JEVNAH
Journal of Evidence Based Nursing and Public Health
JEVNAH 1(2):42–49
ISSN 3028 - 2615

Institute for Advanced Science, Social and Sustainable Future
MORALITY BEFORE KNOWLEDGE

The Effect of Delayed Examination on the Erythrocyte Sedimentation Rate in EDTA Blood Samples

Ivan Benny Lino Jeremia[1]*
[1] *Regional General Hospital of Sabu Raijua, Sabu Raijua District, East Nusa Tenggara Province.;*
*Correspondence: linojeremia24@gmail.com

Received date: July 2, 2024 Revised date: July 29, 2024 Accepted date: August 22, 2024

ABSTRACT

Introduction: Blood is an essential component of living organisms, ranging from primitive animals to humans. Under physiological conditions, blood always remains within blood vessels to carry out its functions (Bakta, 2020). The Erythrocyte Sedimentation Rate (ESR), also known as blood sedimentation rate, is an examination to determine the rate at which erythrocytes settle in non-coagulated blood (blood containing anticoagulants) in a vertical tube over a specific period. The Westergren method for ESR measurement is the most satisfactory method currently used in hospitals and clinics. This study aims to determine the difference in Erythrocyte Sedimentation Rate values between EDTA blood samples examined immediately and those delayed for 3 hours at room temperature. **Method:** This analytical descriptive research compares the results of Erythrocyte Sedimentation Rate examinations conducted immediately and those delayed for 3 hours using the Westergren method at room temperature. The study involved 30 samples obtained from outpatient and inpatient patients by venous blood collection. **Findings:** The statistical analysis using Paired t-Test showed that the P Value was 0.00 ($P < 0.05$), indicating a significant difference between immediate and delayed examinations for 3 hours at room temperature using the Westergren method. **Conclusion:** Based on the research findings, it is recommended to conduct Erythrocyte Sedimentation Rate examinations with EDTA blood samples immediately as it is more effective.

KEYWORDS: blood sedimentation rate; Westergren method, analytical descriptive research.

1. Introduction

The hematological examination is one of the tests conducted to assess blood conditions, including blood cells and the plasma in which the blood is dissolved. It is used to monitor health status (Gilang Nugraha, 2021). Hematological tests encompass various examinations, such as routine blood tests, which include hemoglobin, leukocyte count, leukocyte differential count, erythrocyte sedimentation rate (ESR), and specialized blood tests, such as peripheral blood smears, erythrocyte count, hematocrit, erythrocyte indices, reticulocyte count, and platelet count (Bakta, 2020).

The Erythrocyte Sedimentation Rate (ESR), also referred to as the blood sedimentation rate, is a test to determine the speed at which erythrocytes settle in anticoagulated blood within a vertical tube over a specified period. ESR testing has several benefits, as it allows physicians to monitor suspected diseases. When a disease worsens, the ESR value increases, whereas, as the disease improves, the ESR value decreases (Rukman Kiswari, 2020).

Cite This Article:
Nurdiansyah, R. (2024). The Effect of Delayed Examination on the Erythrocyte Sedimentation Rate in EDTA Blood Samples. *Journal of Evidence Based Nursing and Public Health, 1*(2), 1-15. https://doi.org/10.61511/jevnah.v1i2.2024.771

An elevated ESR value does not specifically diagnose a particular disease but serves as an indicator of disease presence. It can also detect inflammation or malignant conditions such as rheumatic fever and heart attacks. Despite its nonspecific nature, it is highly beneficial in detecting tuberculosis, tissue necrosis or death, bone damage, or other diseases that do not present with symptoms (Rukman Kiswari, 2020).

The Westergren method for measuring ESR is currently the most satisfactory and widely used in hospitals and clinics. According to Wijayanti (2019), in her research, it is recommended that the Westergren method be used for ESR tests due to its greater accuracy. However, this method has drawbacks, such as air bubbles, blood clots, the condition of the environment (tilting of the ESR tube), the lengthy duration of the test (approximately one hour), and a higher risk of exposure to infectious materials. Additionally, the reading is still done manually. However, the cost of this method is relatively low (Riswanto, 2019).

ESR testing should ideally be conducted immediately to prevent sample degradation, which could affect the test results. In practice, field conditions such as a shortage of human resources often delay ESR testing due to an accumulation of patient specimens. In addition, staff members may need to travel long distances to collect specimens, making timely testing difficult. Prolonged storage of blood samples also increases the time required for testing. Ideally, ESR samples should be tested within two hours of blood collection (Solichul, 2020).

In Surahmi's 2019 study, there was a significant difference in ESR values when testing was done immediately compared to when delayed by one or two hours, with p-values of 1.959 > 0.05. Candrakirana's 2018 study found a significant difference in ESR results when testing was delayed by six hours, yielding a p-value of 0.01 < 0.05. These findings indicate that ESR values vary depending on the duration of blood exposure to EDTA.

2. Methods

2.1 Type and Design of Research

This research is a descriptive analytic study. The design was chosen to compare the results of erythrocyte sedimentation rate (ESR) tests immediately examined with those delayed by 3 hours at room temperature.

2.2 Time and Location of Research

The research was conducted from January to March 2024 at RSUD Sabu Raijua.

2.3 Research Sample

The sample consists of 30 outpatient and inpatient individuals at RSUD Sabu Raijua who underwent ESR testing in the laboratory during January, February, and March 2024.

2.4 Data Analysis

The data analysis aims to determine differences in the ESR results using the Westergren method, comparing immediate testing with a 3-hour delay at room temperature. A paired statistical analysis was conducted using the Statistical Program for Social Science (SPSS).

2.5 Univariate Analysis

Univariate analysis was performed to observe the frequency distribution of each variable, including the immediate ESR test and the 3-hour delayed test at room temperature.

2.6 Bivariate Analysis

Bivariate analysis was conducted to examine differences between immediate ESR tests and those delayed for 3 hours at room temperature. The paired t-test criterion is as follows: if the probability is < α (0.05), then Ho is rejected, and H1 is accepted, indicating a difference in ESR results between immediate testing and a 3-hour delay at room temperature using the Westergren method. If the probability is > α (0.05), then Ho is accepted, and H1 is rejected, meaning no significant difference in ESR results between the immediate test and the 3-hour delayed test at room temperature.

2.7 Research Procedure

The research instruments used in this study include various tools and reagents required for blood collection and testing for the erythrocyte sedimentation rate (ESR). The equipment consists of a 5 ml syringe, alcohol swabs, a tourniquet, and a test tube with a purple cap containing EDTA as an anticoagulant. Additionally, Westergren tubes and racks are used to hold the blood samples, along with a timer to monitor the test duration. A suction bulb is employed to draw the blood into the Westergren pipette, and micropipettes of different sizes (1000 µl, 500 µl, 200 µl, and 100 µl) are used for precision in measuring the samples. The process also involves using yellow and blue pipette tips for different volumes, along with a test tube rack and reaction tubes to hold the samples during the procedure. Tissue is also utilized to clean the equipment and remove any excess blood from the exterior of the pipette or tubes to ensure a contamination-free environment.

The reagents required for the procedure include the patient's blood sample and a 0.9% NaCl solution. The blood is mixed with the NaCl solution at a ratio of 1:4, which aids in preparing the sample for the Westergren ESR test. This method ensures accuracy and consistency when assessing the ESR levels in the blood samples.

3. Result and Discussion

3.1 Research characteristics

This study was conducted in the laboratory of RSUD Sabu Raijua from January to March 2024. The sample consisted of 30 specimens from both outpatient and inpatient patients at RSUD Sabu Raijua. The research process involved two conditions: some specimens were examined immediately, while others were delayed for 3 hours at room temperature, using the Westergren method to measure erythrocyte sedimentation rate (ESR).

3.2 Difference in ESR results between immediate and 3-hour delayed testing

3.2.1 ESR test results

Table 1. ESR test results

Sample Code	ESR Results (mm/hour)	
	Immediate	Delayed 3 Hours
1	16	10
2	40	49
3	60	65
4	14	8
5	10	7
6	15	8
7	33	20
8	13	8
9	15	14
10	25	23

11	3	2
12	50	65
13	70	60
14	15	5
15	44	25
16	9	5
17	36	38
18	35	34
19	17	13
20	15	5
21	5	2
22	35	40
23	10	5
24	2	1
25	85	70
26	50	65
27	25	17
28	30	39
29	23	12
30	25	17

Based on the results of the Erythrocyte Sedimentation Rate (ESR) examination, there is a noticeable difference between the results obtained from the immediate examination and those obtained after a 3-hour delay at room temperature. The average result of the immediate ESR examination was 27.50 mm/hour, whereas the average result after the 3-hour delay was 24.40 mm/hour. This indicates that delaying the examination by 3 hours affects the ESR value, leading to a slight decrease in the sedimentation rate over time.

Results of Paired t-Test Statistical Analysis

Tabel 2. Hasil Statistic *Paired t - Test*

	Paired Differences					T	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 Immediate - Delayed	4.71429	2.94641	.78746	3.01308	6.41549	5.987	13	.000

Based on Table 4.2, the results of the statistical test using the Paired t-Test indicate a P Value of 0.00 ($P < 0.05$), suggesting that there is a significant difference between the immediate examination and the examination postponed for 3 hours.

3.2 Discussion

In this study, blood with the anticoagulant EDTA was diluted with 0.9% NaCl for the examination of Erythrocyte Sedimentation Rate (ESR) using the Westergren method with two treatments: immediate examination and examination postponed for 3 hours at room temperature. Based on the statistical test results using the Paired t-Test, the P Value was found to be 0.00 ($P < 0.05$), indicating a significant difference between the immediate examination and the examination postponed for 3 hours. This means that the null hypothesis (H0) is rejected and the alternative hypothesis (H1) is accepted. Thus, there is a significant difference in the results of the erythrocyte sedimentation rate between the immediate examination and the one postponed for 3 hours at room temperature using the Westergren method.

Blood is an essential component of living organisms, ranging from primitive animals to humans. Under physiological conditions, blood is always contained within blood vessels,

allowing it to perform its functions (Bakta, 2019). Blood also contains various immune system components aimed at defending the body against pathogens. Endocrine hormones are also circulated through the blood. Blood appears bright red when oxygenated and dark red when deoxygenated. Its color is due to hemoglobin, a respiratory protein containing iron in the form of heme, which serves as the binding site for oxygen molecules (Gandasoebrata, 2020).

Blood is distributed through blood vessels from the heart to the entire body and then returns to the heart. This system functions to meet the nutritional and oxygen needs of cells or tissues and to transport metabolic waste out of the body. Blood consists of two components: cellular and non-cellular components. The cellular component, often referred to as corpuscles, comprises about 45% and consists of three types of cells: erythrocytes, leukocytes, and platelets. The non-cellular component is a liquid known as plasma, which makes up 55% of the blood (Sugeng Jitowiyono, 2018).

Erythrocyte Sedimentation Rate (ESR) reflects the comparative composition between erythrocytes and plasma. Blood with anticoagulants placed in a small-lumen tube and positioned vertically will show sedimentation of erythrocytes at a rate determined by the surface ratio. This sedimentation of cells, referred to as the erythrocyte sedimentation rate, increases when the weight of the cells rises, but the rate decreases when the cell surface area is larger. Smaller cells sediment more slowly than clumped cells because, in the case of clumping, the increase in weight of the clump is greater than the increase in surface area. In normal blood, the ESR is relatively small because the sedimentation of erythrocytes due to gravitational pull is balanced by upward pressure from plasma movement. Normal blood has a relatively low ESR because the sedimentation of erythrocytes is counterbalanced by upward pressure due to movement. If plasma viscosity is high or cholesterol levels increase, the upward pressure may neutralize the downward pull on each cell or cell clump. Conversely, any condition that increases clumping or settling of cells will elevate the ESR (Sumarata, C. A. P., 2010).

The working principle of the Westergren method is similar to that of the Wintrobe method, with differences in the type of tube used, the blood examined, and the normal ESR values produced. The tube used is the Westergren tube, which is 300 mm long with an inner diameter of 2.5 mm. This tube has millimeter lines from a scale of 0 to 200, whereas the Wintrobe method uses a shorter tube. In the Westergren method, the blood used for examination must be diluted, while the Wintrobe method uses undiluted blood. The results of the ESR examination using the Westergren and Wintrobe methods are not significantly different when the sedimentation rate is within normal limits. However, if the ESR value increases, the results obtained using the Wintrobe method are less reliable. The Westergren method can yield higher results because the Westergren pipette is twice the length of the Wintrobe pipette. This fact causes clinicians to prefer the Westergren method over the Wintrobe method (Gandasoebrata, 2020).

Based on observations during the study, blood samples that were delayed for two hours or more resulted in changes to the red blood cells after sampling; they became more spherical and had difficulty forming rouleaux, leading to a slower ESR and a tendency for the ESR value to decrease. The change in red blood cell shape to spherical and the difficulty in forming rouleaux is caused by a decrease in ATP or energy within the cells, disrupting the function of the Na+, K+ pump that maintains volume. The influx of sodium ions and calcium ions into the cells and the efflux of potassium ions result in water osmosis into the cells.

Generally, in normal blood, the increase in ESR is lower because the sedimentation of erythrocytes due to gravitational pull is balanced by the upward pressure from plasma movement; however, this has been mitigated by homogenizing the blood sample before diluting it with 0.9% sodium chloride.

Other technical factors that can influence the results include the position of the tube not being vertical or the tube being tilted at a 3° angle, which will accelerate the ESR, shaking or vibrating test tubes will speed up sedimentation, erasure of the scale lines on the tube wall, and any cracks in the tube can affect the ESR results.

4. Conclusion

Based on the research results and discussion regarding the differences in the Erythrocyte Sedimentation Rate (ESR) in EDTA blood samples examined immediately after collection and those postponed for 3 hours at room temperature at RSUD Sabu Raijua, it can be concluded that the average ESR result for the immediate examination was 27.50 mm/hour, while for the samples postponed for 3 hours, it was 24.40 mm/hour. The statistical test results using the Paired t-Test showed a P Value of 0.00 (P < 0.05), indicating a significant difference between the immediate examination and the examination postponed for 3 hours at room temperature using the Westergren method.

Author Contribution

All author contributed fully to the writing of this article.

Ethical Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The author declare no conflict of interest.

Open Access

References

Agung, A. (2019). *Differences in Serum Glucose Levels and Sodium Fluoride Plasma with Delayed Examination*. Scientific Writing, Medical Education, Faculty of Medicine, Diponegoro University, Semarang. http://jurnal.kedokteran.pdf

Andiyanto, E. (2019). *Introduction to Blood Diseases Through Blood Imaging*. Surabaya Institute of Technology. http://jurnal.kesehatan.pdf

Bakta, I. M. (2020). *Clinical Hematology in Brief. 1st Edition.* EGC, Jakarta.

Department of Health of the Republic of Indonesia. (2020). *Guidelines for Proper Laboratory Practices*. Jakarta.

Fatimah, R., Surur, M. A., A'tourrohman, M., Rohman, A., & Khumaesa, F. (2019). *Coagulation and Blood Composition,* 1-12.

Gandasoebrata, R. (2020). *Clinical Laboratory Guide*. Dian Rakyat, Jakarta.

Kiswari Rukman. (2020). *Hematology & Transfusion*. Jakarta: Erlangga.

Kurniawan, F. B. (2019). *Hematology Practicum for Health Analysts*. Jakarta: EGC Medical Publishing.

Nasir, Abd., et al. (2020). *Health Research Methodology. Mulia Medika*, Yogyakarta.

Nugraha, Gilang. (2019). *Basic Hematology Laboratory Examination Guide, 2nd Edition*. CV. Trans Info Media, East Jakarta.

Riswanto. (2019). *Hematology Laboratory Examination. Yogyakarta*: Alfa Media & Kanal Medika.

Santi Kurnia, Maya Ni Wayan, Ap Santa Ngurah Agung Anak, & Hadi Fathol. (2019). *Differences in Erythrocyte Sedimentation Rate Examination Results Using EDTA Anticoagulant at Temperature Variations of 16°C, 20°C, and 27°C by the Westergren Method*. https://www.google.com/search?q=perbedaan hasil pemeriksaan laju endap darah dengan antikoagulan edta terhadap variasi suhu

Setiawan, A., Suryani, E., & Winarto. (2019). *Segmentation of Red Blood Cell Images Based on Cell Morphology to Detect Iron Deficiency Anemia*, 3(1), 1.

Solichul, Hadi, S. (2019). *Simple Routine Hematology Laboratory Examination*, Clinical Pathology Laboratory, Faculty of Medicine, Airlangga University, Surabaya.

Surahmi. (2019). *Comparison of Erythrocyte Sedimentation Rate (ESR) Results Between Immediate Examination and Postponement for 1 and 2 Hours at Santa Anna Hospital, Kendari*, Scientific Writing, Ministry of Health of the Republic of Indonesia, Kendari Health Analyst Polytechnic, Kendari.

Wijayanti, W. A. (2019). *Differences in Erythrocyte Sedimentation Rate Measurements Using Manual and Automatic Westergren Methods*. Scientific Writing, Diploma Program in Health Analysis, Insan Cendekia Medika Health Sciences Institute, Jombang.

Biographies of Author(s)

Ivan Benny Lino Jeremia, Regional General Hospital of Sabu Raijua, Sabu Raijua District, East Nusa Tenggara Province. .

- Email: linojeremia24@gmail.com
- ORCID: -
- Web of Science ResearcherID: -
- Scopus Author ID: -
- Homepage: -