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COMPARISON OF ERYTHROCYTE SEDIMENTATION RATE (ESR) VALUES USING EDTA AND 3.8% SODIUM CITRATE ANTICOAGULANTS

PERBANDINGAN NILAI LAJU ENDAP DARAH (LED) MENGGUNAKAN ANTIKOAGULAN EDTA DAN NATRIUM SITRAT 3,8%

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Abstract

Erythrocyte Sedimentation Rate (ESR) is a hematological test widely used as a nonspecific indicator of inflammation or certain diseases. The type of anticoagulant used in blood samples can affect ESR results. This study aimed to determine the differences in ESR values between venous blood samples using Ethylenediaminetetraacetic Acid (EDTA) and 3.8% sodium citrate anticoagulants. This was a descriptive quantitative study with a total sampling method involving 33 respondents at the Clinical Pathology Laboratory, Politeknik Indonusa Surakarta. ESR was measured using the standard Westergren method, and data were analyzed using the Wilcoxon test. The results showed that the mean ESR value in samples with EDTA anticoagulant was 18 mm/hour, while those with 3.8% sodium citrate had a mean value of 14 mm/hour. The Wilcoxon test indicated a significant difference ($p = 0.000$; $p < 0.05$), suggesting that the type of anticoagulant significantly affects ESR results. Overall, EDTA tended to produce higher ESR values compared to 3.8% sodium citrate. These findings emphasize the importance of anticoagulant selection in ESR testing and highlight the need for standardization in laboratory practice. Further studies are recommended to compare other anticoagulants and to evaluate automated ESR methods for more comprehensive insights.

Keywords : Erythrocyte Sedimentation Rate, EDTA, Sodium Citrate 3.8%, Anticoagulant

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Abstrak

Laju Endap Darah (LED) merupakan salah satu pemeriksaan hematologi yang digunakan sebagai indikator nonspesifik adanya proses inflamasi atau penyakit tertentu. Jenis antikoagulan yang digunakan dalam sampel darah dapat memengaruhi hasil pemeriksaan LED. Penelitian ini bertujuan untuk mengetahui perbedaan nilai LED pada sampel darah vena yang menggunakan antikoagulan Ethylenediaminetetraacetic Acid (EDTA) dan natrium sitrat 3,8%. Penelitian ini menggunakan desain deskriptif kuantitatif dengan metode total sampling pada 33 responden di Laboratorium Patologi Klinik Politeknik Indonusa Surakarta. Pemeriksaan LED dilakukan dengan metode standar Westergren, kemudian data dianalisis menggunakan uji Wilcoxon. Hasil penelitian menunjukkan bahwa rata-rata nilai LED pada sampel dengan antikoagulan EDTA adalah 18 mm/jam, sedangkan pada sampel dengan natrium sitrat 3,8% adalah 14 mm/jam. Uji Wilcoxon menghasilkan nilai signifikansi $p = 0,000$ ($p < 0,05$), yang menandakan terdapat perbedaan bermakna antara kedua antikoagulan. Secara umum, penggunaan EDTA cenderung menghasilkan nilai LED yang lebih tinggi dibandingkan natrium sitrat 3,8%. Temuan ini menunjukkan bahwa pemilihan jenis antikoagulan berpengaruh terhadap hasil pemeriksaan LED, sehingga perlu adanya standardisasi dalam praktik laboratorium. Penelitian lebih lanjut disarankan untuk membandingkan jenis antikoagulan lain maupun penggunaan metode otomatis agar diperoleh gambaran yang lebih komprehensif.

Kata Kunci : Laju Endap Darah, EDTA, Natrium Sitrat 3,8%, Antikoagulan

1. INTRODUCTION

Erythrocyte Sedimentation Rate (ESR) is one of the hematological examinations that is still widely used in clinical practice. This test measures the rate at which erythrocytes settle in plasma within a given period of time and serves as a nonspecific indicator of inflammation, infection, or chronic disease. Although ESR is not a specific diagnostic parameter, it is frequently used as a supporting tool for clinicians to monitor disease progression and evaluate treatment response (Wahab, 2020). ESR remains relevant due to its simplicity, relatively low cost, and good sensitivity to both physiological and pathological changes in the patient's body.

However, ESR results are influenced by various intrinsic and extrinsic factors. Intrinsic factors include age, sex, erythrocyte count, hematocrit, and levels of fibrinogen or other acute-phase proteins that increase during inflammation. Extrinsic factors involve technical aspects of the examination, such as the method used, room temperature, storage time of blood samples, and the type of anticoagulant added (Nazarudin et al., 2019). Among these variables, the anticoagulant plays a crucial role in ensuring the validity of ESR results because it may affect erythrocyte morphology, plasma protein concentration, and the rate of erythrocyte sedimentation (Wenty & Umar, 2024).

Two anticoagulants commonly used in ESR testing are Ethylenediaminetetraacetic Acid (EDTA) and 3.8% sodium citrate. EDTA prevents coagulation by irreversibly binding calcium ions without significantly altering blood volume, thereby maintaining plasma concentration and producing relatively higher ESR values. In contrast, 3.8% sodium citrate is a liquid anticoagulant that dilutes blood samples, reducing plasma protein concentration. This dilution effect delays the formation of erythrocyte aggregates (rouleaux formation), which in turn results in lower ESR values compared to EDTA (Rahmawati et al., 2019).

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Several studies have compared these two anticoagulants, yet their findings remain inconsistent. Lestari et al. (2021) reported that EDTA yielded higher ESR values compared to sodium citrate in menstruating women. Hilda Salenda Rahayaan (2022) observed significant differences, with sodium citrate producing slightly higher mean ESR values than EDTA. Nazarudin & Kartika Sari (2021) demonstrated that dilution with NaCl in EDTA blood samples affected ESR results, whereas Hardyansa et al. (2020) found no significant differences between sodium citrate 3.8% and 5% dextrose solutions. More recently, Aisyah Hakim et al. (2024) compared automated ESR using K3EDTA with manual Westergren ESR using sodium citrate and reported significant discrepancies between the two methods. These varied outcomes indicate that anticoagulant selection remains a critical issue that warrants further investigation to ensure reliable ESR results.

Globally, ESR testing continues to be an essential component of laboratory medicine, particularly in developing countries where access to advanced diagnostic modalities may be limited. Despite its limitations as a nonspecific test, ESR remains valuable for screening and monitoring chronic inflammatory conditions such as tuberculosis, autoimmune diseases, and malignancies. The accuracy of ESR, however, depends on strict adherence to standardized procedures, including anticoagulant choice, as recommended by the International Council for Standardization in Haematology (ICSH) and the World Health Organization (WHO). Inconsistent use of anticoagulants across laboratories may lead to variability in results, thereby affecting clinical interpretation and patient management.

This study was conducted to bridge the existing gap in knowledge by providing direct evidence on the comparative impact of EDTA and 3.8% sodium citrate on ESR values using the Westergren method. Unlike previous research that often involved additional diluents, alternative solutions, or different measurement techniques, this study specifically focuses on the two most widely used anticoagulants in routine hematology. The findings are expected to contribute to the scientific discourse on laboratory standardization and support the establishment of more uniform guidelines for ESR testing. Such efforts are crucial to ensure the reliability of laboratory results, enhance diagnostic decision-making, and ultimately improve patient care.

2. RESEARCH METHOD

This research employed a descriptive quantitative approach with a total sampling method. The study was designed to compare the values of the Erythrocyte Sedimentation Rate (ESR) using two different anticoagulants, namely Ethylenediaminetetraacetic Acid (EDTA) and 3.8% sodium citrate, through the standard Westergren method. The design was chosen to allow direct observation of differences in ESR values based on the type of anticoagulant used in venous blood samples.

The study was conducted at the Clinical Pathology Laboratory of Politeknik Indonusa Surakarta between November 2024 and January 2025. The location was selected due to the availability of facilities and laboratory equipment required for the standard Westergren ESR method. During the study period, all procedures were carried out under controlled laboratory conditions to minimize technical errors and ensure compliance with safety protocols.

The subjects of this research consisted of 33 respondents drawn from students of the Medical Laboratory Technology program who met the inclusion and exclusion criteria. The

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inclusion criteria were individuals aged 18–60 years, both male and female, who were clinically healthy and not suffering from chronic illnesses. Exclusion criteria included individuals with a history of chronic disease, those under medication that could influence ESR results, pregnant or menstruating women, and participants involved in other research projects. All respondents provided informed consent prior to blood collection.

Venous blood samples were collected using standard venipuncture techniques, with each participant providing approximately 2 mL of blood. The blood was then divided equally into two separate tubes: one containing EDTA and the other containing 3.8% sodium citrate. Each sample was gently inverted 8–10 times to ensure homogeneity and subsequently transferred into Westergren tubes. The tubes were placed in a vertical position and observed after 60 minutes to determine ESR values, recorded in millimeters per hour (mm/h). The same standardized procedure was applied to all samples to avoid bias and maintain reliability.

Data collection was conducted through direct measurement and documentation of ESR values obtained from both groups. The results were compiled and tabulated for further analysis. Descriptive statistics, including mean, minimum, maximum, and standard deviation, were used to describe the distribution of ESR values. Inferential analysis was performed using the Wilcoxon Signed Ranks Test to assess whether there were statistically significant differences between ESR values obtained from EDTA and 3.8% sodium citrate samples. The Wilcoxon test was selected because the data were paired samples that did not meet the assumption of normality.

The overall research cycle included problem identification, formulation of objectives, sample collection, laboratory analysis, data recording, statistical processing, and interpretation of results. Ethical considerations were observed throughout the study, with confidentiality and anonymity of participant data maintained at all times. Blood collection was carried out by trained personnel using sterile techniques to ensure participant safety and compliance with ethical standards.

3. RESULTS AND DISCUSSION

This study involved 33 respondents from the Medical Laboratory Technology Study Program at Politeknik Indonusa Surakarta who met the inclusion criteria. The characteristics of the respondents are presented in detail in Table 1. The most dominant age group was 15–20 years, comprising 72.73% of the total sample. Meanwhile, the gender distribution showed a significant dominance of female participants, accounting for 87.88%, compared to 12.12% male participants. These demographics reflect the general composition of students in the study program and ensure consistency within the research population.

Tabel 1. Distribution of respondents based on age and sex

Variable		Frequency	Percentage (%)
Usia	15 – 20 Tahun	24	72,73
	21 – 25 Tahun	7	21,21
	> 25 Tahun	2	6,06
Total		33	100
Jenis Kelamin	Perempuan	29	87,88

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	Laki - laki	4	12,12
Total		33	100

The erythrocyte sedimentation rate (ESR) results obtained using the Westergren method showed variations between two types of anticoagulants, namely EDTA and 3.8% sodium citrate. As presented in Table 2, samples treated with EDTA produced a minimum ESR value of 1 mm/hour and a maximum of 55 mm/hour, with an average value of 18 mm/hour. Meanwhile, samples treated with 3.8% sodium citrate showed a slightly higher minimum value of 2 mm/hour and a lower maximum value of 41 mm/hour, with an average of 14 mm/hour. These findings indicate that EDTA tends to produce higher ESR values compared to sodium citrate.

Tabel 2. ESR values based on anticoagulant type

Antikoagulan	N	Minimum (mm/h)	Maximum (mm/h)	Mean (mm/h)
EDTA	33	1	55	18
Sodium citrate 3.8%	33	2	41	14

To further analyze the differences in ESR values between the two types of anticoagulants, the Wilcoxon Signed Ranks Test was employed. This test was chosen due to the paired and non-parametric nature of the data. The results, presented in Table 3, show a Z-value of -3.718 with a significance level (p-value) of 0.000 ($p < 0.05$). This indicates that there is a statistically significant difference between the ESR values in samples treated with EDTA and those treated with sodium citrate.

Tabel 3. Wilcoxon Signed Ranks Test for ESR values between EDTA and sodium citrate

Comparison	Z	Asymp. Sig. (2-tailed)
Sodium citrate – EDTA	-3.718[b]	.000

Of the 33 paired samples analyzed, 27 samples showed higher ESR values when using EDTA, 5 samples produced higher values with sodium citrate, and 1 sample showed no difference between the two anticoagulants. These findings strongly suggest that the type of anticoagulant used plays a critical role in ESR results, with EDTA consistently producing higher values. This difference is likely attributable to EDTA's ability to maintain plasma protein concentration without causing dilution, in contrast to sodium citrate, which may dilute the sample and consequently lower the ESR values.

This study aimed to determine the difference in erythrocyte sedimentation rate (ESR) values between venous blood samples treated with two types of anticoagulants: EDTA and 3.8% sodium citrate, using the Westergren method. The results showed that samples treated with EDTA produced a higher mean ESR value (18 mm/hour) compared to those treated with sodium citrate (14 mm/hour). The Wilcoxon Signed Ranks Test confirmed a

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statistically significant difference between the two groups with a p-value of 0.000 ($p < 0.05$). This confirms the hypothesis that the choice of anticoagulant affects ESR results.

These findings are consistent with research conducted by Lestari et al. (2021), which found that EDTA tends to produce higher ESR values than sodium citrate. This difference is attributed to the mechanism by which each anticoagulant interacts with blood components. EDTA functions by chelating calcium ions in the blood, preventing coagulation without significantly diluting the plasma. This preserves the concentration of plasma proteins such as fibrinogen and globulin, which play a vital role in the formation of rouleaux—stacks of red blood cells that sediment more rapidly. In contrast, sodium citrate is a liquid anticoagulant and tends to dilute the blood sample, which can reduce protein concentrations and slow down rouleaux formation, ultimately leading to lower ESR values.

Other studies, however, report contrasting findings. For example, Hardyansa et al. (2020) and Hasanah et al. (2023) found no significant differences between ESR values using EDTA and sodium citrate, particularly when paired with isotonic diluents like NaCl. These discrepancies could be explained by differences in methodology, such as variations in the ratio of blood to anticoagulant, handling techniques, environmental conditions (e.g., room temperature), or differences in study populations. This highlights the need for standardized procedures and consistent anticoagulant selection in clinical practice.

The present study contributes to the body of knowledge by controlling key variables: both anticoagulants were tested on the same blood donors using the same methodology in a controlled laboratory environment. This design reduces biological variability and strengthens the validity of the findings. Additionally, using a paired-sample design and an appropriate statistical method (Wilcoxon test) provides a robust analysis of the differences observed.

An important strength of this study lies in its practical application to hematology laboratories. ESR testing is widely used as a screening and monitoring tool for various conditions, including infections, autoimmune diseases, and malignancies. The results of this study support the recommendation that laboratories should be consistent in their choice of anticoagulant when monitoring ESR over time in the same patient. Variations in anticoagulant type may lead to misinterpretation of inflammation severity or treatment progress.

Despite its strengths, this study has several limitations. The sample size, although calculated using the Slovin formula, was relatively small and focused solely on a specific population students of a health sciences institution. This limits the generalizability of the results to other populations, such as elderly patients or individuals with inflammatory or hematological disorders. Moreover, the study did not investigate the influence of other factors that may affect ESR results, such as hematocrit, plasma viscosity, or specific disease states.

Additionally, the study relied solely on the manual Westergren method, which, while considered a gold standard, is also known for its susceptibility to pre-analytical and analytical variability, such as tube angle, time measurement accuracy, and human error. In recent years, automated ESR analyzers have been developed to address some of these limitations by providing faster, more standardized results. Future research may benefit from comparing manual and automated methods, as well as including a wider range of anticoagulants and patient conditions.

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Furthermore, no biochemical or protein analyses were conducted to correlate ESR values with fibrinogen or CRP levels, which are key mediators of rouleaux formation. Including such parameters in future studies could provide a more comprehensive understanding of the relationship between anticoagulant type, plasma protein composition, and ESR.

4. CONCLUSION

This study confirmed that the type of anticoagulant has a significant effect on the Erythrocyte Sedimentation Rate (ESR). Blood samples treated with EDTA consistently produced higher ESR values than those treated with 3.8% sodium citrate, and statistical analysis using the Wilcoxon test indicated a significant difference ($p < 0.05$). These findings support the hypothesis that anticoagulant selection influences the reliability of ESR results.

The strength of this study lies in its direct comparison of the two most commonly applied anticoagulants in hematology laboratories, using the standardized Westergren method under controlled conditions. This provides valuable evidence for laboratory practitioners and clinicians, highlighting that variations in anticoagulant type may result in different ESR outcomes and could potentially affect clinical interpretation. However, the study has limitations, particularly the relatively small sample size and the lack of comparison with automated ESR methods, which may have influenced the generalizability of the findings.

In conclusion, the results emphasize the need for standardization of anticoagulant use in ESR testing to ensure accuracy and consistency across laboratories. Further research involving larger populations, diverse clinical conditions, and automated technologies is recommended to validate these findings and expand their clinical applicability. By addressing these aspects, ESR testing can continue to serve as a reliable supporting tool in diagnosis and monitoring of inflammatory and chronic diseases.

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