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ERYTHROCYTE MORPHOLOGY ON PERIPHERAL BLOOD SMEAR USING AN EDTA ANTICOAGULANT TUBE AT 0, 3,5, AND 7 HOURS AT ROOM TEMPERATURE AT UPTD LABKESDA OF GORONTALO PROVINCE

Chindi Olyvia Manihiya[1)], Rita Amini Warastuti[2)], and Natan Pasiga[3)]
[1,2)]BinaMandiriUniversity Gorontalo, Indonesia
[3)]Suwawa Health Center
E-mail: cindymanihiya29@gmail.com

ABSTRACT

Peripheral Blood Smear (SADT) is one of the laboratory tests used to see blood morphology one of them is erythrocyte blood morphology, then microscopically looked at the shape, size, color, and blood cell disorders with blood samples using the EDTA anticoagulant tube. EDTA blood sample *(Etilen Diamine Tetra Asetat)* stored at room temperature for too long can cause cell morphological abnormalities namely krenase cell. This study aims to determine the description erythrocyte morphology on peripheral blood smear using an EDTA anticoagulant tube based on variations in inspection time.

This type of study is descriptive with an experimental approach. The sampling technique is purposive Sampling with a sample of 16 respondents and checked at 0, 3, 5, and 7 hours then the results were analyzed using SPSS with the cross-tabulation test. The results showed that there was a change in the shape of erythrocytes in the form of krenase cells at 0 hours which had good criteria, at 3 hours 37.5% good, medium category 62.5%, at 5 hours it was good category 6.2%, medium category 37.5%, bad category 56.2%, at 7 hours 37.5% were categorized as bad, 62.5% was very bad. So it can be concluded that there is a change in the erythrocytes morphology that form cell krenase, the formation of krenase cells occurs as the delay in the examination time is increased as evidenced by the cross-tabulation test.

Keywords: Erythrocyte Morphology, EDTA Blood, Postponementof Examination Time

INTRODUCTION

Blood is the main component in the body as a supplier of oxygen that is distributed to body tissues, blood is in the form of fluid consisting of 3 types of blood cells, namely erythrocytes, platelets, and leukocytes which have their respective roles in the body. Erythrocytes are red blood cells that contain hemoglobin as a transport for oxygen and carbon dioxide throughout the body's tissues that contain iron, where iron is obtained from good and healthy food intake such as vegetables, namely spinach. Leukocytes or white blood cells act as antibodies or the body's first defense against disease, which if there is inflammation or infection in the body, the leukocytes will increase to fight disease attacks, leukocytes consisting of monocytes, lymphocytes, eucinophils, basophils, and neutrophils[4].

In laboratory testing, the test results greatly affect the medical diagnosis and therapy applied. In a clinical laboratory setting, the sequence of each test begins with patient preparation, continues with sample collection, sample processing and analysis then ends with results reporting[1].

A number of error factors that often occur, namely in pre-analytic about 60-70%, analytic about 10-15% and post-analytic about 15-20% in the diagnostic laboratory. Pre-analytic accounts for the largest error, this percentage is generally a problem that arises from patient preparation, sample collection, sample delivery and storage[13].

Delays in examination often occur in the field due to delays in sending samples, besides the long distance in the process of sending samples to the laboratory also contributes to delays in sample examination[17].

In the blood morphological examination, it is carried out using a venous blood sample that is inserted into an EDTA (Ethylene Diamin Tetra Acetate) tube which contains anticoagulants to inhibit blood clotting by binding to calcium ions so that it does not dissolve in blood[10].

EDTA (Ethylene Diamin Tetra Acetate) is available in the form of powdered di-Potassium (K2) and liquid tri-Potassium (K3), Potassium Ethylene Diamin Tetra Acetate (K3EDTA) is a type of anticoagulant that is often used to prevent the coagulation process by binding calcium ions so that an insoluble salt is formed [7]. The ratio of blood with EDTA (Ethylene Diamin Tetra Acetate) is 1: 1 meaning 1-1.5 mg EDTA / ml blood. A 3 ml blood sample requires 4.5 mg of EDTA (Ethylene Diamin Tetra Acetate) powder, when given in 10% solution it takes 45 µl[9].

EDTA (Ethylene Diamin Tetra Acetate) is hypertonic to blood cells, so the concentration must be right because of the effect of increasing the EDTA (Ethylene Diamin Tetra Acetate) concentration above normal values which will affect erythrocytes, causing damage to cell membranes or hemolysis. The erythrocyte count shows low results due to the inaccurate ratio of the volume of K3EDTA (Tri-Potassium Ethylene Diamine Tetra Acetate) with blood because the excessive volume of anticoagulants will cause shrinkage and degenerative changes of erythrocytes.' causing a decrease in the number of erythrocytes because the anticoagulant EDTA (Ethylene Diamin Tetra Acetate) is hyperosmolar[15].

Storage of EDTA (Ethylene Diamin Tetra Acetate) blood at room temperature for too long can cause a series of changes in erythrocytes such as the rupture of the erythrocyte membrane (hemolysis) so that free hemoglobin into the surrounding medium or plasma. This can lead to errors in the results[15].

The length of time the samples are stored causes the blood cells to undergo biochemical, biomechanical, and immunological changes that cause structural / morphological damage known as storage lesions. Crenation is a form of erythrocytes that shrink and develop bumps on the surface. Krenation usually forms in blood that is left for a long time, which means it is also exposed to anticoagulants for a long time[19].

Variations in storage time and temperature of blood samples can affect the quality of the sample which results in changes in the shape and size of erythrocyte cells through examination of the peripheral blood smear, the temperature of 4^0C is stored for a maximum of 2 hours in a cooling cabinet while at room temperature of 25^0C it is stored for a maximum of 1 hour[14].

Previous research by Ajeng Galih Wedhaswara in 2018 with the title Effect

of Delay in Making Edible Blood Smear Preparations in EDTA Samples on Red Blood Cell Morphology, the results showed that in smear preparations the morphology of red blood cells was 100% good, with a delay of 3 hours, 8 preparations were found (88.9 %) moderate and 1 preparation (11.1%) was bad, while at a delay of 9 hours, 6 samples of bad preparations (66.67%) and 3 preparations (33.33%) were very bad. red blood cell morphology.

RESEARCH METHODS

The type of research used is descriptive with an experimental approach, namely the type of research that aims to obtain a complete picture of the morphology of erythrocytes in peripheral blood smears at 0, 3, 5, and 7 hours at room temperature[3].

The location of the research was carried out at the Regional Health Laboratory Center of Gorontalo Province. The population in the study were students of class 2017 D-3 Health Analyst Study Program, Bina Mandiri University Gorontalo, as many as 58 populations with a total sample of 16 calculated based on the Sudjatmiko formula.

The results of the examination obtained are primary data, which is then made in the form of a table using univariate data analysis techniques by describing or describing the research variables then tested using the crosstabulation test in the SPSS application for two or more variables by looking at the column or row percentages[12].

The variable in this study, namely the independent variable (independent), is a variable that affects the dependent variable (dependent). What acts as the independent variable in this study is the variation of inspection at 0 hours, 3 hours 5 hours, and 7 hours at room temperature. The dependent variable is the variable that is influenced by the independent variable (independent). What acts as the dependent variable in this study is the morphological description of erythrocytes.

The instruments used in this research were EDTA tubes, tourniquet, writing instruments, tube racks, glass objects, deck glass, dropper pipettes, sample racks and microscopes. The materials used in this study were blood samples, dry cotton, alcohol. swabs, plaster, syringes, Giemsa, and methanol. Then the venous blood was taken after that made the preparations of the peripheral smear using Giemsa's stain 10% then carried out the examination under a microscope with a 10x magnification of the ocular lens and 100x objective lens, and seen the color, size and shape of the erythrocytes.

The sampling technique in this study was purposive sampling, which was selected based on inclusion and exclusion criteria.

A. The inclusion criteria in the study are:
  a. Respondents who are willing to be research subjects
  b. Respondents who were not included as post-splenectomy patients
  b. Respondents who did not take anti-inflammatory drugs and chemotherapy

B. The exclusion criteria in this study were:
  a. Respondents who were included as post splenectomy patients
  b. Respondents who took anti-inflammatory drugs and chemotherapy

Operational definitions in this study are:

a. Erythrocyte morphology is the structure or shape of erythrocytes that can be examined under a microscope to see any abnormalities in erythrocytes by looking at the shape,

size, color, and cell abnormalities in erythrocytes.

b. Peripheral blood smear is an examination that is able to assess various elements of peripheral blood cells such as cell morphology (erythrocytes, leucocytes, platelets), determine the number and type of leukocytes, estimate the number of platelets and identify the presence of parasites.
c. Variations in storage time and temperature of blood samples can affect the quality of these samples resulting in changes in erythrocyte cell shape through examination of peripheral blood smears performed at 0 hours, 3 hours, 5 hours and 7 hours.

RESEARCH RESULT

Table 1.

Distribution of Respondents by Gender

Gender	Erythrocyte morphology examination at 0, 3, 5, and 7 hours at room temperature	
	N	%
Male	6	37.5
Women	10	62.5

Source: Data processed (2020)

Examination of erythrocyte morphology at 0, 3, 5, and 7 hours at room temperature based on the distribution of respondents according to gender, which is presented in table 1, there are 16 respondents with 6 male respondents (37.5%) and 10 female respondents(62.5%).

Table 2.

Crosstab test to check the size of erythrocytes

Erythrocyte Size	Postponement of Examination Time				Total
	0 hours	3 hours	5 hours	7 hours	
Normocytic	100.0%	100.0%	100.0%	100.0%	100.0%
Microcytic / macrocytic	.0%	.0%	.0%	.0%	.0%

Source: Data processed (2020)

The results of the morphological examination of erythrocyte size showed that 64 samples that were examined at 0 hours, 3 hours, 5 hours, 7 hours did not show a change in erythrocyte size, that is, all samples were normal or normocytic with a percentage of 100%.

Table 3.

Crosstab Test, Morphological Examination of Erythrocyte Color

Erythrocyte Color	Postponement of Examination Time				Total
	0 hours	3 hours	5 hours	7 hours	
Normochrome	100.0%	100.0%	100.0%	100.0%	100.0%
Hypochromic / Polychrome	.0%	.0%	.0%	.0%	.0%

Source: Data processed (2020)

The results of the erythrocyte color morphological examination showed that 64 samples that were examined at 0 hours, 3 hours, 5 hours, 7 hours did not show a change in erythrocyte color, i.e. all samples were normal or normochromic with a percentage of 100%.

Table 4.

Erythrocyte morphology examination crosstab test

Categorical Form of Erythrocytes	Postponement of Examination Time				Total
	0 hours	3 hours	5 hours	7 hours	
Good	100.0%	37.5%	6.2%	.0%	35.9%
Moderate	.0%	62.5%	37.5%	.0%	25.0%
Bad	.0%	.0%	56.2%	37.5%	23.4%
Very bad	.0%	.0%	.0%	62.5%	15.6%
Total	100.0%	100.0%	100.0%	100.0%	100.0%

Source: Data processed (2020)

Information :

Table 5.

Description of Research Results

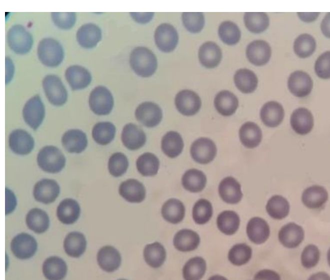
Sample	Research result	Categorical
S = No. Sample	1	Good: No chrenase cells were found
	2	Medium: Found 1-3 krenase / Lp cells
	3	Bad: Found 4-6 cells krenase / Lp
	4	Very Bad: Found> 6 cells krenase / Lp

Source: Data processed (2020)

Based on the results of the morphological examination, the shape of erythrocytes was categorized as good at 0 hours 100.0%, 3 hours 37.5%, 5 hours 6.2%, 7 hours .0% with a total of 35.9%. Medium category at 0 hours .0%, 3 hours 62.5%, 5 hours 37.5%, 7 hours .0% with a total of 25.0%. Bad category at 0 hours .0%, 3 hours .0% 5 hours 56.2%, 7 hours 37.5% with a total of 23.4%. And very bad category at 0 hours .0%, 3 hours .0% 5 hours .0%, 7 hours 62.5% with a total of 15.6%.

Picture 1

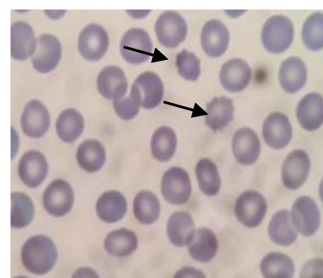
Ertyrocyte Morphology Observation at 0 Hours



Good: No chrenase cells were found

Figure 2

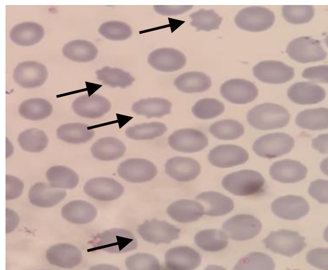
Erythrocyte morphology observation at 3 hours



Medium: Found 1-3 cells krenase / LP

Figure 3

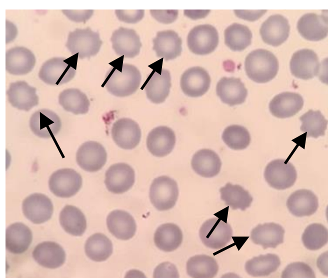
Observation of Ertirocyte Morphology at 5 Hours



Bad: 4-6 cells krenase / LP were found

Figure 4

Observation of Ertirocyte Morphology at 7 Hours



Very bad: Found> 6 cells krenase / LP

DISCUSSION

This study took a case description of the results of erythrocyte morphology on the peripheral blood smear (SADT) using the anticoagulant EDTA (Ethylene Diamin Tetra Acetate) at 0, 3, 5, and 7 hours at room temperature. Respondents in this study were 16 people, 6 respondents were male (37.5%) and 10 female respondents (62.6%) were selected based on sample criteria, namely respondents who were not included as post-splenectomy patients, respondents who did not take anti Inflammation and chemotherapy were used as research samples using purposive sampling technique, which were selected based on sample criteria, namely respondents who were not included as post-splenectomy patients, respondents who did not take anti-inflammatory drugs and chemotherapy were used as research samples.

Post-splenectomy respondents will experience a disruption in the erythrocyte glycolysis system which causes reduced ATP (Adenosine Triphosphate) or increased intracellular calcium content, increased intracellular calcium causes loss of potassium and water in the erythrocyte membrane resulting in hemolysis which results in changes in erythrocyte shape shrinking or forming cell crenase[6] . In someone who takes anti-inflammatory drugs or chemotherapy will experience swelling of the erythrocyte membrane causing fluid to come out of the cells so that the erythrocytes experience shrinkage (krenase)[6].

Based on table 2, which is processed using the crosstabulation test, the results of the morphological examination of erythrocyte size show that 16 samples that were examined at 0 hours, 3 hours, 5 hours, 7 hours did not show a change in erythrocyte size, that is, all samples were in normal condition. Microscopic image of erythrocyte size, namely erythrocytes appear round and 7-8 μ in diameter. Changes in erythrocyte size can be caused by disruption of the mitosis process, hemoglobin synthesis, and abnormalities of cell organelles[17].

Based on table 3, which is processed using the crosstabulation test, the results of the erythrocyte color morphology examination show that 16 samples that were subjected to a delay of examination at 0 hours, 3 hours, 5 hours, 7 hours did not show a change in erythrocyte color, i.e. all samples were in normal condition The microscopic image of erythrocyte color morphology is that the erythrocytes appear red in color and appear paler in the middle with a diameter of approximately one third of the total erythrocyte diameter. Color variations indicate cytoplasmic

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content which can be caused by a lack of iron or describes cell immaturity[17].

Based on table 4, which is processed using the crosstabulation test, the results of erythrocyte morphological examination with a delay of 0 hours, 3 hours, 5 hours, and 7 hours at room temperature show that the morphological results of the erythrocyte shape at 0 hours were not found to be any deformities of erythrocytes, meaning 16 samples in good condition, at 3 hours it was found 6 samples were in good condition and 10 samples were in the moderate category, at 5 hours 1 sample was in the good category, 6 samples were in the moderate category and 9 samples were in the bad category, at 7 hours, 6 samples were in the bad category and 10 samples were categorized as very poor. This means that the morphological examination of the shape of erythrocytes is based on the variation in examination time (0 hours, 3 hours, 5 hours,and 7 hours) at room temperature the longer the delay in the examination will affect the morphology of erythrocyte shape, namely the formation of krenase cells so that the number of good percentages decreases and the number of bad percentages increases.

The formation of krense cells occurs with the length of time delay in the examination, the crenase cells are in the form of bumps on the erythrocyte surface due to drying, and storage of samples that are too long, and due to osmotic pressure imbalance[2].

In the S07 sample, the morphological results of the shape of erythrocytes with a time delay of 0 hours, 3 hours and 5 hours were still in good condition and at 7 hours there were 5 cells krenase / LP with the anticoagulant EDTA (Ethylene Diamin Tetra). Acetate), which has a pH stability close to the pH of the blood, which is about 6.4 to prevent clots and changes in blood morphology[16]. However, K3EDTA (Potassium Ethylene Diamine Tetra Acetate) is a hypertonic solution to blood cells, so that the longer the sample is exposed to anticoagulants, it will affect blood morphology, causing shrinkage of red blood cells[16].

Red blood cells or erythrocytes have semi-permeable properties, which are able to absorb water or certain substances, if in a hypertonic state it will cause osmotic pressure disturbances that occur from inside the cells out of the cells so that red blood cells experience shrinkage (krenase)[19].

Delay in examining the EDTA (Ethylene Diamine Tetra Acetate) blood sample for too long at room temperature causes a series of erythrocyte changes such as hemolysis or the rupture of the cell membrane in the erythrocytes so that the cells are dehydrated and change shape to become crenated. Rupture of the erythrocyte membrane causes fluid displacement from the intracellular to the extracellular part, thereby disrupting the osmotic balance[18].

EDTA (Ethylene Diamin Tetra Acetate) blood samples that are not checked for more than 2 hours can be stored in a refrigerator, because the blood concentration in the specimen can change as a result of various metabolic processes[8].

The making of the Peripheral Blood Smear (SADT) is carried out on a clean glass object using a venous blood sample, then wiped off and then dried and fixed, which functions to glue the smear so that the smear absorbs color properly, the fixation process uses absolute methanol to lyse the cell walls and stop the metabolic process cells without changing the shape or structure of these blood cells. Poor fixation can also cause changes in the morphology of blood cells, too long inundation causes methanol to evaporate and has a water content of> 3% in it which can lead to chrenase morphology

because the solution becomes hypertonic[11].

The fringing blood smear (SADT) staining uses giemsa staining because it has dye resistance properties and the staining results are clearer, which is diluted first so that the acidity or pH of the diluent is maintained, the pH of the Giemsa solution should be between 6.8-7.0 . If the pH of Giemsa is not suitable, it will affect the quality of the staining so that the erythrocyte morphology is unclear, it is better to use giemsa immediately after the dilution because changes in pH in the solution can also affect peripheral blood cells, one of which is erythrocyte morphology, which affects the resultsexamination[14].

Factors that can affect the results of other examinations are the quality of the peripheral blood smear, including delayed examinations that cause distortion or damage to blood cells, slow smearing after blood is dripped on the slide which causes a disproportion of large cells, too many drops or too little causes the smear to be too thick and long or too thin and short, the angle of the slide is too large so that the smear is too thick or too small causes the smear to be too long, and too slow the swab causes uneven cell distribution[7].

Improper sample handling includes wearing the dam bond for too long or too hard causing hemoconcentration, clotting in the syringe due to slow work, clotting in the tube due to improperly mixed with dry oxalate or other anticoagulants[7].

CONCLUSION

Based on the research results that have been described, it can be concluded that:

1. The results of the morphological examination of the size and color of erythrocytes were 100% good.
2. The results of the morphological examination of the shape of erythrocytes at 0 hours have good criteria, at 3 hours it is 37.5% good, 62.5% moderate, at 5 hours good 6.2%, 37.5% moderate, 56.2% bad, at 7 hours it is bad 37.5%, 62.5% very bad.
3. There is a change in the morphology of erythrocytes that form krenase cells, the formation of chrenase cells occurs as the delay in the examination time increases as evidenced by the crosstabulation test

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