs also depicted that K+ concentration markedly increased in the 0.5ml group compared with the1ml group (12±0.03vs9.4±0.08mmol/L. respectively) (Fig. 2B).

Discussion:

This study's results showed that HCT and MCV significantly increased in 0.5mL blood volume compared with 1 and 2 ml blood volume but other parameters didn't changed. Also, potassium concentration of plasma and lysed RBC significantly increased in 0.5mL blood volume compared with 1ml blood volume. The previous studies has stated that the CBC indexes, reticulocyte counts and white blood cell differentials with K3EDTA glass versus K2EDTA plastic tubes on normal blood volume are minimum [6,7]. In the other study, Goossens W et al. compared the effect of different concentrations of both K2 and K3 anticoagulants. They reported that HCT decreased with increasing K3.EDTA concentration but, MCV did not influence up to ten times K3.EDTA normal and MCV increased slightly by high K2.EDTA [8]. It has also been reported that excessive Na2.EDTA concentrations decrease RBC, Hb, HCT, and MCHC significantly while, MCV and RDW values significantly increase. MCH was stable among groups [9]. In a report by Thompson, it has been shown that in the first two hours after blood collection MPV increase then remain stable up to eight hours at room temperature in K2.EDTA and Na2.EDTA while, WBC, RBC and PLT count in both anticoagulants will be invariant [10, 11].

Contrary to these results, our findings showed that HCT and MCV increased in 0.5ml blood volume. It could be the result of high potassium concentration in external environment that leads to decreased efflux of potassium via kcc1 and small conductance potassium (SK) channels from RBCs or increased influx of potassium via leaky potassium channels in RBCs. To explore it, K+ concentration in plasma and lysed RBCs were measured. Results showed that K+ concentration of lysed RBCs and plasma in 0.5ml group was higher than 1ml group. It probably states ions accumulation and water osmosis in RBCs and finally, an increase in erythrocyte volume. Further studies are needed to support this hypothesis.

In conclusion, our findings showed that increased K2.EDTA volume to blood sample ratio can be lead to falsely increased HCT and MCV.

Table1: Analysis of CBC indexes values between different groups. Mean±SD

CBC indexes	0.5mL	1mL	2mL	P value
HGB	13.21±0.15	13.1±0.14	13.04±0.14	0.61
RBC	4.72±0.43	4.71±0.44	4.70±0.44	0.98
WBC	7.21±0.21	7.34±0.21	7.34±0.22	0.90
PLT	246±6.87	242±6.87	242±6.87	0.85
MCH	28.2±0.32	27.9±0.3	27.82±0.3	0.66
MCHC	31.5±0.25	31.85±0.26	31.9±0.28	0.47
MPV	9.7±0.12	9.9±0.12	10±0.12	0.19

Figure legends:

Fig. 1. A: HCT percent in different groups. Mean ± SD. * p < 0.05 compared to 1 ml and 2 ml groups.
B: MCV value in different groups. Mean ± SD. * p < 0.05 compared to 1 ml and 2 ml groups.

Fig. 2. A: Plasma K+ concentration in different groups. Mean ± SD. * p < 0.05 compared to 1 ml group.
B: Lysed RBCs K+ concentration of lysed RBCs in different groups. Mean ± SD. * p < 0.05 compared to 1 ml group.

REFERENCES:

1. Bourner G, Dhaliwal J, Sumner J. Performance evaluation of the latest fully automated hematology analyzers in a large, commercial laboratory setting: a 4-way, side-by-side study. Lab Hematol 2005;11(4):285-97.
2. Xu M, Robbe VA, Jack RM, Rutledge JC. Under-filled blood collection tubes containing K2EDTA as anticoagulant are acceptable for automated complete blood counts, white blood cell differential, and reticulocyte count. Int J Lab Hematol 2010 Oct;32(5):491-7.
3. Sears D, Charachie S, Perlstein M. Electronic blood cell counters: Faulty calibration due to type and amount of anticoagulant in collection tubes. Arch Pathol Lab Med 1985;109:247-9.
4. Lampasso JA. Error in hematocrit value produced by excessive ethylenediaminetetraacetate. Am J ClinPathol 1965;44(1):109-10.
5. White GC, Scarborough DE, Brinkhous KM. Morphological study of early phases of platelet adhesion to foreign surfaces: effect of calcium. Ann NY AcadSci 1983;416:351-62.
6. Van Cott EM, Lewandrowski KB, Patel S, Grzybek DY, Patel HS, Fletcher SR, Kratz A. Comparison of glass K3EDTA versus plastic K2EDTA blood-drawing tubes for complete blood counts, reticulocyte counts, and white blood cell differentials. Lab Hematol2003;9(1):10-4.
7. Lewis SM, Stoddart CT. Effects of anticoagulants and containers (glass and plastic) on the blood count. Lab Pract 1971;10(10):787-92.
8. Goossens W, Van Duppen V, Verwilghen RL. K2- or K3-EDTA: the anticoagulant of choice in routine haematology? Clin Lab Haematol 1991;13(3):291-5.
9. Ratnaningsih T, Sukorini U, Gumilang RA. The effects of excessive disodium ethylene diaminetetraacetic acid (Na2.EDTA) anticoagulant concentration toward hematology profile and morphology of erythrocytes in peripheral blood examination. Medical Journal of Indonesia 2006;15(3):157-64.
10. Thompson CB, Diaz DD, Quinn PG, Lapins M, Kurtz SR, Valeri CR. The role of anticoagulation in the measurement of platelet volumes. Am J ClinPathol 1983;80(3):327-32.
11. Kennedy JB, Machara KT, Baker AM. Cell and platelet stability in disodium and tripotassium EDTA. Am J Med Technol 1981;47:89.