

COMPARISON OF COMPLETE BLOOD COUNT PARAMETERS USING ETHYLENE DIAMINE TETRAACETIC ACID (EDTA) ANTICOAGULANT WITH LITHIUM HEPARIN AND SODIUM CITRATE ANTICOAGULANTS AMONG HEALTHY VOLUNTEERS: A LABORATORY-BASED CROSS-SECTIONAL ANALYTICAL STUDY

Nazia Hussain^{1*}, Sai Sridevi Krishna², G. Shobana Megala³, Vinoth Thanikachalam⁴

¹Assistant Professor in the Department of Pathology, APS Medical College Hospital and Research Institute, Mevalurkuppam, Sriperumbudur 602105, Tamil Nadu, India

²Associate Professor in the Department of Pathology, Government Medical College, Ariyalur 621713, Tamil Nadu, India

³Assistant Professor in the Department of Pathology, Chengalpattu Medical College, Chengalpattu 603001, Tamil Nadu, India

⁴Assistant Professor in the Department of Community Medicine, Tagore Medical College and Hospital, Chennai-600127, Tamil Nadu, India
Emails: ajaravindevi@gmail.com² gandhi.vaishnavi93@gmail.com³ Vinoththanikachalam09@gmail.com⁴

*Correspondence: najjuhussain@gmail.com¹

ABSTRACT

Introduction: The Complete Blood Count (CBC) test is the most commonly requested laboratory test that has significantly contributed to the diagnosis and treatment of diseases in the fields of hematology and general medicine. However, though K₂EDTA is the anticoagulant suggested for hematological examination of blood, some other anticoagulants such as Lithium Heparin and Sodium Citrate have been used as an alternative to K₂EDTA when it cannot be used. In this research, there will be a comparison of parameters of CBC in K₂EDTA, Lithium Heparin and Sodium Citrate anticoagulated blood samples in order to assess their similarity and compatibility.

Methodology: An analytical laboratory study was performed involving 110 healthy participants from February 2024 to March 2025. Blood samples from the participants were collected in K₂EDTA, Lithium Heparin, and 3.2% Sodium Citrate vacutainers and then tested with the Sysmex XP-300 automated hematology analyzer. The parameters tested included WBC count, RBC count, Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), MCH, MCHC, and platelet count. Data analysis was performed using SPSS version 25.0 and R software. Comparisons were made using one-way ANOVA, while ICC and CCC were used to assess reliability and agreement.

Results: Excellent reliability was reported for most CBC parameters, with ICC values ranging from 0.916 to 0.977, and Platelet count showed good reliability (ICC=0.806). A significant difference was noted between the three anticoagulants in relation to WBC, RBC, Hb, HCT, and platelet count ($p < 0.05$), whereas MCV, MCH, and MCHC showed no significant difference. Sodium citrate had a better correlation with K₂EDTA than lithium heparin in most of the hematological parameters, especially WBC, RBC, Hb, HCT, and platelet count. Red cell indices had excellent agreement in all the anticoagulants.

Conclusion: K₂EDTA is still the preferred choice for CBC. However, sodium citrate is more consistent with K₂EDTA than lithium heparin and can be an alternative anticoagulant in circumstances when EDTA is not available. Lithium heparin should be used with caution due to increased variability in certain hematological parameters.

KEYWORDS: Complete Blood Count, K₂EDTA, Lithium Heparin, Sodium Citrate

1. INTRODUCTION

A Complete Blood Count (CBC) is among the most frequently requested investigations performed in laboratories. It has a great importance in diagnosis, evaluation, and management of numerous hematologic and systemic diseases such as anemia, infections, thrombocytopenia, leukemia, and inflammatory processes[1,2]. Some of the parameters that are measured during CBC are “WBC (white blood cells) count, RBC (red blood cells) count, Hb (hemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), platelet count, differential leukocyte count, RDW (red cell distribution width)”[1,3].

Modern automated hematology analyzers deliver fast, accurate and reproducible results through the use of various techniques, including electrical impedance, optical light scatter, flow cytometry, fluorescence, and volume-conductivity-scatter analysis[2,4]. The accuracy of CBC results is dependent not only on the performance of the analyzer but also on the pre-analytic factors, such as the type of anticoagulant[5,6].

Among the various anticoagulants available, Ethylene Diamine Tetraacetic Acid (EDTA) is considered the gold standard anticoagulant for haematological investigations because of its excellent preservation of cellular morphology and prevention of clot formation through irreversible chelation of calcium ions[2,3]. As a result, the use of K₂EDTA or K₃EDTA is highly recommended by the International Council for Standardization in Hematology (ICSH) and the Clinical and Laboratory Standards Institute (CLSI) as the anticoagulant for the complete blood count test[2,4]. Yet, EDTA has certain drawbacks too. Too much EDTA, storage for a long period of time, or delayed analysis could lead to cells shrinking, leukocyte degeneration, platelets swelling, and pseudothrombocytopenia due to EDTA dependency[3,5].

Lithium heparin prevents coagulation by potentiating the activity of antithrombin III and is routinely used for biochemical investigations. Although it preserves several hematological parameters, it may cause leukocyte and platelet clumping, making it less suitable for routine CBC analysis[6,7]. Sodium citrate acts by reversible calcium chelation and is the preferred anticoagulant for coagulation studies. It has also been proposed as an alternative anticoagulant in patients with EDTA-induced pseudothrombocytopenia; however, its liquid formulation introduces a dilution factor that may influence CBC parameters[6].

Several studies have compared CBC parameters obtained using EDTA, lithium heparin, and sodium citrate. While most investigators continue to recommend EDTA as the anticoagulant of choice, conflicting evidence exists regarding the suitability of lithium heparin and sodium citrate as alternative anticoagulants[6,7]. Therefore, the present study was carried out to evaluate the parameters of complete blood count in EDTA, lithium heparin, and sodium citrate anticoagulants among healthy individuals. The purpose of this study was to evaluate if the parameters, such as “RBC count, WBC count, hematocrit, MCV, MCH, MCHC, and platelet counts, differ among the three anticoagulants and whether lithium heparin and sodium citrate can substitute for EDTA for complete blood counts”.

2. METHODS

2.1 Study Design, Subjects, and Inclusion/Exclusion Criteria

The study was a cross-sectional laboratory study that was carried out in the Department of Pathology from February 2024 to March 2025. All the selected individuals were given information regarding the objective and methods of the study and obtained written consent. A total number of 110 healthy volunteers were enrolled in the study through universal sampling. The study included individuals of either gender who had no history of hypertension, diabetes mellitus, epilepsy, tuberculosis, HIV infection, hepatitis B, hepatitis C, or hematological disorder.

2.2. Determination of Sample Size

Calculation of the sample size was carried out using the G Power statistical software version 3.1.9.7. It was estimated using a repeated measures ANOVA test for hematological parameters with respect to three different anticoagulants. Considering an effect size of 0.35, alpha at 0.05, and 95% statistical power, the minimum sample size would be 102 individuals. In order to improve the reliability of results, 110 healthy individuals were used in the experiment.

2.3. Sample Collection and Statistical Analysis

Blood samples from each individual were taken carefully under aseptic conditions by the laboratory staff in sterile disposable syringes and needles. “Venous blood of about 8 mL was extracted from the median cubital vein or any other clearly visible peripheral vein and placed in three separate tubes with anticoagulants as follows: 2 mL in a K₂EDTA tube, 2 mL in a 3.2% sodium citrate tube, and 4 mL in a lithium heparin tube”. After a few inversions of these tubes to ensure proper mixing of the samples with anticoagulants, all blood samples were tested in the Sysmex XP-300 hematology analyzer machine following standard operating procedure.

Parameters that were determined using the CBC test include “WBC count, RBC count, Hb, HCT, MCV, MCH, MCHC, and platelet count”. These hematological parameters were determined using the blood samples collected in the K₂EDTA, sodium citrate, and lithium heparin tubes of each participant to determine the effect of different anticoagulants on the CBC parameters.

Data entry in MS Excel was done for cleaning, verification, and confirmation of completeness before being exported into Statistical Package for the Social Sciences (SPSS) version 25.0 and R Software to do the statistical analysis. Descriptive statistics including mean and standard deviation for the continuous variable, while frequency and percentage for the categorical variable was used. A one-way ANOVA test was done to compare the mean of hematological parameters for all three anticoagulants. Agreement of the three anticoagulants was analyzed through the Intraclass Correlation Coefficient (ICC). “Intra-class correlation coefficient (ICC) was analyzed to determine the consistency of the three anticoagulants whose interpretation was as follows: <0.50 = poor consistency, 0.50 – 0.75 = moderate, 0.75 – 0.90 = good, >0.90 = excellent consistency” and “Lin’s Concordance Correlation Coefficient (CCC) done by R software was done with K₂EDTA as reference standard whose interpretation was as follows: ≥0.99 = almost perfect agreement, 0.95 – 0.99 = significant agreement, 0.90 – 0.95 = moderate agreement and <0.90 = poor agreement”. The statistical significance was taken as p value < 0.05.

3.RESULTS

The total number of 110 healthy subjects was chosen for the cross-sectional and analytical study performed in the laboratory. The blood samples were collected from all subjects by using K2 EDTA, lithium heparin, and sodium citrate anticoagulant tubes and the CBC parameters were analyzed by using Sysmex XP-300 hematology analyzer. The following table depicts the characteristics of the anticoagulant tubes that were used in this study. The three anticoagulant tubes had the same volume and the same size but the specifications were different.

For the reliability determined by the ICC, it was excellent for all hematological parameters except the platelet count (Table 2). The greatest agreement was noted for MCV (ICC = 0.977; 95% CI: 0.968 – 0.983), followed by MCH (ICC = 0.973; 95% CI: 0.963 – 0.981), Hb (ICC = 0.941; 95% CI: 0.919 – 0.958), and WBC (ICC = 0.933; 95% CI: 0.908 – 0.952). The least agreement was observed for platelet count (ICC = 0.806; 95% CI: 0.734 – 0.861). Nevertheless, it is still within the threshold of reliability. Statistical significance was found in different values of WBC count, RBC count, Hb, hematocrit, and platelet count ($p > 0.05$) between the three types of anticoagulants. On the other hand, MCV ($p = 0.572$), MCH ($p = 0.636$), and MCHC ($p = 0.388$) did not show significant differences.

Analysis of the results (Table 4) revealed statistically significant differences between EDTA and lithium heparin regarding the following parameters: WBC, RBC, Hb, HCT, and platelets ($p > 0.05$). On the other hand, no significant differences were established for MCV, MCH, and MCHC. Analysis of the results for EDTA and sodium citrate (Table 5) showed that only one parameter, which is WBC count, demonstrated a statistically significant difference. In the case of RBC, Hb, HCT, MCV, MCH, MCHC, and platelets ($p > 0.05$), there were no significant differences between these anticoagulants.

The comparison of lithium heparin and sodium citrate (Table 6) was characterized by statistically significant differences in WBC, RBC, Hb, HCT, and platelet count ($p > 0.05$). Agreement analysis (CCC) between EDTA and the other two anticoagulants was done and is presented in Table 7. Sodium citrate showed significantly higher concordance compared to lithium heparin in almost all of the parameters. In the case of WBC, sodium citrate showed better agreement (CCC = 0.960) than lithium heparin (CCC = 0.853). Similar results were obtained in the cases of RBC (0.957 vs. 0.991 accuracy), Hb (0.986 vs. 0.973), HCT (0.921 vs. 0.980), and platelet count (0.984 vs. 0.979). Excellent agreement was seen in the red cell indices (MCV, MCH, and MCHC) with both the anticoagulants, but sodium citrate showed better concordance in general.

Table 1: Characteristics and Specifications of the Anticoagulant Blood Collection Tubes Used in the Study

Parameter	EDTA Tube	Heparin Tube	Citrate Tube
Tube Type	K2 EDTA	Lithium Heparin	3.2% Sodium citrate (PT)
Tube Dimension	13 × 75 mm	13 × 75 mm	13 × 75 mm
Temperature range for storage	2°C to 25°C	20°C to 25°C	15°C to 25°C
Expiry Date	15.6.2026	11.5.2026	20.6.2026
Volume of tube	5 mL	5 mL	5 mL
Volume of sample needed	1.5 – 2.0 mL	1.5 – 2.0 mL	1.8 mL
Company name	AV Labotube	AV Labotube	Hemo Vac
Origin of company	India	India	India
Anticoagulant concentration	1.8 mg /mL of blood	17 IU /mL of blood	3.2% (0.109 M)
Tube material	Plastic	Plastic	Plastic

Table: 2 Intraclass Correlation Coefficient (ICC) and 95% Confidence Intervals for CBC Parameters Among the Three Anticoagulants

Parameter	Intra class correlation coefficient	95% (CI)	
		Lower	Upper
WBC	0.933	0.908	0.952
RBC	0.827	0.763	0.876
HB	0.941	0.919	0.958

HCT	0.916	0.885	0.940
MCV	0.977	0.968	0.983
MCH	0.973	0.963	0.981
MCHC	0.921	0.892	0.944
PLATELETS	0.806	0.734	0.861

Table: 3 Comparing CBC Parameters Among K2 EDTA, Lithium Heparin and Sodium Citrate Anticoagulants

Parameter	K2 EDTA	LITHIUM HEPARIN	SODIUM CIRATE	P value
	Median (Min – Max)	Median (Min – Max)	Median (Min – Max)	
WBC	7.45 (4.1 – 17.6)	6.30 (1.0 – 15.1)	4.34 (3.4 – 16.6)	0.001
RBC	4.34 (2.3 – 5.7)	4.50 (2.5 – 8.8)	4.20 (2.3 – 5.7)	0.001
HB	12.05 (6.2 – 16.7)	12.60 (6.7 – 20.9)	11.55 (6.2 – 16.6)	0.004
HCT	38.10 (23.0 – 50.3)	39.40 (25.2 – 59.2)	36.40 (22.6 – 52.6)	0.001
MCV	87.20 (55.2 – 102.7)	87.25 (63.8 – 104.6)	86.35 (63.7 – 102.6)	0.572
MCH	28.40 (16.6 – 34.3)	28.40 (15.8 – 38.2)	28.15 (15.9 – 33.5)	0.636
MCHC	32.20 (25.1 – 35.2)	32.10 (24.6 – 39.0)	31.60 (24.1 – 36.5)	0.388
PLATELETS	3.04 (1.1 – 6.1)	2.32 (0.8 – 5.3)	2.88 (1.0 – 5.8)	0.001

Table: 4 Comparing CBC Parameters Between K2 EDTA and Lithium Heparin Anticoagulant

Parameter	K2 EDTA	LITHIUM HEPARIN	P value
	Median (Min – Max)	Median (Min – Max)	
WBC	7.45 (4.1 – 17.6)	6.30 (1.0 – 15.1)	0.001
RBC	4.34 (2.3 – 5.7)	4.50 (2.5 – 8.8)	0.006
HB	12.05 (6.2 – 16.7)	12.60 (6.7 – 20.9)	0.045
HCT	38.10 (23.0 – 50.3)	39.40 (25.2 – 59.2)	0.050
MCV	87.20 (55.2 – 102.7)	87.25 (63.8 – 104.6)	0.943
MCH	28.40 (16.6 – 34.3)	28.40 (15.8 – 38.2)	0.944
MCHC	32.20 (25.1 – 35.2)	32.10 (24.6 – 39.0)	0.479
PLATELETS	3.04 (1.1 – 6.1)	2.32 (0.8 – 5.3)	0.001

Table: 5 Comparing CBC Parameters Between K2 EDTA and Sodium Citrate Anticoagulants

Parameter	K2 EDTA	SODIUM CIRATE	P value
	Median (Min – Max)	Median (Min – Max)	
WBC	7.45 (4.1 – 17.6)	4.34 (3.4 – 16.6)	0.001
RBC	4.34 (2.3 – 5.7)	4.20 (2.3 – 5.7)	0.058
HB	12.05 (6.2 – 16.7)	11.55 (6.2 – 16.6)	0.180
HCT	38.10 (23.0 – 50.3)	36.40 (22.6 – 52.6)	0.060
MCV	87.20 (55.2 – 102.7)	86.35 (63.7 – 102.6)	0.339
MCH	28.40 (16.6 – 34.3)	28.15 (15.9 – 33.5)	0.420
MCHC	32.20 (25.1 – 35.2)	31.60 (24.1 – 36.5)	0.489
PLATELETS	3.04 (1.1 – 6.1)	2.88 (1.0 – 5.8)	0.171

Table: 6 Comparing CBC Parameters Between Lithium Heparin and Sodium Citrate Anticoagulants

Parameter	LITHIUM HEPARIN	SODIUM CIRATE	P value
	Median (Min – Max)	Median (Min – Max)	
WBC	6.30 (1.0 – 15.1)	4.34 (3.4 – 16.6)	0.007
RBC	4.50 (2.5 – 8.8)	4.20 (2.3 – 5.7)	0.001
HB	12.60 (6.7 – 20.9)	11.55 (6.2 – 16.6)	0.001
HCT	39.40 (25.2 – 59.2)	36.40 (22.6 – 52.6)	0.001
MCV	87.25 (63.8 – 104.6)	86.35 (63.7 – 102.6)	0.391
MCH	28.40 (15.8 – 38.2)	28.15 (15.9 – 33.5)	0.397
MCHC	32.10 (24.6 – 39.0)	31.60 (24.1 – 36.5)	0.183
PLATELETS	2.32 (0.8 – 5.3)	2.88 (1.0 – 5.8)	0.001

Table: 7 Lin's Concordance Correlation Coefficient (CCC) Demonstrating Consistency of Lithium Heparin and Sodium Citrate With K2 EDTA for CBC Parameters

Parameter	K2 EDTA LITHIUM HEPARIN	Pearson (precision)	Accuracy	K2 EDTA SODIUM CIRATE	Pearson (precision)	Accuracy
WBC	0.72 (0.61 – 0.79)	0.853	0.984	0.94 (0.92 – 0.96)	0.960	0.998
RBC	0.55 (0.40 – 0.67)	0.635	0.991	0.86 (0.80 – 0.90)	0.885	0.957
HB	0.82 (0.75 – 0.88)	0.865	0.973	0.93(0.90 – 0.95)	0.947	0.986
HCT	0.77 (0.67 – 0.84)	0.811	0.980	0.88 (0.83 – 0.92)	0.912	0.921
MCV	0.93 (0.90 – 0.95)	0.935	0.949	0.92 (0.89 – 0.95)	0.932	0.963
MCH	0.94 (0.91 – 0.96)	0.938	0.995	0.94 (0.92 – 0.96)	0.950	0.991
MCHC	0.83 (0.77 – 0.88)	0.843	0.984	0.83 (0.76 – 0.88)	0.835	0.946
PLATELETS	0.42 (0.26 – 0.56)	0.589	0.979	0.90 (0.86 – 0.94)	0.918	0.984

4.DISCUSSION

“Complete blood count (CBC) is one of the most performed laboratory investigations and serves as an essential tool in the diagnosis, evaluation, and management of various hematological and systemic disorders”. Although automated hematology analyzers provide accurate and reliable results, several pre-analytical factors, including the type of anticoagulant used for blood collection, can significantly influence hematological parameters. “Ethylenediaminetetraacetic acid (EDTA) is the anticoagulant of choice for routine hematological investigations because it preserves cellular morphology and maintains reliable blood cell measurements”. However, under certain circumstances, sodium citrate and lithium heparin may be considered as alternative anticoagulants to EDTA [10–14].

In the present study, complete blood count (CBC) parameters were analyzed using three anticoagulants: K₂EDTA, lithium heparin, and sodium citrate in 110 volunteers. Very good agreement was observed among the results obtained with the three anticoagulants for most hematological parameters, with ICC values exceeding 0.90 for WBC count, hemoglobin, MCV, and MCH. The lowest ICC value was observed for platelet count (0.806), which still indicated good agreement. These findings suggest that erythrocyte indices are minimally influenced by the type of anticoagulant used, whereas cellular counts, particularly platelet count, are more susceptible to anticoagulant-related variations [8,9,13].

ANOVA demonstrated statistically significant differences among the three anticoagulants for WBC count, RBC count, hemoglobin, hematocrit, and platelet count, whereas no significant differences were observed for MCV, MCH, and MCHC. These findings suggest that the choice of anticoagulant has a greater influence on certain quantitative hematological parameters, particularly leukocyte and platelet measurements, while red cell indices remain relatively stable. These observations are consistent with previous studies, which also reported minimal variation in erythrocyte indices among different anticoagulants, while demonstrating greater effects on leukocyte and platelet parameters [6,14,15].

Comparison of K₂EDTA with lithium heparin in the present study demonstrated statistically significant differences in WBC count, RBC count, hemoglobin, hematocrit, and platelet count, whereas MCV, MCH, and MCHC showed no significant differences. These findings suggest that lithium heparin may influence certain quantitative hematological parameters, while its effect on red cell indices (MCV, MCH, and MCHC) appears to be minimal. Similarly, Akorsu et al. reported that lithium heparin showed better agreement with EDTA than sodium citrate for several complete blood count parameters. However, as differences were observed in some hematological indices, the authors recommended cautious use of lithium heparin as an alternative to EDTA, as the results may not be completely interchangeable [6].

Importantly, comparison of K₂EDTA- and sodium citrate-anticoagulated samples in the present study revealed a statistically significant difference only in the white blood cell count, whereas no significant differences were observed in red blood cell count, hemoglobin, hematocrit, platelet count, or red cell indices. Furthermore, CCC demonstrated better agreement between sodium citrate and K₂EDTA than between lithium heparin and K₂EDTA for most CBC parameters. High concordance was observed for red blood cell count, hemoglobin, hematocrit, platelet count, MCV, MCH, and MCHC, suggesting that sodium citrate may provide CBC results comparable to those obtained with EDTA for several hematological parameters [6,9].

The findings of the present study differ from those reported by Akorsu et al., who observed significantly lower values for several complete blood count (CBC) parameters in sodium citrate-anticoagulated samples compared with EDTA and lithium heparin. They attributed these reductions primarily to the dilutional effect of sodium citrate and concluded that it may not be an ideal anticoagulant for routine CBC analysis because it can underestimate hematological parameters [6]. The discrepancy between the findings of the two studies may be explained by differences in hematology analyzers, study population, blood collection and handling procedures, anticoagulant concentrations, and the interval between sample collection and analysis.

Similarly, the findings of the present study are partially consistent with those of Putra et al., who compared complete blood count parameters in blood samples anticoagulated with EDTA, sodium citrate, and heparin using the Sysmex XN-1000 analyzer. They reported no significant differences in hemoglobin concentration, red blood cell count, hematocrit, MCV, MCH, RDW, and white blood cell count among the three anticoagulants. However, platelet count and platelet indices were significantly lower in sodium citrate- and heparin-anticoagulated samples than in EDTA-anticoagulated samples. Based on these findings, the authors concluded that although sodium citrate and heparin may be used as alternative anticoagulants under certain circumstances, EDTA remains the preferred anticoagulant for routine hematological analysis because it provides more reliable platelet measurements [7].

However, the reduced reliability of platelet count observed in the present study is in agreement with previous reports. Platelet count is one of the CBC parameters most affected by the choice of anticoagulant because platelets are highly susceptible to anticoagulant-induced aggregation and swelling. As a result, platelet counts may vary between automated hematology analyzers depending on the anticoagulant used, with EDTA remaining the preferred anticoagulant for accurate platelet estimation in routine hematological analysis [3,14,16,17].

Several animal studies have demonstrated impact of anticoagulants on hematological parameters. Guzman et al. reported significant differences between lithium heparin- and K₂EDTA-anticoagulated samples, with lower packed cell volume and plasma protein levels but higher lymphocyte counts in lithium heparin samples. In contrast, total leukocyte counts did not differ significantly between the two groups. The authors also observed leukocyte and thrombocyte clumping in heparinized samples, which could lead to inaccurate cellular counts [18]. Similarly, Muro et al. reported alterations in hematological variables in lithium heparin-anticoagulated blood from Hermann's tortoises compared with EDTA-anticoagulated samples [19]. Although these findings are based on animal studies, they support the concept that lithium heparin may influence cellular structure and function.

On the other hand, Ciepliński et al. reported no statistically significant differences between EDTA- and lithium heparin-anticoagulated samples with respect to hemoglobin, red blood cell (RBC) count, white blood cell (WBC) count, and mean corpuscular hemoglobin (MCH) in brown trout [20]. Similarly, Oliveira et al. found no significant differences in RBC count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) among EDTA-, heparin-, and sodium citrate-anticoagulated samples from freshwater stingrays [21]. These findings suggest that erythrocyte parameters remain relatively stable across different anticoagulants and are consistent with the results of the present study.

Ali et al. evaluated the effect of K₃EDTA, lithium heparin, and 3.2% sodium citrate on complete blood count parameters in rats. They reported significantly lower hematocrit, mean corpuscular volume (MCV), total leukocyte count, selected differential leukocyte parameters, and mean platelet volume (MPV) in sodium citrate-anticoagulated samples compared with EDTA- and lithium heparin-anticoagulated samples. Based on these findings, the authors concluded that EDTA and lithium heparin were more suitable than sodium citrate for routine complete blood count analysis [15]. Although the present study demonstrated a statistically significant difference only in the white blood cell (WBC) count between EDTA and sodium citrate, the findings of both studies suggest that leukocyte parameters are among the hematological indices most susceptible to the choice of anticoagulant.

One of the major strengths of the present study is the combined assessment of reliability using intraclass correlation coefficients (ICCs) and agreement using Lin's concordance correlation coefficient (CCC), providing a comprehensive evaluation of the interchangeability of different anticoagulants. Furthermore, blood samples from all participants were analyzed using a single automated hematology analyzer under standardized laboratory conditions, thereby minimizing analytical variability. However, the present study included only healthy

participants, which may limit the generalizability of the findings to patients with hematological disorders. Additionally, platelet indices such as MPV, PDW, P-LCR, and PCT were not evaluated, and the effect of delayed sample processing was not investigated [2,22,23].

In general, this study has demonstrated the effectiveness of K₂EDTA as the anticoagulant of choice for complete blood count analysis, as it provided consistent results across all evaluated blood parameters. However, for most CBC parameters, including RBC count, hemoglobin, hematocrit, and platelet count, 3.2% sodium citrate showed good agreement with K₂EDTA compared with lithium heparin. Therefore, sodium citrate may be considered a suitable alternative to K₂EDTA in certain clinical situations where K₂EDTA cannot be used. The red cell indices, including MCV, MCH, and MCHC, demonstrated high reproducibility irrespective of the anticoagulant used, indicating that these parameters are minimally influenced by anticoagulant selection [6,8,9,15]. Lithium heparin, on the other hand, showed good reliability for selected parameters but demonstrated greater variability and should be used cautiously [6,8].

Conclusion:

K₂EDTA continues to be the best anticoagulant for routine CBC analysis since it produces the most consistent hematological parameters. Despite the variation in white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), and platelets (Platelet) among the anticoagulants tested, the red cell indices (MCV, MCH, and MCHC) were consistent irrespective of the anticoagulant used. Sodium citrate was more comparable to K₂EDTA than lithium heparin with respect to most of the CBC parameters, and hence, it is possible to use it as an alternative to EDTA where EDTA cannot be used, especially in pseudothrombocytopenia due to EDTA.

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