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THE EFFECT OF K3EDTA AND K2EDTA ANTICOAGULANTS ON COMPLETE BLOOD COUNT

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ABSTRACT

Background: Complete blood counts are done to monitor overall health, to screen for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments. In laboratory medicine, pre- analytical quality control is very important, Blood-drawing tubes made from plastic containing K2EDTA as an anticoagulant are gaining widespread use in clinical hematology. Ethylene diamine tetra acetic acid (EDTA), is a poly amino carboxylic acid and a colourless, water-soluble solid. Its conjugate base is named ethylene diamine tetra acetate. It is widely used to dissolve timescale. Its usefulness arises because of its role as a hexadentate ("six-toothed") ligand and chelating agent, i.e. its ability to "sequester" metal ions. **Objective:** This study aimed to assess the effect of both K3EDTA glass tubes and Vacutainer K2EDTA plastic tubes on fresh and after six hours on Complete Blood Count Parameters. **Materials and Methods:** This was a comparative cross-sectional study conducted in Khartoum state, Sudan in 2016. It included 100 samples and 400 tests have done, The CBC was done with plastic tubes Vacutainer K2EDTA on fresh sample and after 6 hours and glass tubes Vacutainer K3EDTA on fresh sample and after 6 hours, Sysmex® Kx-21N hematological analyzer used to estimate CBC. **Results:** There was a significant difference in HCT, MCHC, MXD%, MXD#, RDW_sd, PDW, and P-LCR, with P.values (.001),(.006) (.049), (.019),(.018) and (.033) respectively. **Conclusion:** This study concluded that effect of K3EDTA and K2EDTA on Complete Blood Count Parameters caused significant difference in HCT, MCHC, MXD%, MXD#, RDW_sd, PDW, and P- LCR, (p-value < 0.05).

KEYWORDS: Complete blood count, K3EDTA, K2EDTA.

1. INTRODUCTION

Ethylene diamine tetra acetic acid, widely abbreviated as EDTA, is a polyamino carboxylic acid and a colourless, water-soluble solid. Its conjugate base is named ethylene diamine tetra acetate. It is widely used to dissolve timescale. Its usefulness arises because of its role as a hexadentate ("six- toothed") ligand and chelating agent, i.e. its ability to "sequester" metal ions such as Ca^{2+} and Fe^{3+}. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium EDTA.[1]

In general, hematology testing is performed on EDTA-(lavender top tube) anticoagulated blood. This is the only type of anticoagulant that can be assayed with our hematology analyzer, therefore all hematology tests performed with this analyzer (routine hemograms, red and white cell counts, etc) will only be done from EDTA tubes. [1]

1.1. Synthesis

The compound was first described in 1935 by Ferdinand Munz, who prepared the compound from ethylenediamine and chloroacetic acid. Today, EDTA is mainly synthesised from ethylenediamine (1,2-diaminoethane), formaldehyde, and sodium cyanide. This route yields the sodium salt, which can be converted in a subsequent step into the acid forms.[2]

EDTA (ethylenediaminetetraacetic acid) is the most commonly used anticoagulant in evacuated tubes. It inhibits the clotting process by removing calcium from the blood. This chemical has been used to prevent clotting in blood specimens since the early 1950s and has certain advantages over other anticoagulants.4 EDTA's most distinct characteristic is that it does not distort blood cells, making it ideal for hematology use.

Enough EDTA must be present to prevent coagulation, but excessive amounts cause morphological changes in blood cells.2 When K2EDTA is present in a

concentration of 1.5 to 2.0 mg/ml of blood, it does not have any significant effect on the blood count parameters. All tubes should be inverted several times (8-10) to ensure thorough mixing and, therefore, proper anticoagulation.[3]

1.2. Effect of EDTA on Red Blood Cells (RBCs)

High quality blood smears can be made from the EDTA tube as long as they are made within 2-3 hours of the blood draw.6Smears made from EDTA tubes that sit at room temperature for more than 5 hours often have unacceptable artifact of the blood cells (echinocytic RBCs, spherocytes, and necrobiotic leukocytes).[4] If a tube is not filled to its full volume of draw, the additive to blood specimen ratio is affected, resulting in too high a concentration of EDTA.8 In high concentrations, EDTA causes red cells to shrink because of hypertonicity of the plasma with increased ionic concentration and may create artifacts that make RBC morphology difficult to interpret. An excess of EDTA affects both erythrocytes and leukocytes, causing membrane damage. [5]

1.3. Effect of EDTA on Platelets

EDTA reduces platelet activation by protecting the platelets during contact with the glass tube that may initiate platelet activation. Activation causes platelets to clump in the presence of calcium and platelets adhere to the glass surface at a rapid rate. Chelation of calcium using EDTA results in decreased platelet adhesion or retention to glass.[6]

Pseudothrombocytopenia can complicate an accurate determination of a platelet count in a patient with an underlying thrombocytopenic disorder.[7] Platelet clumping may be a result of poor mixing too little and/or too late, and/or a small, whole blood clot or very small fibrin clots in the EDTA- anticoagulated specimen. Additionally, the improper collection of the blood sample may cause thrombin release and a falsely low platelet count due to platelet aggregation.[8] Clotting can also be the result of insufficient EDTA, usually caused by overfilling the vacuum tube, or poor solubility of EDTA (most commonly disodium EDTA).

It is important to be able to distinguish between reduced platelet counts due to technique related variables or due to a patient's medical condition. There are two patient conditions in which the presence of EDTA causes the platelets to clump.[9]

The first patient condition, platelet satellitism, is observed on a differential smear as a "halo" or ring of platelets surrounding white blood cells (WBCs). If the specimen is collected in an anticoagulant other than EDTA, this ring does not occur. Antibodies in most cases probably cause this *in vitro* phenomenon.

The second patient condition is pseudothrombocytopenia due to EDTA. This occurs in certain patients who have antibodies that can bind to platelets. When EDTA is added to the blood, the antibodies are activated and cause platelet clumping. This phenomenon is sometimes temperature dependent, with more EDTA prevents platelets from clumping on the slide, making it easier to more accurately estimate platelet counts. When a test result shows a low platelet count, a differential smear should be made to help determine if the cause of the low platelet count is due to a patient condition or mixing of blood with EDTA.

1.4. Effects of EDTA on Leukocytes (WBCs)

Studies have demonstrated that the WBC count remained stable for at least 3 days when EDTA anticoagulated blood was stored at room temperature.[10]

Neutrophils and monocytes appeared to be the cells most sensitive to storage in EDTA, whereas lymphocytes were the most stable.[15] With respect to the WBC morphologic characteristics of EDTA-anticoagulated blood on storage at ambient (20-24°C) temperatures, a slight vacuolization of monocytes was found after one hour, progressing to moderate after four hours; a slight vacuolization of neutrophilic granulocytes was found after three to four hours, progressing to moderate after six hours.16 Only minimal changes in the WBC morphologic characteristics have been reported on storage at 4°C for as long as 12 hours.[11]

1.5. EDTA Choices

There are three different forms of EDTA. EDTA is available in disodium (Na2EDTA), dipotassium (K2EDTA) and tripotassium (K3EDTA) salts. K2EDTA and Na2EDTA salts are commonly used in dry form; K3EDTA is normally used in liquid.

K3EDTA is dispensed as a liquid and thus causes a slight dilution of the specimen. This salt also has been shown to affect the red blood cell size more at increased concentrations and on storage than the dipotassium salt. Therefore, K2EDTA is recommended as the anticoagulant of choice in specimen collection for blood cell counting and sizing. K2EDTA is spray-dried on the wall of the tube and will not dilute the sample and is recommended by ICSH (International Council for Standardization in Haematology) and CLSI (Clinical and Laboratory Standards Institute) for hematology testing.[12]

2. Literature Review

A study was done by V. Wiwanitkit in Thailand on 2011. The results were some evidence indicating the EDTA K2 might affect the MCV result. Lower MCV has been observed with EDTA K3 anticoagulation (typically a -0.1 to -1.3% difference is mentioned).Gossans et al noted that "MCV is not influenced by K3EDTA concentrations up to ten times normal, while K2EDTA, at high concentrations, results in a slight increase in MCV, as measured with three of the instruments In addition, it has also been reported that "the difference in MCV between

K2EDTA and K3EDTA was more marked under the condition of lower blood pH Hence, in cases with sepsis or severe infection with a trend towards decreased blood pH, a significant difference in MCV between measurements using EDTA K2 and EDTA K3 might be expected.[13]

Another study was done in Saudi Arabia 2008 by Mamdooh A. Gari. The conclusion of this study defined that the differences between results obtained with K3EDTA glass tubes versus K2EDTA plastic tubes are minimal and unlikely to be of any clinical significance.[14]

MATERIALS AND METHODS

This study is a comparative cross-sectional study, conducted in Khartoum state, Sudan, 2016. It included 100 volunteers and 400 tests have been done, Blood samples were collected from all subjects into K2EDTA and K3EDTA containers for measurement of complete blood count using Sysmex® Kx-21N hematological analyzer. 6 ml of Blood sample was drawn, 3 ml of Blood sample was drawn into plastic tubes Vacutainer K2EDTA and 3 ml into glass tube Vacutainer K3EDTA investigated immediately on fresh, then the rest 3 ml of same samples investigated again after six hours in same plastic tubes Vacutainer K2EDTA and glass tubes Vacutainer K3EDTA.

This study was approved by ethical committee of the faculty of medical laboratory sciences, Alneelin University, and informed consent was obtained from each participant before sample collection.

Haematological assay

CBC which contained the follow parameters; WBCs, RBCs, HGB, HCT, MCV, MCH, MCHC, PLT, Lymp%, MXD%, NEUT%, Lymp#, MXD#, NEUT#, RDWsd, RDWcv, PDW, MPV and P-LCR which have been investigated by using Sysmex® Kx-21N hematological analyzer.

Each CBC parameters were counted using the direct current detection method with coincidence correction. Automatic discriminators separate the cell populations based on complex algorithms. The intensity of the electronic pulse from each analyzed cell is proportional to the cell volume. The hematocrit (HCT) is directly determined based on the red cell count and volume detection of each individual RBC. Even with samples at extremely low or unusually high concentrations.

Data analysis

All statistical analyses were performed by SPSS software version (20). Continuous variables were expressed as mean and standard deviation. In the analytical phase, One way Annova was applied to compare means and make Comparison between groups. Correlations was determined and p-value < 0.05 was considered significant.

RESULTS

In total of 100 cases collected in this study 400 CBC tests were done , Complete Blood Count was estimated for all samples using Sysmex® Kx-21N hematological analyzer. The CBC was done with K2EDTA on fresh sample and after 6 hours and K3EDTA on fresh sample and after 6 hours.

The results found that WBCs (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 5.3 (+/-1.7),5.2 (+/-1.6), 5.2(+/-1.64), and 5.3 (+/-1.7), respectively. No statistically significant difference in WBCs count (r=-0.008, P=0. .919). The red blood cells (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hour were 4.9 (+/-.41), 4.9 (+/-0.4), 5.0 (+/-0.4) and 5.0 (+/-0.4), respectively. The difference between both K2EDTA and K3EDTA on fresh sample and after 6 hours on red blood cell was insignificant (r=.097, P=0.198). Hb (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hour were 14.9 (+/- 1.11), 14.9 (+/- 1.2), 15.1 (+/-1.1) and 15.03 (+/- 1.07), respectively. Also no statistically significant difference appeared on Hb (r=.057 P=0..449). Hematocrit (mean+/-SD) were; 41.9 (+/- 2.53), 42.0 (+/-2.6), 43.86 (+/-2.6), and 43.3 (+/-2.7) respectively. There was a strong statistically significant difference on hematocrit results (r=.247 P=0. .001) there was increase in hematocrit between K2EDTA and K3EDTA. Mean cell volume (mean+/- SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 85.9 (+/- 5.1), 85.7 (+/-5.2), 87.85 (+/-5.3) and 87.1 (+/-5.3), respectively. No statistically significant difference appeared on MCV (r=.127, P=0.094). Mean cell hemoglobin (mean+/-SD) on fresh sample, 24 hours, 48 hours and 72 hours were 30.5 (+/- 2.8), (+/-2.7), 30.43 (+/-3.0) and 30.3 (+/-2.4), respectively, there was no significant difference in mean cell hemoglobin (r= -.033p= .666)). Mean cell hemoglobin concentration (mean+/-SD) on on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 35.5 (+/- 1.9), 35.6 (+/-1.9), 34.4 (+/-1.6) and 34.7 (+/-1.2), respectively, there was significant difference in mean cell hemoglobin concentration (r= -.223, p=.003 P=0.01) showed decrease in concentration with time.

Platelets (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 242.4 (+/- 78.1), 246.02 (+/-73.4), 246.2 (+/-77.04) and 238.6 (+/-76.5) respectively, no significant difference in Platelets showed (r= -.016, p=.829).

Lymphocytes% showed difference in (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 38.2 (+/- 10.6), 37.75 (+/-10.7), 37.8 (+/-10.2) and 37.96(+/- 10.9), respectively. There was no significant difference (r= -.007, p=.928). in addition, MXD% (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 7.9 (+/- 5.1), 6.2 (+/-4.7), 9.6 (+/-5.2) and 9.5 (+/-6.7), respectively, there was strong significant difference (r= .206, p= .006)

showed increase MXD% with time. NEUT# (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 3.1 (+/- 1.4), 3.01 (+/-1.4), 2.9 (+/-1.4) and 2.9 (+/-1.5), respectively, there was no significant difference (r= -.036, p= .637).

RDW_sd (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 44.03 (+/- 3.25), 43.1 (+/-3. 3), 45.7 (+/-3.9) and 45.3(+/-3.4), respectively, there was strongly significant difference (r= .177, p= .019) showed increase RDW_sd with time.

RDW_cv (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were13.6 (+/- .9), 13.6 (+/-0.9), 13.5 (+/-0.96) and 13.7 (+/-0.96), respectively, there was no significant difference (r= .028, p= .709). Platelet distribution width also showed difference in (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 12.2 (+/-1.6), 12.2 (+/-1.6), 2.52 (+/-1.66) and 12.6 (+/-1.7), respectively, there was no significant difference (r= .111, p= .144). Mean platelet volume (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 9.8 (+/-.84), 9.8 (+/-0.83),10.0 (+/-0.8) and 10.1 (+/-0.9), respectively, there was strongly significant difference (r=.178, p= .018) showed increase MPV with time.

Finally P-LCR also showed difference in (mean+/-SD) on both K2EDTA and K3EDTA on fresh sample and after 6 hours were 23.8(+/- 6. 4), 24.04 (+/-6.23), 25.3 (+/-6.2) and 26.4 (+/- 6.6), respectively, there was weak significant difference (r= .161, p= . .033) showed increase P-LCR with time.

Table 1. the mean and SD of CBC parameters and relation between K2EDTA and K3EDTA on fresh and after six hours.

	K2EDTA fresh Mean(+/-SD)	K3EDTA fresh Mean(+/-SD)	K2EDTA after 6 hrs Mean(+/-SD)	K3EDTA after 6 hrs Mean(+/-SD)	Correlation	P-Value
WBCs	5.3 (+/-1.7)	5.2 (+/-1.6)	5.2(+/-1.64)	5.3 (+/-1.7)	-0.008	.919
RBCs	4.9 (+/- .41)	4.9 (+/-0.4)	5.0 (+/-0.4)	5.0 (+/-0.4)	.097	.198
HGB	14.9 (+/- 1.11)	14.9 (+/- 1.2)	15.1 (+/-1.1)	15.03 (+/-1.07)	.057	.449
HCT	41.9 (+/- 2.53)	42.0 (+/-2.6)	43.86 (+/-2.6)	43.3 (+/-2.7)	.247	.001
MCV	85.9 (+/- 5.1)	85.7 (+/-5.2)	87.85 (+/-5.3)	87.1 (+/-5.3)	.127	.094
MCH	30.5 (+/- 2.8)	30.5 (+/-2.7)	30.43 (+/-3.0)	30.3 (+/-2.4)	-.033	.666
MCHC	35.5 (+/- 1.9)	35.6 (+/-1.9)	34.4 (+/-1.6)	34.7 (+/-1.2)	-.223	.003
PLT	242.4 (+/- 78.1)	246.02 (+/-73.4)	246.2 (+/-77.04)	238.6 (+/-76.5)	-.016	.829
Lymp%	38.2 (+/- 10.6)	37.75 (+/-10.7)	37.8 (+/-10.2)	37.96(+/-10.9)	-.007	.928
MXD%	7.9 (+/- 5.1)	6.2 (+/-4.7)	9.6 (+/-5.2)	9.5 (+/-6.7)	.206	.006
NEUT%	56.1 (+/- 11.4)	56.1 (+/-12.46)	52.65(+/-13.4)	52.65 (+/-14.5)	-.081	.285
Lymp#	1.9 (+/- .53)	1.9 (+/-0.55)	1.9(+/-0.53)	1.9 (+/-0.6)	.001	.985
MXD#	.4 (+/-.36)	0.33 (+/-0.3)	0.5(+/-0.3)	0. 5 (+/-0.4)	.148	.049
NEUT#	3.1 (+/- 1.4)	3.01 (+/-1.4)	2.9 (+/-1.4)	2.9 (+/-1.5)	-.036	.637
RDWsd	44.03 (+/- 3.25)	43.1 (+/-3. 3)	45.7 (+/-3.9)	45.3 (+/-3.4)	.177	.019
RDWcv	13.6 (+/- .9)	13.6 (+/-0.9)	13.5 (+/-0.96)	13.7 (+/-0.96)	.028	.709
PDW	12.2 (+/-1.6)	12.2 (+/-1.6)	12.52 (+/-1.66)	12.6 (+/-1.7)	.111	.144
MPV	9.8 (+/- .84)	9.8 (+/-0.83)	10.0 (+/-0.8)	10.1 (+/-0.9)	.178	.018
P-LCR	23.8(+/- 6. 4)	24.04 (+/-6.23)	25.3 (+/-6.2)	26. 4(+/-6.6)	.161	.033

DISCUSSION

In this study it is compared the performance of glass K3EDTA and plastic K2EDTA tubes for complete blood counts on fresh and after six hours, the results found statistically significant differences occurred between these sampling systems.

Firstly, according to our study the findings revealed a significant differences between HCT on both K2EDTA and K3EDTA on fresh sample and after 6 hours, increase HCT level appeared with K2EDTA and K3EDTA after six hours. On the opposite, MCHC showed significant differences revealed decrease in MCHC level with both K2EDTA and K3EDTA after six hours.

Furthermore both MXD% and MXD# showed sharply increase with significant association between both K2EDTA and K3EDTA after six hours and slight increasing by using glass K2EDTA on fresh time. In same results, RDWsd showed significant increase between both K2EDTA and K3EDTA after six hours with slight increasing by using glass K2EDTA on fresh time. MPV results showed significant association between K2EDTA and K3EDTA especially slight increase by using plastic K3EDTA after six hours. Finally, P-LCR results revealed significant association between K2EDTA and K3EDTA P-LCR levels increased after six hours. This results showed similarity with another study was done in Saudi Arabia 2008 by Mamdooh A. Gari. The conclusion of this study defined that the differences between results obtained with K3EDTA glass tubes versus K2EDTA plastic tubes are minimal and unlikely to be of any clinical significance. [14]

On the opposite, MCV values did not showed significant differences between K2EDTA and K3EDTA on fresh time and after six hours, This findings in contrast with A study done by V. Wiwanitkit in Thailand on 2011, which some evidence indicating the EDTA K2 might affect the MCV result. Lower MCV has been observed with EDTA K3 anticoagulation. And in consist with Gossans et, al. which revealed that "MCV is not influenced by K3EDTA concentrations up to ten times normal, while K2EDTA, at high concentrations, results in a slight increase in MCV, as measured with three of the instruments In addition, Gossans et al. reported that "the difference in MCV between K2EDTA and K3EDTA was more marked under the condition of lower blood pH Hence, in cases with sepsis or severe infection with a trend towards decreased blood pH, a significant difference in MCV between measurements using EDTA K2 and EDTA K3 might be expected.[13]

The difference between our results and other results may be due environmental factors, different instruments use or technical difference.

CONCLUSION

This study concluded that there was significant increasing when using K2EDTA and K3EDTA after six hours with HCT, MXD%, MXD#,RDW_sd, PDW, and P-LCR values. And significant decreasing after six hours with MCHC value (p-value < 0.05). it is recommended to perform CBC test using K2EDTA and K3EDTA on fresh samples without delay to avoid disturbance of results.

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