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Cell Morphology Differences in EDTA Tube Blood Between Immediate Examination and 4 Hours Delayed Examination at 20 - 25°C

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Abstract: ***Laboratory examination consists of three main processes including pre - analytic, analytical and post - analytic. These three processes are mutually sustainable and need to be considered because they greatly influence the results that are issued. The pre - analytical stage refers to all the steps that must be carried out before the sample can be analyzed. Delay in processing samples that are too long will cause physical and chemical changes which can be a source of errors in the examination. This study aims to analyze the differences in cell morphology in EDTA blood which was examined immediately and delayed for 4 hours at a temperature of 20 - 25°C. The type of research used is an experiment. The population is volunteer patients who carry out a complete blood count using EDTA blood who meet the inclusion criteria set by the researcher. Samples were taken using non - probability sampling technique purposive sampling method and analyzed using the Paired Sample T - Test. The results showed that there were differences in cell morphology that were treated immediately and delayed 4 hours, with p - values for erythrocytes, leukocytes and platelets respectively, namely 0.000, 0.002 and 0.000. This value indicates that the p - value <0.05 so that the hypothesis is accepted. Erythrocyte morphological changes that occur are the discovery of burr cells. In leukocytes, the cytoplasm fades, the nucleus breaks and there is cytoplasmic vacuolization. The changes that occur are platelets enlarge to become macro platelets and giant thrombocytes or often called giant platelets. Processing time affects changes in cell morphology, namely the longer the delay time, the greater the number of samples that experience morphological changes compared to samples that are processed immediately.***

Keywords: erythrocytes, leukocytes, platelets

1. Introduction

A health laboratory is a facility that performs measurements, determinations, and tests on materials originating from humans or non - human sources to assess health conditions, identify types of diseases, establish diagnoses, provide treatment, evaluate treatment results, and make other decisions (Wedhaswara, 2018). Laboratory examinations are needed for screening, diagnosis, disease monitoring, and treatment monitoring. Therefore, the laboratory results must be precise, accurate, and reliable.

Laboratory examinations consist of three main processes: pre - analytical, analytical, and post - analytical. These three processes are interconnected and must be carefully considered as they significantly impact the results produced. The pre - analytical stage contributes 61% of the total errors, the analytical stage 25%, and the post - analytical stage 14% (Wedhaswara, 2018).

The pre - analytical stage refers to all the steps that must be taken before a sample can be analyzed. This stage includes aspects related to patient variables (such as diet, age, gender, etc.), specimen collection and labeling techniques, specimen preservatives and anticoagulants, specimen transportation, as well as processing and storage. Delays in processing samples can lead to pre - analytical errors. Prolonged delays in sample processing can cause physical and chemical changes that may result in errors during examination. The maximum allowed storage time at room temperature for peripheral blood smear preparations is less than one hour (Kiswari R, 2014).

The examination of peripheral blood smear preparations is an important part of the series of hematology tests. The advantages of the peripheral blood smear examination include the ability to assess various elements of peripheral blood cells, such as cell morphology (erythrocytes, leukocytes, platelets), determine the number and type of leukocytes, estimate the number of platelets, and identify the presence of parasites (Riswanto, 2013). The purpose of staining peripheral blood smear preparations is to facilitate the observation of different types of cells and to evaluate the morphology of these cells (Rodak, et al., 2007). The International Council for Standardization in Haematology (ICSH) recommends the Romanowsky staining method because it provides satisfactory results on peripheral blood smears (Bain, 2014).

Based on another research journal by John Wiley and Sons (2013), it is stated that the stability of samples in EDTA tubes is maintained for less than 4 hours; after 4 hours, significant changes in cell morphology occur. The most relevant changes include cytoplasmic fragmentation, degranulation, Pelger - Huët anomaly in neutrophils, vacuolization in monocytes, and echinocytes in red blood cells. Morphological changes in platelets are less clear but are characterized by an increase in size.

According to Warsita (2019), there are several factors causing delays in examinations, such as delays in the delivery of referral samples from other hospitals or clinics, and the process of collecting a large number of samples while the number of phlebotomists is limited. When processing samples, attention must be paid to various aspects, starting

from equipment preparation, preparation of the blood collection site, sample collection method, sample volume, actions taken after sample collection, and handling of the collected samples (Sujud, 2015).

Laboratory examinations are necessary for screening, diagnosis, disease monitoring, and treatment monitoring. A laboratory examination is considered high - quality if the results are precise and accurate. This examination consists of three stages: the pre - analytical stage contributes 61% of total errors, analytical errors contribute 25%, and post - analytical errors contribute 14%. In the pre - analytical stage, several factors can cause errors in the examination results, such as delays in processing EDTA samples, which can lead to physical and chemical changes. These changes can affect the morphology of blood cells, such as erythrocytes, leukocytes, and platelets. If the examination is delayed, erythrocytes may show an increase in abnormal cells, such as swelling and bursting cells. Leukocytes may undergo cell degeneration and changes in cell shape integrity, while platelets may become damaged, and their count may decrease.

If there is a delay in processing the samples, it will affect the examination results. Additionally, there has been limited research on the types of blood cells (erythrocytes, leukocytes, and platelets). Therefore, the researcher is interested in conducting a study on the “differences in cell morphology in EDTA blood that is examined immediately and after a 4 - hour delay at a temperature of 20 - 25°C. ”

2. Analysis and Method

This research was conducted at Virtu DigiLab Nusa Dua Main Clinic, located at Jl. Bypass Ngurah Rai No.220, Benoa, South Kuta, Badung Regency. The clinic has four laboratory units, consisting of a Clinical Pathology Laboratory, Anatomical Pathology Laboratory, Microbiology Laboratory, and PCR Laboratory. The research was carried out in two rooms: the Sampling Room for sample collection and the Clinical Pathology Laboratory for sample processing. The Clinical Pathology Laboratory is staffed by one clinical pathology specialist doctor and two laboratory analysts. The most common test performed in the Clinical Pathology Laboratory is a complete blood count (CBC), with an average of 30 samples examined per day. Laboratory personnel follow pre - analytical, analytical, and post - analytical stages. These stages must be carried out correctly to obtain accurate results in line with the patient's medical condition.

The study, titled "Differences in Cell Morphology in EDTA Blood Immediately Examined and Delayed by 4 Hours at 20 - 25°C, " involved two treatments: EDTA blood samples were processed immediately (0 hours) and after being left for 4 hours at room temperature (20 - 25°C). The samples were then prepared for peripheral blood smears and observed under a microscope. The research utilized an Olympus CX23 Binocular Microscope, which had been calibrated to ensure accurate observation of the samples.

The number of male patients obtained was 22 (44%), while the number of female patients was 28 (56%), making the total sample size for the study 50 people (100%). The characteristics of the research subjects were obtained from volunteer patients who underwent a complete blood count and met the inclusion criteria determined by the researchers.

Results of Erythrocyte Morphology Examination

The erythrocyte morphology examination was conducted using the peripheral blood smear method with Giemsa staining on EDTA blood from patients, with two treatments: immediate examination and a 4 - hour delay. The results of the erythrocyte morphology examination at 20 - 25°C are as follows:

Table 2.1: Erythrocyte Morphology Examination Results

Time	No Changes (samples)	Percentage (%)	Changes (samples)	Percentage (%)
0 Hour	50	100	0	0
4 Hours	08	16	42	84

Based on Table 2.1 it can be seen that in the erythrocyte examination performed immediately (0 hours), no cells exhibited morphological changes. In contrast, after a 4 - hour delay, 42 samples (84%) showed morphological changes, while 8 samples (16%) did not exhibit any changes. The observed morphological change in erythrocytes was the presence of burr cells.

Results of Leukocyte Morphology Examination

Table 2.2: Leukocyte Morphology Examination Results

Time	No Changes (samples)	Percentage (%)	Changes (samples)	Percentage (%)
0 Hour	12	24	38	74
4 Hours	03	06	47	94

Based on Table 2.2, it can be seen that in the leukocyte examination performed immediately (0 hours), 38 samples (76%) showed morphological changes, while 12 samples (24%) did not exhibit any changes. In contrast, after a 4 - hour delay, 47 samples (94%) showed morphological changes, and 3 samples (6%) did not. The observed morphological changes in leukocytes included cytoplasmic fading, nuclear fragmentation, and cytoplasmic vacuolization.

Results of Platelet Morphology Examination Results

Table 2.3: Platelet Morphology Examination Results

Time	No Changes (samples)	Percentage (%)	Changes (samples)	Percentage (%)
0 Hour	13	26	37	74
4 Hours	0	0	50	100

Based on Table 2.3 it can be seen that in the platelet examination performed immediately (0 hours), 37 samples (74%) showed morphological changes, while 13 samples (26%) did not. In contrast, after a 4 - hour delay, all samples (100%) exhibited morphological changes. The observed changes included platelets enlarging into macroplatelets and giant thrombocytes, often referred to as giant platelets.

Data Analysis Results

The examination data in this study is presented in tables and analyzed statistically using Statistical Product and Service Solutions (SPSS) version 25.0. The analysis was performed using a parametric test, specifically the Paired Sample T -

Test, to observe differences in cell morphology between samples processed immediately and those delayed by 4 hours. The results of the Paired Sample T - Test analysis are presented in the following table:

Table 2.4: Results of Cell Morphology Data Analysis

Morphology Examination	p- value
Erythrocytes	0.000
Leukocytes	0.002
Platelets	0.000

Based on Table 2.4, the results for erythrocyte morphology show a p - value of 0.000, indicating a p - value < 0.05, which means there is a significant difference in erythrocyte morphology between the samples examined immediately and those delayed by 4 hours. For leukocyte morphology, the p - value is 0.002, also indicating a p - value < 0.05, showing a significant difference in leukocyte morphology between the two conditions. Similarly, the results for platelet morphology show a p - value of 0.000, indicating a significant difference in platelet morphology between immediate and 4 - hour delayed examinations.

3. Discussion

3.1 Results of Erythrocyte, Leukocyte, and Platelet Morphology Examination in EDTA Blood Immediately Examined at 20 - 25°C

Based on Table 2.1, it can be observed that in the erythrocyte examination conducted on 50 samples, no morphological changes were found in the samples processed immediately. The erythrocytes observed were in good condition. According to the literature, erythrocytes are non - nucleated blood cells that have a biconcave disc shape. These cells appear red due to the presence of hemoglobin. Under the microscope, erythrocytes appear round, red, and paler in the center (Kiswari, 2014).

Based on Table 2.2, in the leukocyte examination conducted on 50 samples, 12 samples (24%) showed no changes, while 38 samples (76%) exhibited morphological changes. According to the literature, leukocytes have a colorless nucleus and are larger in size compared to erythrocytes (Maharani, 2018). This study's findings show that a larger number of leukocytes underwent changes, which contrasts with the study by Asiyah et al. (2018), where no significant changes were observed. This discrepancy may be due to differences in research variables, methods, and tools used.

Based on Table 2.3, in the platelet examination conducted on 50 samples, 13 samples (26%) showed no changes, while 37 samples (74%) exhibited morphological changes. Platelets are derived from the cytoplasmic fragmentation of megakaryocytes and do not have a nucleus. Platelets have adhesive and aggregative properties, meaning they must be examined immediately, and delays beyond one hour should be avoided (Harjo, 2011).

3.2 Results of Erythrocyte, Leukocyte, and Platelet Morphology Examination in EDTA Blood Delayed for 4 Hours at 20 - 25°C

Based on Table 2.1, in the erythrocyte examination conducted on 50 samples, 8 samples (16%) showed no changes, while 42 samples (84%) exhibited morphological changes after a 4 - hour delay. Normal erythrocytes are always biconcave, non - nucleated, and contain hemoglobin, which gives blood its red color. The morphological changes observed were the presence of abnormal erythrocytes with protrusions around the cell surface. When sample processing is delayed, the anticoagulant in the EDTA tube reduces the surface tension of the erythrocyte membrane, making the membrane weak and unstable. The erythrocytes swell, forming surface protrusions, leading to a shape change from disc - shaped to echinocyte.

This finding is consistent with a study by Yunus et al. (2022), which observed that erythrocyte morphology in EDTA blood smears, based on storage time, showed morphological abnormalities in size, staining, and shape after a 2 - hour delay. Both studies found morphological changes occurred.

The 4 - hour delay caused morphological changes in erythrocytes, where they appeared swollen with protrusions on their surface. EDTA tubes contain K3EDTA anticoagulant, which prevents blood clotting, so EDTA tube samples must be processed quickly to reduce examination errors.

Cinthia's (2018) research also produced similar results. After a 3 - hour delay, 12.5% of samples were categorized as good, while 87.5% were categorized as poor, indicating that more damage occurs when EDTA blood examination is delayed. This can be attributed to chemical, biomechanical, and immunological changes in blood cells during storage, which may cause structural or morphological damage.

The findings are further supported by a study conducted by Isti et al. (2018), which observed samples over storage periods of 0, 7, 14, 21, and 28 days. The study reported that abnormal erythrocyte morphology increased with prolonged storage time.

Based on Table 2.2, it can be seen that in the leukocyte examination conducted on 50 samples, after a 4 - hour delay, 3 samples (6%) showed no changes, while 47 samples (94%) exhibited morphological changes. Normal leukocytes are larger than erythrocytes, with a colorless nucleus and clear cytoplasm. The changes observed included cytoplasmic fading, nuclear fragmentation, and cytoplasmic vacuolization. These changes occurred due to the delayed examination of EDTA samples, which can cause blood cells to undergo morphological alterations.

These findings align with a study by Zalinna (2017), which states that delaying examination for more than 2 hours can lead to leukocyte lysis, vacuolization, degranulation, hypersegmentation, and the disintegration of leukocytes into smaller sizes, potentially affecting the results of the examination.

The study is also supported by research by Erlin (2014), which found significant differences in leukocyte counts in EDTA blood stored at room temperature and examined after 2, 4, and 6 hours. Delaying EDTA blood examination for 1 - 3 hours causes swelling in the leukocyte nucleus, leading to structural changes such as nuclear fragmentation, cytoplasmic fading, and vacuolization. If automated cell counting is performed, the results may not accurately reflect the sample condition.

Delaying the examination time can affect leukocyte morphology; the longer the delay, the more the cells' morphology deteriorates due to hemolysis or cell death. Blood cells undergo biochemical, biomechanical, and immunological changes during storage, causing morphological damage. Storage can result in lobulation, disintegration, and vacuolization in leukocytes. Additionally, research by Ekanem (2012) supports that delays in examination time reduce the leukocyte count, as cells are damaged (hemolysis) or die. If the examination is delayed beyond 2 hours, the blood specimen may undergo degeneration of blood elements. This pre - analytical phase is the most significant contributor to examination errors.

Based on Table 2.3, it can be seen that in the platelet examination delayed by 4 hours, all 50 samples (100%) exhibited morphological changes. These changes included platelets enlarging into macroplatelets and the presence of giant thrombocytes.

This finding is consistent with research by Wirawan (2011), which states that delaying platelet counts in EDTA blood for 1 hour can cause platelets to easily adhere to each other (aggregation) or attach to foreign objects (adhesion).

Another study by Merta et al. (2014) noted that delaying blood collection for 1 - 3 hours causes swelling in the cell nucleus, changes in nuclear chromatin, and eventually disintegration of the cells. The decrease in platelet count following a delay in examination is due to the swelling of platelets, resulting in the presence of giant platelets.

3.3 The Effect of Delayed Processing of EDTA Blood on Erythrocyte, Leukocyte, and Platelet Morphology When Examined Immediately and After a 4 - Hour Delay at 20 - 25°C

Based on Table 2.4, the Paired Sample T - Test results indicate the differences in cell morphology between samples examined immediately and those delayed by 4 hours. The results for erythrocyte morphology showed a p - value of 0.000, indicating $p < 0.05$, meaning there is a significant difference in erythrocyte morphology between samples examined immediately and after a 4 - hour delay. The results for leukocyte morphology showed a p - value of 0.002, again indicating $p < 0.05$, showing a significant difference in leukocyte morphology between the two conditions. Similarly, the platelet morphology results showed a p - value of 0.000, also indicating a significant difference in platelet morphology between immediate and delayed examinations.

The observed changes in erythrocyte morphology included the presence of burr cells. In leukocyte morphology, changes such as cytoplasmic fading, nuclear fragmentation, and cytoplasmic vacuolization were observed. In platelets, after a 4 - hour delay, macroplatelets and giant thrombocytes were identified. These results demonstrate microscopically that the delay in processing affects the quality of erythrocyte, leukocyte, and platelet morphology. Each cell type has different stability levels, so if delays are unavoidable, the maximum allowable delay time should be carefully considered.

A study by John Wiley & Sons (2013) found that sample stability in EDTA tubes is maintained for less than 4 hours, after which significant morphological changes occur. This aligns with research by Kiswari (2014), which stated that the maximum storage time for EDTA blood samples, particularly concerning leukocyte count, is 2 hours at room temperature. According to Hardiasari et al. (2015), platelet examinations should be performed accurately and within less than 1 hour after blood collection. Therefore, delays in processing can significantly impact the quality of blood cell morphology.

Challenges encountered during the research process can lead to less valid results, so it is important to carefully consider the factors that can affect research outcomes.

4. Conclusion

Based on the results and discussion of this study, the following conclusions can be drawn:

1) The results of cell morphology examination in EDTA blood samples that were examined immediately showed that 100% of erythrocyte samples had no morphological changes, indicating that the erythrocytes in all samples were in good condition. For leukocytes, 24% showed no changes, while 76% exhibited morphological changes. For platelets, 26% showed no changes, while 74% exhibited morphological changes.
2) The results of cell morphology examination in EDTA blood samples delayed for 4 hours showed that 16% of erythrocytes had no changes, while 84% exhibited morphological changes. For leukocytes, 6% showed no changes, while 94% exhibited morphological changes. In platelets, all samples (100%) exhibited morphological changes.
3) There is a significant difference in the morphology of erythrocytes, leukocytes, and platelets between samples examined immediately and those delayed for 4 hours, with each showing a p - value of less than 0.05.

Ethical Clearance

This research has been number of ethical clearance at research ethical comitee with number registered no.61/E1. STIKESWIKA/EC/II/2023.

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