


Original Article

Impact of Common Anticoagulants on Complete Blood Count Parameters Among Humans

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Abstract

Introduction

Among the most frequently used anticoagulants in hematological testing are tetra-acetic acid (EDTA), sodium citrate, and sodium heparin. However, there is a noticeable gap in literature concerning the effects of these anticoagulants on hematological parameters specifically in humans. This study aims to assess the effectiveness of EDTA, sodium citrate, and sodium heparin for conducting complete blood count (CBC).

Methods

This cross-sectional study conducted at Smart Health Tower from January to April 2024 involved 250 participants who underwent CBC using K2EDTA, sodium citrate, and sodium heparin. The acquired data were analyzed using SPSS, with a significance level of $p < 0.05$, employing Intra-class correlation coefficient and one-way ANOVA to assess consistency and agreement among anticoagulants.

Results

A total of 250 participants, with 138(55.2%) males and 112(44.8%) females, underwent CBC testing with di potassium EDTA(K2EDTA), sodium citrate, and sodium heparin. Comparing K2EDTA with sodium heparin showed comparable values in 14 out of 23(60.87%) CBC parameters. Using K2EDTA as the standard, citrate showed perfect or substantial agreement in assessing 8 out of 23 CBC parameters (34.78%). Regarding the comparison of anticoagulants to K2EDTA to determine their agreement levels while sodium heparin was accurate and precise in 13(56.52%) parameters.

Conclusion

Citrate was found to be a less reliable anticoagulant for CBC estimation compared to K2EDTA, potentially leading to inaccurate readings. On the other hand, sodium heparin showed comparable performance to K2EDTA, making it a suitable alternative under specific conditions.

1. Introduction

The Complete Blood Count (CBC) is a widely requested blood test by clinicians, assessing the total quantities and characteristics of cellular constituents within the bloodstream. The CBC parameters include red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). This comprehensive

assessment includes determining the total and differential count of WBCs, also measuring RBC count, hemoglobin (HGB) levels, and hematocrit (HCT), as well as their indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW). Additionally, CBC evaluates platelet (PLT) count indices [1,2].

A CBC serves as an important diagnostic tool for assessing human health, detecting congenital abnormalities, and identifying functional changes due to various pathological factors [3]. Its findings can reveal various conditions such as infections with elevated WBC counts, leukemia with abnormal WBC counts, anemia with low HGB levels, and liver cirrhosis with reduced PLT counts. Recent studies suggest that specific combinations of CBC components, along with derived secondary results, can predict risks of different diseases like cardiovascular disease, cancer, type 2 diabetes, and metabolic syndrome [1,2].

It's widely recognized that collection and sampling of blood, laboratory techniques and storage conditions, and the choice of anticoagulant can substantially impact the outcomes derived from hematological analysis, especially CBC results [4]. Among the various anticoagulants used for both sample collection and routine laboratory analysis, the most commonly utilized ones in hematology are ethylene diamine tetra acetic acid (EDTA), citric acid salts, sodium and lithium oxalates, and heparin [5,6].

The National Committee for Clinical Laboratory Standards has suggested using EDTA for CBC due to its ability to preserve cell structure [7]. However, limited evidence exists on the effects of other anticoagulants on CBC parameters among animal species. Among humans, heparin is typically avoided for blood smears and WBC counts due to staining and clotting issues, respectively. Conversely, EDTA is considered unsuitable for erythrocyte osmotic fragility assessment and may cause cell damage if overused [8].

The majority of studies documented in existing literature have focused on evaluating the impacts of different anticoagulants on CBC results or its specific components across diverse animal species. The current study aims to estimate variations in CBC parameters using different anticoagulants, employing dipotassium EDTA(K2EDTA), sodium citrate, and sodium heparin, among humans to evaluate their effectiveness.

2. Methods

2.1. Study Design, population, and criteria

This cross-sectional laboratory-based study was conducted at Smart Health Tower from January to April 2024. Prior to participation, all individuals were thoroughly briefed about the study and required to provide informed written consent. The study included a total of 250 participants, all of whom underwent complete blood count tests utilizing K2EDTA, sodium citrate, and sodium heparin as anticoagulants. The study population consisted of patients attending Smart Health Tower, representing both genders without any gender bias. Inclusion was restricted to those who had visited the facility, while individuals or their guardians (in case of minors) who declined to provide consent were excluded from the study.

2.2. Determination of the sample size

The effective sample size was determined using G*Power statistic 3.1.9.7, employing linear multiple regression as the statistical test with a two-tailed approach. With an effective sample size of 0.35, an α error probability of 0.01, and a statistical power of 0.99, along with a predictor value of 1, the minimum required sample size was 158. Therefore, a sample size of 250 was utilized for the comparison in CBC parameters between these three different anticoagulants.

2.3. Sample collection and statistical analysis

Trained health workers collected blood samples from participants using sterile syringes and needles, drawing 5 mL from either the median cubital or prominent forearm vein. The samples were distributed as follows: 1.8 mL into sodium citrate tubes and 1.6 mL into K2EDTA and sodium heparin tubes. After gentle mixing, complete blood counts (CBC) were analyzed with the Medonic M51 automated hematology analyzer within 3 to 6 hours post-collection. Tube characteristics are detailed in Table 1. Various hematological parameters were assessed, including WBC, percentages of neutrophils, lymphocytes, monocytes, eosinophils, basophils, as well as RBC, HCT, HGB, MCV, MCHC, RDW-SD, PLT, MPV, PDW, PCT, and PLCR. Participant demographics, such as age and gender, were also recorded. Data were initially processed in Microsoft Excel 2019 for accuracy and completeness before being transferred to SPSS version 25.0 and MedCalc version 20 for statistical analysis. Intra-class correlation coefficient (ICC) analysis was conducted to evaluate consistency among the three anticoagulants, with interpretations as follows: <0.50 for poor consistency, 0.50-0.75

Table 1. Properties of Laboratory Test Tubes Utilized for CBC Evaluation.

Tube details	EDTA	Heparin	Citrate
Type of tube	K2EDTA	Sodium (vacuum blood collection tube)	PT Tube (Sodium citrate)
Dimension	13 x 75 mm	13 x 75 mm	13 x 75 mm
Storage	5- 25°C	5-25°C	5-25°C
Expiration date	31-3-2025	24-11-2027	19-12-2025
Tube capacity (volume)	5 ml	5 ml	5ml
Required volume	1.5-2 ml	1.5-2ml	1.8ml
Tube material	Plastic	glass	glass
Manufacturer	Vacutest kima sri	MR+	MR+
Origin/country	Italy	China	China
Anticoagulant concentration	5.4 mg	18iu	3.2%

for moderate, 0.75-0.90 for good, and >0.90 for excellent consistency. A p-value of <0.05 was considered significant. One-way ANOVA assessed variations in CBC parameters among samples collected in K2EDTA, sodium citrate, and sodium heparin tubes. Additionally, the concordance correlation coefficient (CCC) was used to evaluate agreement, with K2EDTA as the standard, and interpreted as follows: ≥ 0.99 for almost perfect agreement, 0.95-0.99 for significant agreement, 0.90-0.95 for moderate agreement, and <0.90 for poor agreement [9].

3. Results

Among the 250 participants involved, 138 (55.2%) were male, and 112 (44.8%) were female. The participants had an average age of 41.20 ± 16.51 years (5-91). Consistency in CBC results using sodium heparin, K2EDTA, and sodium citrate indicated excellent consistency in the determination of WBC, %Neu, %Lymph, Neu, Lymph, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, MPV, PDW, and PLCR among these anticoagulants with ICC >0.90 (Table.2).

Regarding variation in CBC parameters using K2EDTA, sodium citrate, and sodium heparin, no statistically significant variation was found in the median %Lymph, Eos, MCV, and MCH among these three different anticoagulants (Table 3).

Regarding variation in estimation of CBC parameters using the results of two anticoagulated blood such as K2EDTA-sodium citrate, K2EDTA-sodium heparin, sodium citrate-sodium heparin, the results of the comparison of K2EDTA-sodium citrate indicated comparable results in median %Neu, %Lymph, %Eos, Neu, Bas, MCV, MCH, and MCHC with a p-value of ≥ 0.05 . Comparison of K2EDTA-sodium heparin results indicated comparable results in median WBC, %Lymph, %Eos, Neu, Lymph, RBC, HGB, HCT, MCV, MCH, RDW-SD, MPV, PDW, and PLCR with a p-value of ≥ 0.05 . In comparing the results of CBC between sodium citrate-sodium heparin, the result indicated a nonsignificant difference in median WBC, %Lymph, %Eos, Lymph, MCV, MCH, RDW-SD, and PCT (Table 4).

The agreement levels between different anticoagulants, using K2EDTA as the standard, were evaluated. Sodium citrate showed perfect agreement in assessing MCV and MCH (CCC = 0.990) but displayed significant agreement in determining WBC, %Neu, %Lymph, Neu, Lymph, and Eos (CCC between 0.95 and 0.99). Moderate agreement was observed in assessing MCHC (CCC = 0.929), while poor agreement was found in all other parameters with CCC <0.90 . Similarly, sodium heparin demonstrated perfect agreement in determining MCV (CCC=0.994) and MCH (CCC=0.990), with substantial agreement in other parameters such as WBC, %Lymph, Neu, Lymph, RBC, and HGB (CCC between 0.95 and 0.99), but poor agreement in parameters with CCC <0.90 . Regarding the comparison of K2EDTA and sodium citrate, citrate was highly precise and accurate in the estimation of WBC, %Neu, %Lymph, Neu, Lymph, Eos, MCV, MCH, and MCHC. While comparing sodium heparin to K2EDTA, it was highly precise in

the estimation of WBC, %Neu, %Lymph, Neu, Lymph, Eos, RBC, HGB, HCT, MCV, MCH, MCHC, and PLCR (Table.5).

4. Discussion

The choice of anticoagulants and storage time significantly affect blood sample analysis [10]. In a study by Akorsu et al. involving 55 healthy individuals, consistency in blood parameters across three anticoagulants was observed: K3EDTA, sodium citrate, and lithium heparin [3]. Similarly, a current study utilized K2EDTA, sodium citrate, and sodium heparin,

Table 2. CBC results consistency using K2EDTA, Sodium heparin, Sodium citrate.

CBC parameters	Intra class correlation coefficient	Confidence interval 95%	
		Lower	Upper
WBC	0.991	0.972	0.996
%Neu	0.961	0.880	0.981
%Lymph	0.987	0.984	0.990
%Mon	0.494	0.038	0.717
%Eos	0.869	0.838	0.895
%Bas	0.733	0.636	0.801
Neu	0.988	0.961	0.994
Lymph	0.987	0.973	0.993
Mon	0.612	0.120	0.802
Eos	0.182	0.038	0.367
Bas	0.803	0.719	0.858
RBC	0.922	0.328	0.976
HGB	0.923	0.321	0.976
HCT	0.902	0.449	0.964
MCV	0.998	0.994	0.999
MCH	0.996	0.992	0.998
MCHC	0.963	0.921	0.979
RDW-SD	0.924	0.901	0.941
PLT	0.536	0.276	0.689
MPV	0.915	0.843	0.948
PDW	0.921	0.880	0.945
PCT	0.563	0.104	0.763
PLCR	0.930	0.856	0.960

Table 3. Variations among CBC parameters using different anticoagulants.

CBC parameters	Sodium Heparin Median (Min-Max)	Citrate Median (Min-Max)	K2EDTA Median (Min-Max)	P-value
WBC	7.54(2.52-26.26)	7.21(2.26-23.85)	7.51(2.54-26.10)	0.046
%Neu	61.15(37.2-92.6)	56.70(37.60-90.90)	56.30(30.70-91.80)	<0.001
%Lymph	33.2(3.8-50.30)	33.55(4-50.50)	33.45(3.90-52.60)	0.718
%Mon	1.9(0.0-12.20)	6(0.20-13)	6.45(0.80-14.10)	<0.001
%Eos	2.5(0.10-22.50)	2.50(0.20-24.30)	6.45(0.80-14.10)	<0.001
%Bas	0.65(0.20-3.10)	0.50(0.10-2.20)	0.50(0.10-1.50)	<0.001
Neu	4.92(0.94-24.31)	4.40(0.99-21.68)	4.65(0.78-23.96)	0.015
Lymph	2.41(0.39-5.76)	2.31(0.37-5.40)	2.49(0.45-5.99)	0.049
Mon	0.15(0.00-0.93)	0.43(0.01-1.06)	0.49(0.05-1.17)	<0.001
Eos	0.20(0.01-2.08)	0.18(0.01-2.19)	0.16(0.01-2.55)	0.730
Bas	0.05(0.01-0.23)	0.04(0.01-0.17)	0.04(0.01-0.13)	<0.001
RBC	5.12(2.47-7.65)	4.62(2.21-6.35)	5.13(2.48-7.05)	<0.001
HGB	14.2(6.90-21.30)	12.6(6.20-16.9)	14.10(6.90-18.20)	<0.001
HCT	43.15(21.4-64.8)	38.90(19-51.20)	43.45(3.72-54.60)	<0.001
MCV	85.45(56.90-108.5)	85.15(56.8-108.4)	85.9(57.3-108.6)	0.534
MCH	28.4(18.10-35.70)	28.10(18-37.5)	28.25(18.10-36.40)	0.425
MCHC	33(30.50-37.60)	32.70(30.4-39.10)	32.60(30-38.40)	<0.001
RDW-SD	43.5(34.70-63.10)	43.40(34.60-64.00)	44.10(35.30-82.20)	0.016
PLT	159(32-424)	176(21-1584)	250(86-482)	<0.001
MPV	9.30(6.90-12.10)	8.80(4.40-11.80)	9.10(7.10-13.00)	<0.001
PDW	11.85(7.30-21.10)	11.10(2.60-20.00)	11.60(8.10-23.60)	<0.001
PCT	0.15(0.03-0.34)	0.15(0.02-0.70)	0.22(0.09-0.37)	<0.001
PLCR	31.75(15.30-51.10)	28.15(5.20-48.80)	30.40(15.40-57.90)	<0.001

finding excellent consistency in various blood parameters, with ICC values exceeding 0.90.

Regarding variation in CBC parameters using different anticoagulants, in a study which is conducted on 30 clinically healthy dogs from different breeds, no significant variation between sodium citrate and K3EDTA was found in 4 out of 8 CBC parameters (50%) including HGB, HCT, PLT, and PCT [11]. Similarly, in a study conducted on humans, in which variation in the estimation of CBC parameters was evaluated using three different anticoagulants, namely, K3EDTA, sodium citrate, and lithium heparin, no statistically significant difference was observed in 5 out of 14 CBC parameters (35.7%) including MCV, MCH, MCHC, %Lymph, and %Neu among the three anticoagulants examined [3]. In the present study, regarding variation in CBC parameters using K2EDTA, sodium citrate, and sodium heparin, no statistically significant variation was found in 4 out of 23 CBC parameters (17.40%) including

%Lymph, Eos, MCV, and MCH among these three different anticoagulants. The significant variations observed in other CBC parameters underscore the need for careful consideration when selecting anticoagulants, particularly in clinical settings where precise and consistent CBC measurements are crucial for accurate diagnosis and monitoring of conditions [12].

In a study of 50 healthy dogs comparing EDTA and sodium citrate, no comparable results were found among 9 CBC parameters, suggesting citrate may lead to inaccurate results compared to EDTA [13]. Another study of 55 healthy individuals comparing heparin and citrate revealed significant differences in 5 out of 14 CBC parameters (35.71%), with the remaining parameters showing variations. Similar patterns were observed when comparing citrate to EDTA. Comparing heparin to K3EDTA showed significant variations in three parameters (21.43%) [3]. In the current study, comparing K2EDTA to sodium citrate showed similar results in 8 out of 23 CBC

Table 4. Variation in estimation of CBC using two anticoagulants' results

CBC parameters	Sodium Citrate Median (Min-Max)	Sodium Heparin Median (Min-Max)	P-value	K2EDTA Median (Min-Max)	Sodium Heparin Median (Min-Max)	P-value	K2EDTA Median (Min-Max)	Sodium Citrate Median (Min-Max)	P-value
WBC	7.21(2.26-23.85)	7.54(2.52-26.26)	0.121	7.51(2.54-26.10)	7.54(2.52-26.26)	0.941	7.51(2.54-26.10)	7.21(2.26-23.85)	0.05
%Neu	56.70(37.60-90.90)	61.15(37.2-92.6)	<0.001	56.30(30.70-91.80)	61.15(37.2-92.6)	<0.001	56.30(30.70-91.80)	56.70(37.60-90.90)	0.788
%Lymph	33.55(4-50.50)	33.2(3.8-50.30)	0.922	33.45(3.90-52.60)	33.2(3.8-50.30)	0.695	33.45(3.90-52.60)	33.55(4-50.50)	0.903
%Mon	6(0.20-13)	1.9(0.0-12.20)	<0.001	6.45(0.80-14.10)	1.9(0.0-12.20)	<0.001	6.45(0.80-14.10)	6(0.20-13)	0.003
%Eos	2.50(0.20-24.30)	2.5(0.10-22.50)	0.801	6.45(0.80-14.10)	2.5(0.10-22.50)	0.994	6.45(0.80-14.10)	2.50(0.20-24.30)	0.740
%Bas	0.50(0.10-2.20)	0.65(0.20-3.10)	<0.001	0.50(0.10-1.50)	0.65(0.20-3.10)	<0.001	0.50(0.10-1.50)	0.50(0.10-2.20)	0.07
Neu	4.92(0.94-24.31)	4.92(0.94-24.31)	0.011	4.65(0.78-23.96)	4.92(0.94-24.31)	0.288	4.65(0.78-23.96)	4.92(0.94-24.31)	0.345
Lymph	2.41(0.39-5.76)	2.41(0.39-5.76)	0.282	2.49(0.45-5.99)	2.41(0.39-5.76)	0.633	2.49(0.45-5.99)	2.41(0.39-5.76)	0.040
Mon	0.15(0.00-0.93)	0.15(0.00-0.93)	<0.001	0.49(0.05-1.17)	0.15(0.00-0.93)	<0.001	0.49(0.05-1.17)	0.15(0.00-0.93)	<0.001
Eos	0.20(0.01-2.08)	0.20(0.01-2.08)	<0.001	0.16(0.01-2.55)	0.20(0.01-2.08)	<0.001	0.16(0.01-2.55)	0.20(0.01-2.08)	<0.001
Bas	0.05(0.01-0.23)	0.05(0.01-0.23)	<0.001	0.04(0.01-0.13)	0.05(0.01-0.23)	<0.001	0.04(0.01-0.13)	0.05(0.01-0.23)	0.547
RBC	5.12(2.47-7.65)	5.12(2.47-7.65)	<0.001	5.13(2.48-7.05)	5.12(2.47-7.65)	0.993	5.13(2.48-7.05)	5.12(2.47-7.65)	<0.001
HGB	14.2(6.90-21.30)	14.2(6.90-21.30)	<0.001	14.10(6.90-18.20)	14.2(6.90-21.30)	0.816	14.10(6.90-18.20)	14.2(6.90-21.30)	<0.001
HCT	43.15(21.4-64.8)	43.15(21.4-64.8)	<0.001	43.45(3.72-54.60)	43.15(21.4-64.8)	0.999	43.45(3.72-54.60)	43.15(21.4-64.8)	<0.001
MCV	85.45(56.90-108.5)	85.45(56.90-108.5)	0.874	85.9(57.3-108.6)	85.45(56.90-108.5)	0.808	85.9(57.3-108.6)	85.45(56.90-108.5)	0.503
MCH	28.4(18.10-35.70)	28.4(18.10-35.70)	0.391	28.25(18.10-36.40)	28.4(18.10-35.70)	0.773	28.25(18.10-36.40)	28.4(18.10-35.70)	0.807
MCHC	33(30.50-37.60)	33(30.50-37.60)	0.009	32.60(30-38.40)	33(30.50-37.60)	<0.001	32.60(30-38.40)	33(30.50-37.60)	0.502
RDW-SD	43.5(34.70-63.10)	43.5(34.70-63.10)	0.709	44.10(35.30-82.20)	43.5(34.70-63.10)	0.110	44.10(35.30-82.20)	43.5(34.70-63.10)	0.014
PLT	159(32-424)	159(32-424)	0.001	250(86-482)	159(32-424)	<0.001	250(86-482)	159(32-424)	<0.001
MPV	9.30(6.90-12.10)	9.30(6.90-12.10)	<0.001	9.10(7.10-13.00)	9.30(6.90-12.10)	0.211	9.10(7.10-13.00)	9.30(6.90-12.10)	<0.001
PDW	11.85(7.30-21.10)	11.85(7.30-21.10)	<0.001	11.60(8.10-23.60)	11.85(7.30-21.10)	0.207	11.60(8.10-23.60)	11.85(7.30-21.10)	0.019
PCT	0.15(0.03-0.34)	0.15(0.03-0.34)	0.022	0.22(0.09-0.37)	0.15(0.03-0.34)	<0.001	0.22(0.09-0.37)	0.15(0.03-0.34)	<0.001
PLCR	31.75(15.30-51.10)	31.75(15.30-51.10)	<0.001	30.40(15.40-57.90)	31.75(15.30-51.10)	0.143	30.40(15.40-57.90)	31.75(15.30-51.10)	0.001

Table 5. Concordance correlation coefficient (CCC) for the estimation of the level of agreement between K2EDTA with sodium citrate and sodium heparin.

CBC parameters	K2EDTA-Citrate	Pearson ρ (precision)	Accuracy	K2EDTA-Sodium heparin	Pearson ρ (precision)	Accuracy
WBC	0.97(0.9571 -0.9718)	0.988	0.977	0.985(0.981- 0.989)	0.986	0.999
%Neu	0.972(0.964-0.978)	0.974	0.998	0.847(0.814-0.875)	0.925	0.916
%Lymph	0.984(0.980- 0.988)	0.985	0.999	0.951(0.937- 0.961)	0.954	0.997
%Mon	0.663(0.593 - 0.723)	0.705	0.941	0.157(0.111 -0.201)	0.440	0.355
%Eos	0.636(0.567 - 0.697)	0.688	0.926	0.594(0.525 - 0.656)	0.675	0.881
%Bas	0.460(0.361 -0.548)	0.480	0.958	0.305(0.209 - 0.396)	0.371	0.822
Neu	0.980(0.975 - 0.984)	0.990	0.990	0.972(0.964 - 0.978)	0.980	0.991
Lymph	0.956(0.949 - 0.966)	0.984	0.974	0.969(0.961 - 0.976)	0.973	0.997
Mon	0.733(0.675 - 0.782)	0.795	0.922	0.209(0.157 - 0.261)	0.500	0.419
Eos	0.968(0.960 - 0.975)	0.973	0.995	0.927(0.909 - 0.941)	0.942	0.983
Bas	0.565(0.477 - 0.642)	0.576	0.990	0.458(0.371 -0.537)	0.543	0.848
RBC	0.723(0.691 - 0.764)	0.983	0.742	0.973(0.966-0.979)	0.974	0.999
HGB	0.742(0.705 - 0.775)	0.988	0.752	0.977(0.971 - 0.982)	0.979	0.998
HCT	0.670(0.620 - 0.714)	0.911	0.735	0.907(0.882 - 0.926)	0.909	0.998
MCV	0.990(0.987 - 0.992)	0.995	0.995	0.994(0.992 - 0.995)	0.995	0.998
MCH	0.990(0.987 - 0.992)	0.991	0.998	0.990(0.987 - 0.992)	0.992	0.998
MCHC	0.929(0.910 - 0.944)	0.933	0.995	0.874(0.844 - 0.898)	0.932	0.937
RDW-SD	0.721(0.661 - 0.772)	0.762	0.946	0.738(0.681- 0.786)	0.771	0.958
PLT	0.313(0.236 - 0.386)	0.470	0.668	0.290(0.235 - 0.343)	0.648	0.448
MPV	0.784(0.734 - 0.826)	0.830	0.945	0.873(0.841 - 0.899)	0.887	0.985
PDW	0.790(0.740 - 0.832)	0.815	0.970	0.819(0.774 - 0.856)	0.832	0.983
PCT	0.319(0.256 - 0.380)	0.598	0.534	0.238(0.187- 0.289)	0.585	0.408
PLCR	0.833(0.794- 0.866)	0.879	0.948	0.876(0.847- 0.901)	0.901	0.973

parameters (34.78%), while comparing K2EDTA to sodium heparin showed comparable values in 14 out of 23 CBC parameters (60.87%).

Comparing PLT results between K2EDTA and sodium citrate with sodium heparin, significantly lower PLT counts were found in the latter two in the current study, contradicting findings in existing genuine literature [14-16]. One study suggested that citrate's strong platelet activation in sick animals may lead to decreased PLT counts due to platelet clumping [17]. Additionally, lower HGB and HCT values were observed in citrated blood samples compared to EDTA, consistent with previous studies [3,11]. This discrepancy may be attributed to citrate's interference with HGB oxidation, resulting in higher HGB levels in EDTA samples.

The CBC is commonly conducted on venous blood specimens anticoagulated with EDTA. Among various EDTA subtypes, the dipotassium salt form, K2EDTA, is endorsed by the International Council for Standardization in Hematology as the preferred anticoagulant for blood cell enumeration and sizing [7]. The study evaluated agreement levels between different anticoagulants, using K2EDTA as the standard. Sodium citrate showed substantial agreement in 8 out of 23 CBC parameters (34.78%), including MCV, MCH, WBC, %Neu, %Lymph, Neu, Lymph, and Eos. Similarly, sodium heparin demonstrated substantial agreement in determining MCV, MCH, WBC, %Lymph, Neu, Lymph, RBC, and HGB. These findings align with previous literature, which indicated substantial agreement with heparin in assessing 4 out of 14 CBC parameters (28.57%), including RBC, HGB, HCT, and MCH [3].

5. Conclusion

Citrate was found to be a less reliable anticoagulant for CBC estimation compared to K2EDTA, potentially leading to inaccurate readings. On the other hand, sodium heparin showed comparable performance to K2EDTA, making it a suitable alternative under specific conditions.

Declarations

Conflicts of interest: The authors have no conflicts of interest to disclose.

Ethical approval: The study was approved by the Institutional Ethics Committee and utilized data obtained from hospital archives. Ethical Approval for this study was obtained from the Ksciens ethical committee (Approval Number 43. 2025).

Patient consent (participation and publication): Written informed consent was obtained from all patients (or their legal guardians, where applicable) for participation in the study and for the publication of all associated clinical information and images.

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Authors' contributions: RQS and SQO were major contributors to the conception of the study, as well as to the literature search for related studies. DAH and AMM were involved in the literature review and the writing of the manuscript. SLE, HAY, HSA and MTT were involved in the literature review, the design of the study, the critical revision of the manuscript, and the processing of the tables. QOS and AMM confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Use of AI: AI was not used in the drafting of the manuscript, the production of graphical elements, or the collection and analysis of data.

Data availability statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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