ANALYSIS OF DIFFERENCES IN ERYTHROCYTE MORPHOLOGY IN K_3EDTA AND Na_2EDTA BLOOD CLOTS BASED ON TIME SAMPLE STORAGE

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ABSTRACT

Blood is a liquid system that combines blood plasma and blood cells. Blood cells consist of three types, namely erythrocytes, leukocytes and platelets. For analitical check up, anticoagulant is needed to prevent blood clotting by binding (chelation) or precipitating (precipitation) calcium, or by inhibiting the formation of thrombin needed to convert fibrinogen to fibrin in the freezing process. Check up using EDTA blood tests must be done immediately, if forced to postpone it should be noted the storage time limit for each check up. To make peripheral blood smear preparations can be used EDTA blood stored at least 2 hours at room temperature. There are two types of EDTA salts, namely sodium and potassium. The purpose of this research was to find out the comparison of the erythrocyte morphology in blood smear K_3EDTA and Na_2EDTA based on storage time variations. The type of research conducted is laboratory experiments. Data analysis used t test for erythrocyte form and description for erythrocyte size and color. The results of the t test between the forms of blood cationation Na_2EDTA and K_3EDTA chronology obtained significance values (2 tailed) 0.613> 0.05 (p> α) so that it can be concluded that H_0 is accepted and Ha is rejected which means there are differences but not meaningful, but can be seen from the mean Na_2EDTA (20,1667)> K_3EDTA (17,4167) which means the average Na_2EDTA crenation is higher than the K_3EDTA blood sample. There is no significant to morphological changes between the blood sample of Na_2EDTA and K_3EDTA. There is storage time effects on the erythrocyte shape but there is no effect on the size and color of erythrocyte.

Keywords: Edge Blood Smear, Na_2EDTA and K_3EDTA, erythrocyte morphology

INTRODUCTION

Blood is an essential component of living things, from primitive animals to humans. In physiological conditions, blood is always in the blood vessels so that it can carry out its functions as: (a) oxygen carrier (oxygen carrier); (b) the body's defense mechanism against infection; and (c) the mechanism of hemostasis. (I Made Bakta, 2013).

EDTA blood tests should be done immediately, only if necessary, may be stored in the refrigerator (4˚C). to make the blood smear smear can be used EDTA blood stored for a maximum of 2 hours. (Gandasoebrata, 2011). Referring to the research conducted by Flowerina Bilinda, based on the morphological examination of erythrocytes in EDTA blood smear with sample storage time for 0 hours, 2 hours, 4 hours, 6 hours, the results showed that the erythrocyte morphology in EDTA swab with 0-hour storage contained a percentage of occurrence chronicle as much as 5.37%, the time delay of the 2-hour examination there is a percentage of occurrence of curing as much as 11.28%, the time of delay of 4 hours examination there is a percentage of occurrence of curing as much as 39.89%, while delaying examination at 6 hours is 43 46%.

Factors affecting erythrocytes are: sample storage time, solution concentration, type of anticoagulant, and volume of anticoagulants. Storage of samples that are too long can affect the morphology of erythrocytes such as cataract erythrocytes. The concentration of the solution is very influential because the erythrocyte membrane is semipermiable which can be penetrated by liquids and other substances which will make the erythrocytes swell and rupture. The type of anticoagulant used for the morphological examination of erythrocytes is EDTA because it does not affect the morphology of blood cells. The volume of anticoagulants used in excess can make the erythrocytes shrink.

But the reality found in the field is often not appropriate. Not infrequently in some health services the distance between the sampling room and the laboratory is quite far so that the sample is collected first, then sent to the laboratory. Or because of the large number of patients, so that the examination is delayed and the deadline for examining the sample is less attention. As a result the examination must be postponed and this can affect the final results on reading peripheral blood smear preparations.

The purpose of this study was to determine the differences in the morphology of erythrocytes in blood smear K_3EDTA and Na_2EDTA based on storage time variations.

METHOD

The study used a type of laboratory experimental research. The location of the study was carried out at the Campus Hematology Laboratory of the Health Analyst Department of the Makassar Health Polytechnic. When the study was conducted in May 2019.

The population in this study were all blood. The sample used in this study was blood stored 0 hours, 1.5 hours, 3 hours, 4.5 hours. The sample criteria consist of inclusion criteria, namely: Normal blood. While the exclusion criteria are: abnormal blood. The sampling technique of this study used purposive sampling technique. The sample in this study will be treated in triplo.

DATA COLLECTION

Instrument research

The tools used in this study include disposable syringes, alcohol cotton, tourniquets, masks, handscoons, object glass, test tubes, object glass shelves, test tube racks, Pasteur pipettes, micropipettes and tips.

The research materials used were: Whole Blood, 10% Na_2EDTA Anticoagulant and 10% K_3EDTA, Giemsa Dyes, Methanol.

Work Procedure

1. Pre Analytical

a. Patient preparation: do not require special preparation.
b. Sample preparation: collecting blood samples (1 sample 2 tubes), a tube containing Na_2EDTA and a tube containing K_3EDTA.
c. The principle of smear preparation: blood smear is made on glass objects.
d. The principle of coloring: based on the chemical properties in the cell. Alkaline dyes, and vice versa.

2. Analytic

a. How to get venous blood

1) Clean the cubital vein area with 70% alcohol and let it dry.
2) Attach the damming bond to the upper arms and have the person clench the veins so that they are clearly visible.
3) Tighten the skin over the vein with the fingers of the left hand so that the vein cannot move.
4) Stick the skin with a needle and syringe in the right hand to the tip of the needle into the lumen of the vein.
5) Remove or stretch the dam and slowly pull the suction syringe to the desired amount of blood.
6) Remove the dam if it is still installed.
7) Place the cotton over the needle and pull out the syringe and needle.
8) Ask the person whose blood was taken so that the puncture site was pressed for several minutes with the cotton.
9) Lift the needle from the syringe and drain (do not spray) blood into the container or tube that is available through the wall.

b. Making EDTA blood

1) Provide 2 tubes filled with 10µl Na_2EDTA and 10µl K_3EDTA, respectively.
2) Flow each 1ml of venous blood into the tube from a needle-free syringe.
3) Close the tube and immediately mix the blood with anticoagulants for 60 seconds or more.
4) Take blood for a check directly from each tube.

c. Make smear preparations

1) Touch without touching the small blood skin (the center line does not exceed 2 mm) with the glass, about 2 cm from the tip, and place the glass on the table with the blood tets on the right
2) With the right hand placed a glass of another object to the left of the blood drop and moved to the right until it reaches the drops of blood.
3) Blood drops will spread to the side of the sliding glass. Wait until the blood reaches a point of about ½ cm from the angle of the sliding glass.
4) Immediately slide the glass to the left while holding it tilted at an angle of 30-45 degrees. Don't push the sliding glass down.
5) Let the preparation dry in the air. The identity of the sample was written on the thick part of the pausan with a glass pencil.

d. Coloring the smear

1) Place the smeared mixture on the coloring rack.
2) Fix with methanol for 5-10 minutes until all smear surfaces are covered with solution.
3) Dispose of excess methanol from the glass
4) Foliage of smear prepared with giemsa dyes which have been diluted with aquadest (1: 9 for 30 minutes or 1: 3 for 10)
5) Rinse with tap water, first with slow flow then stronger with the aim of removing all excess dyes.
6) Place the preparation in a vertical manner and let it dry itself.

DATA ANALYSIS

For data analysis used t test for erythrocyte form and description for the color and size of erythrocytes. This hypothesis test will be conducted with the Statistical Product and Service Solutions (SPSS) program.

RESULTS

The results of research conducted from Na_2EDTA blood samples and K_3EDTA blood samples stored at 0 hours, 1.5 hours, 3 hours, and 4.5 hours showed that morphological changes in erythrocytes in blood samples of Na_2EDTA and K_3EDTA only occurred in the form of erythrocytes, i.e. the form of frustration increases every hour of storage. Whereas for color and size remain normal.

Based on the table of t-test results on the morphological forms of blood sample erythrocytes Na_2EDTA and K_3EDTA. Based on the significance value of the results above, it is known that the blood samples Na_2EDTA and K_3EDTA have a significance value (t) 0.613 greater than 0.05 (t> 0.05) so Ha is accepted and H_0 is rejected which means there are no significant differences.

Table 1
Results of examination of blood smear in Na_2EDTA blood samples

Time Storage		Morphology of Erythrocyte		
		Stain	Size	Shape
) hour	1	normokrom	normositik	crenated 2%
	2	normokrom	normositik	crenated 3%
	3	normokrom	normositik	crenated 4%
,5 hour	1	normokrom	normositik	crenated 12%
	2	normokrom	normositik	crenated 14%
	3	normokrom	normositik	crenatedi 14%
3 hour	1	normokrom	normositik	crenated 26%
	2	normokrom	normositik	crenated 28%
	3	normokrom	normositik	crenated 29%
,5 hour	1	normokrom	normositik	crenated 35%
	2	normokrom	normositik	crenated 37%
	3	normokrom	normositik	crenated 38%

Table 2
Results of examination of blood smear in K_3EDTA blood samples

Time Storage		Morphology of Erythrocyte		
		Stain	Size	Shape
0 hour	1	normokrom	normositik	Crenated 1%
	2	normokrom	normositik	Crenated 3%
	3	normokrom	normositik	Crenated 3%
1,5 hour	1	normokrom	normositik	Crenated 9%
	2	normokrom	normositik	Crenated 12%
	3	normokrom	normositik	Crenated 10%
3 hour	1	normokrom	normositik	Crenated 23%
	2	normokrom	normositik	Crenated 22%
	3	normokrom	normositik	Crenated 24%
4,5 hour	1	normokrom	normositik	Crenated 32%
	2	normokrom	normositik	Crenated 36%
	3	normokrom	normositik	Crenated 34%

Table 3
T test results on the morphological forms of blood samples Na_2EDTA and K_3EDTA

	Sampel Darah	n	Mean	T	Sig (2-tailed)
Crenated	Darah Na_2EDTA	12	20.1667	0.513	0.613
	Darah K_3EDTA	12	17.4167	0.513	0.613

DISCUSSION

Blood is a special connective tissue that circulates throughout the body, plays a role in transporting respiratory gases, digestive results, functional components such as enzymes, hormones, and various other molecules, as well as the disposal of waste metabolism. Blood is composed of cell components and a liquid called plasma. Blood cells consist of erythrocytes, leukocytes and platelets. Each cell has an important task to support body activity.

Red blood cells (erythrocytes) are biconcaf cells with a diameter of about 7 microns. Biconcavitation allows the movement of oxygen to enter and exit cells

rapidly with a short distance between the membrane and the cell nucleus. The color is rosy, because it contains a substance called hemoglobin.

Anticoagulants are substances that prevent blood clotting by binding (chelation) or precipitating (precipitation) calcium, or by inhibiting the formation of thrombin needed to convert fibrinogen to fibrin in the freezing process. Frequently used anticoagulant hemtology examination is EDTA. EDTA does not affect the shape and size of erythrocytes and leukocyte forms. There are two types of EDTA salts, namely Sodium (Na_2EDTA) and potassium (K_3EDTA / K_2EDTA).

Edge blood smear preparations include routine Laboratory examinations in addition to examination of hemoglobin levels and examination of leukocyte counts. The examination aims to evaluate the morphology of peripheral blood cells (erythrocytes, leukocytes, and platelets), estimate the number of leukocytes and platelets, and identify parasites such as malaria parasites, falsifarum, microfilariae, and trypanosoma.

Morphological abnormalities of erythrocytes is a morphology of erythocytes that is different from normal erythrocytes. Morphological abnormalities of erythrocytes consist of color abnormalities (Stain), Size abnormalities (size) and shape deformities.

Normochrome is the color of normal erythrocytes. Erythrocyte color abnormalities include hypochromes and hyperchromes. Hypochromes are erythrocyte color abnormalities where the erythrocytes are more pale due to less than normal Hb concentrations. While hyperchromic erythrocyte color abnormalities where erythrocytes are darker due to thickening of the erythrocyte membrane.

Normocytes are a measure of normal erythrocytes. Erythrocyte size abnormalities include macrocytic and microcytic. Macrocytes are erythrocyte size abnormalities larger than their normal size (erythrocytes measuring> 9μm), while microcytes are erythrocyte size abnormalities smaller than their normal size (<7 μm).

Storage time can significantly reduce the number of erythrocytes. The longer the storage, the number of cells counts decreases because the cells are damaged (hemolysis) or die. During storage, blood cells undergo biochemical, biomechanical, and immunological reactions, causing damage structural / morphological known as storage lesion. Erythrocytes are the blood cells that are most easily damaged. Improper anticoagulant concentrations can also cause disturbances in tonicity, cause cell swelling, hemolysis, or crenation.

Crenation is an erythrocyte showing blunt bumps on the entire cell surface. The location is irregular, found in intravascular hemolysis. This is due to the high acidity in the extracurricular fluid which results in the destruction of the consistency of the delicate skin of the red blood. In this case the red blood cells are in a hypertonic solution.

Erythrocyte membranes are semi permeable which means they can be penetrated by liquid and other solutes. Red blood cells will not experience changes if they are in an isotonic solution. Blood cells will swell and break when inserted into a hypotonic solution because the plasma membrane is not strong enough to withstand the pressure inside the erythrocyte itself. Conversely, if erythrocytes are in a hypertonic solution, the fluid in erythrocyte cells will go to the outer medium of erythrocytes, causing the volume of the cytoplasm to decrease resulting in shrinking erythrocytes.

Based on table 4.1 and table 4.2, it was found that the changes in the morphology of erythrocytes in blood samples of Na_2EDTA and K_3EDTA only occur in the form of erythrocytes, which is a form of crenation. Whereas for color and size remain normal. This means that the size and color of the erythrocytes does not change even though the sample is delayed up to 4.5 hours. However, in the form of erythrocytes there is a change in shape, namely crenation which increases every hour of storage. Because of the increase in the percentage of cationation of the Na_2EDTA blood sample and the different K_3EDTA blood sample, a t test was conducted to compare the blood samples of both.

The t-test results on the morphology of blood sample erythrocytes Na_2EDTA and K_3EDTA. Based on the significance value of the results above, it is known that the blood samples Na_2EDTA and K_3EDTA have a significance value (t) 0.613 greater than 0.05 (t> 0.05) so Ha is accepted and H0 is rejected which means there are no

significant differences.

Based on the discussion above, it can be concluded that the results of the research conducted on Na_2EDTA blood samples and K_3EDTA blood samples have no significant differences in morphological changes. As for storage time, there is an effect of storage time on erythrocyte forms but there is no influence on the size and color of erythrocytes.

CONCLUSION

Based on the results of research conducted on Na_2EDTA blood samples and K_3EDTA blood samples it can be concluded that there were no significant differences in morphological changes between Na_2EDTA blood samples and K_3EDTA blood samples.

SUGGESTION

It is expected that laboratory officers do not delay the Hematology examination, especially the Blood Edge Evaluation examination. However, if delayed, you should use K_3EDTA anticoagulants which can be delayed for a maximum of 2 hours for good blood stability.

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