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Erythrocyte Morphology in Women of Reproductive Age (WORA) with Anemia

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ABSTRACT

Anemia is a decrease in the number of erythrocytes in the blood circulation or the level of hemoglobin that is less than normal. The three major body mechanisms that cause anemia are excessive destruction of erythrocytes, blood loss, and decreased erythrocyte production. Based on the Basic Health Research (*Riskesdas*) in 2013, the prevalence of anemic women of reproductive age (WORA) aged 15-44 years in Indonesia was 35.3%. Anemia is classified based on the morphology of erythrocytes, including hypochromic microcytic, normocytic normochromic, and macrocytic. Erythrocyte morphology can be observed using peripheral blood smear examination. The objective of this study was to determine the morphology of erythrocytes in anemic women of reproductive age. This study belongs to descriptive research. The population of the study was 136 women of reproductive age, covering the students of D-IV in Medical Laboratory Technology at Setia Budi University. Forty-one respondents suffering from anemia were taken using a purposive sampling technique. The types of anemia were determined with examination using an Easy Touch hemoglobinometer with the Hb level of less than 12g/dL. Preparation of peripheral blood smear examination using EDTA venous blood and stained with Giemsa. Microscopic examination was performed with 1000x objective magnification. The peripheral blood smear reading showed the erythrocyte morphology that includes normocytic normochromic (38 samples or 93%), microcytic hypochromic (three samples or 7%), and poikilocytosis consisting of teardrop cells, target cells, ellipstocytes, and stomatocytes (five samples or 18%). Further study is required to investigate the correlation of erythrocyte index and peripheral blood smear in anemia.

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INTRODUCTION

Anemia is a decrease in the number of erythrocytes in the blood circulation or a hemoglobin level that is less than normal (Basith, 2017). Three main body mechanisms that cause anemia are excessive red blood cell destruction, blood loss, and decreased red blood cell production (Luju, 2018).

Anemia causes the improper function of blood in binding and transporting oxygen from the lungs to the rest of the body and this condition causes difficulty in concentrating, which results in decreased learning achievement, and low physical endurance that makes the body easily tired and get sick, as well as experience a decrease in physical activity, which then triggers students' laziness to go to school (Depkes, 2008).

Anemia can be influenced by various factors, such as gestational age, low socioeconomic condition, age (20-30 years), and sex (Ardianti et al., 2017). Based on the Basic Health Research (*Riskesdas*) in 2013, the prevalence of anemia in Indonesia for people aged> 1 year was 27.1%. In Indonesia, women had an anemia prevalence of 23.9%, which was 18.4% higher than men (Ardianti et al., 2017). The physiological needs of women that increase during pregnancy, as well as the bleeding through menstruation that occurs every month, can cause anemia (Depkes, 2003). A total of 45.7% of women of reproductive age (WORA) in Southeast Asia and 47.5% in Africa were reported suffering from anemia (WHO, 2008). The percentage of anemia in Indonesia among non-pregnant women (≥ 15 years) in urban areas was 19.7% (*Riskesdas*, 2007). Furthermore, in 2013, the percentage of anemia in women aged 15-44 years was 35.3% (*Riskesdas*, 2013).

Anemia can be classified, among others, into iron deficiency anemia with a morphological picture of a hypochromic microcytic blood smear, aplastic anemia with the morphology of normocytic normochromic erythrocytes, hemolytic anemia with a normocytic normochromic morphology, and megaloblastic anemia with the morphology of hypochromic macrocytic erythrocytes. Iron deficiency anemia is a type of anemia with the morphology of microcytic hypochromic peripheral blood smear and is the most common anemia occurring in the world. Iron deficiency anemia is more common in women aged 21-30 years old (Ardianti et al., 2017). Fifty percent of anemia is caused by iron deficiency (WHO, 2008). Anemia can also be classified based on cell size, namely microcytic anemia that is mainly caused by iron deficiency, thalassemia (hemoglobin disorder), sideroblastic anemia, and anemia from chronic inflammation. Normocytic anemia is commonly attributed to chronic diseases, such as kidney disorders, while macrocytic anemia is mainly caused by myelodysplasia anemia, anemia due to alcohol consumption, and megaloblastic anemia (Ademola and Abiola, 2016).

The morphology of red blood cells can be observed using a peripheral blood smear examination. The peripheral blood smear is one of the laboratory tests in patients with anemia. The examination provides significant information on the classification of anemia and is an important tool in differential diagnosis and indication for further investigation (Ardianti et al., 2017).

Anemia examination should not only be performed at the screening stage by the checking hemoglobin levels; a particular and simple examination is also required by making peripheral blood smears that can be done even in the peripheral areas with minimal health services so that future therapy and prevention can be planned appropriately.

Based on the aforementioned description, the researchers were interested in researching erythrocyte morphology in women of reproductive age (WORA) with anemia.

MATERIALS AND METHODS

Research Design

This study applied a descriptive research method, which aims to present a complete erythrocyte morphology in women of reproductive age with anemia.

Research population and samples

Research population

The population in this study were women of reproductive age, including 136 female students of the Medical Laboratory Technology department.

Research samples

Purposive sampling technique was used by considering inclusion and exclusion criteria.

a. Inclusion criteria:
   1) suffering from anemia; and
   2) willing to be respondents and taken the blood
b. Exclusion criteria:
   1) receiving the last blood transfusion in less than six months;
   2) donating blood in the last three months; and
   3) suffering from a serious disease or having been just discharged from the hospital.

Materials and Equipment

The equipment used in this study includes a syringe, EDTA vacuum tube, alcohol swab, object-glass, deck glass, dry cotton, small tube, POCT test strip, POCT easy touch GCHb equipment, microscope, and Giemsa stain. The materials required were tenous blood samples with EDTA anticoagulants

Working Procedures

Venous blood draw

a) The patient was positioned, the tourniquet was placed, and the patient was asked to make a fist.
b) The vein was selected, the tourniquet strap was opened, and the patient was asked to open the fist.
c) Tourniquet was removed and the area where the veins were twisted out was disinfected.
d) The installation of the tourniquet was repeated and the syringe was prepared.
e) The needle was injected into the vein at an angle of 15-30º.
f) Blood was sucked by pulling the plunger and then the tourniquet was released. Sterile gauze was put over the puncture and the needle was pulled out of the puncture.
g) Blood was put in the tube with the prepared anticoagulant.
h) The sterile gauze was pressed and the tape was applied over the gauze on the patients' hand.
i) The needle was disposed to a proper place.

Hb examination using Point of Care Testing (POCT) Method

a) The tip of the finger (ring finger or middle finger) that would be injected was cleaned with an alcohol swab and let dry.
b) Fingertip that had been cleaned was injected using a lancet.
c) The first drop of blood was removed, and then the next drop was affixed to the attached POCT test strip.
d) The result would be released.
e) The result was recorded on the form.

Making of peripheral blood smear

a) The sample was dropped at about two cm from one edge of the glass slide. The slide was placed on the table with the blood drops on the right.
b) Another slide was placed to the left of the drop of blood using the right hand and moved to the right until it hit the blood drop.
c) The drop of blood would spread on the side of the slide. The blood was let for a

while until reaching a point of ½ cm from the corner of the slide.

d) The slide was shifted to the left at an angle of 30-40 degrees. Never let the slide pressed down.

e) The preparation was let dry and labeled.

Giemsa staining

a) Giemsa solution and methyl alcohol were prepared.

b) The preparation was put with a layer of blood upon the shelf for staining.

c) Alcohol methyl was dropped on the preparation until the part was completely covered with blood, and then let for five minutes or longer.

d) Extra methyl alcohol was removed from the glass slide.

e) The preparation was filled with diluted Giemsa and let for 20 minutes.

f) The preparation was rinsed with running water.

g) The preparation was dried in the air by placing it in a vertical position.

Observation of erythrocyte morphology

The dried blood smear was read under a microscope at 1000X magnification. In the assessment of erythrocyte morphology, 3S principles were considered, including *size* (the size of 6-8 microns that could be compared with small lymphocyte nuclei, with a cell size of 7-10 microns, *shape* (biconcave round), and *staining* (the color that could be seen at the center pallor or pale area of erythrocytes).

1. Erythrocyte size can provide an initial profile of the quality of erythropoiesis in the body. Erythrocyte size is commonly classified as follows:
   a) Normocytic; the size of normal erythrocytes (6-8 microns) is nearly the same as the size of lymphocytes.
   b) Microcytic; the size of erythrocyte cells is smaller (<6 microns).
   c) Macrocytic; the size of erythrocyte cells is greater (> 8 microns).
   d) Anisocytosis; there are various sizes of erythrocytes. Anisocytosis indicates anemia but it is not specific.
2. The normal erythrocyte shape is round, disc-like, and sometimes irregular. Abnormal erythrocytes can have a variety of shapes and some contain inclusion materials.
3. The normal erythrocyte color is reddish because of the pigment hemoglobin and a pale area/central pallor. Pallor area covers approximately ½ - 1/3 of the total area of the cell. Erythrocyte color can be valued as follows:
   a) Normochromic; normal erythrocyte color with a pale area of ½-1/3 erythrocytes.
   b) Hypochromic; a pale color that is larger than ½ of the erythrocyte.
   c) Hyperchromic; erythrocyte is darker than normal and no area is pale.
   d) Polichromasia; there are various colors of the erythrocyte.

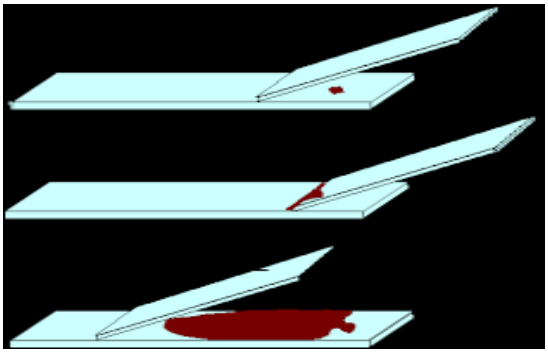

Figure 1. Procedures for making peripheral blood smear preparation (Lember et al., 2015)

RESULT AND DISCUSSION

This study was carried out in the Laboratory of Hematology, Universitas Setia Budi, in February-March 2019 with 41 respondents. Table 1 presents that the minimum age of the respondent was 19 years old, while the maximum age was 24 years old. The table also shows the maximum hemoglobin level of 11.9 g/L and a minimum level of 6.0 g/dL.

Table 1. Characteristics of Subjects

Characteristic	Mean	Maximum	Minimum
Age (year)	21	24	19
Hb level (g/dL)	10.9	11.9	6.0

. Table 2 shows that the erythrocyte morphology includes normochromic (38 samples of 93%), microcytic hypochromic (three samples or 7%), and poikilocytosis consisting of teardrop cells, target cells, ellipstocytes, and stomatocytes (five samples or 18%).

Table 2. The prevalence of anemia based on the size and color of erythrocytes

Erythrocyte Profile	Number	%
Normocytic Normochromic	38	93%
Microcytic Hypochromic	3	7%
Macrocytic	-	0%
Total	41	100%

The research population was 136 female university students of reproductive age, 44 (32.4%) suffering from anemia and the 41 respondents met the criteria of samples. According to Fitrah et al. (2011), the prevalence of anemia in Indonesia was 57.1% in female teenagers, 27.95% in women of reproductive age, and 40.1% in pregnant women. The Basic Health Research (*Riskedas*) in 2013 reported the anemia occurrence in women of reproductive age was 32.9% and the cause of iron deficiency was 50%. The prevalence of anemia in Central Java Province was 57.7% (Aulia et al.: 2017). Meanwhile, the prevalence of this health problem in women of reproductive age in Sukoharjo Regency, as reported by Salam (2012), was 48.5% in 2008, 33.84% in 2009, and 48% in 2010.

The main causes of anemia are nutrition and infection. Iron deficiency is one of the factors contributing to anemia because the consumption of food is monotonous but rich in substances that inhibit iron absorption so that iron cannot be utilized properly by the body (Puslitbangkes, 2016). Routine laboratory tests to detect anemia include the examination of hemoglobin, hematocrit, erythrocyte count, and erythrocyte index. Anemia is characterized by hemoglobin, hematocrit, and erythrocyte cell counts less than normal (Basith, 2017). Meanwhile, the erythrocyte index examination is commonly performed to determine the type of anemia. This test can be confirmed using a blood smear examination.

In this study, researchers examined hemoglobin levels using a hemoglobinometer Easy Touch Point of Care Testing. A hemoglobin level below 12 g/dL was considered a case of anemia. This examination used capillary blood. As the name suggests, microcytic hypochromic anemia is a type of anemia with a low hemoglobin concentration, characterized by a pale red cell color and a smaller erythrocyte size than normal. The main mechanism in microcytic hypochromic anemia is attributed to an iron loss in chronic bleeding, insufficient nutritional intake, as well as increased iron requirements during growth and pregnancy (Kiswari, 2014).

Normocytic normochromic anemia is a type of anemia with both normal cell hemoglobin concentration and cell size. This category of anemia is found in anemia caused by acute bleeding and excessive blood destruction so that the spinal cord has to work more intensively. The causes of this anemia are acute blood loss, hemostasis disorders, chronic diseases including infections, and bone marrow failure (Kiswari, 2014).

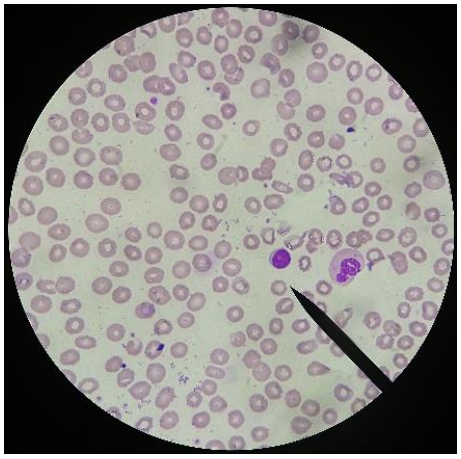

Figure 3. Normocytic Normochromic Erythrocyte Morphology in a Woman of Reproductive Age with Anemia Giemsa Stain Method with 1000x Magnification

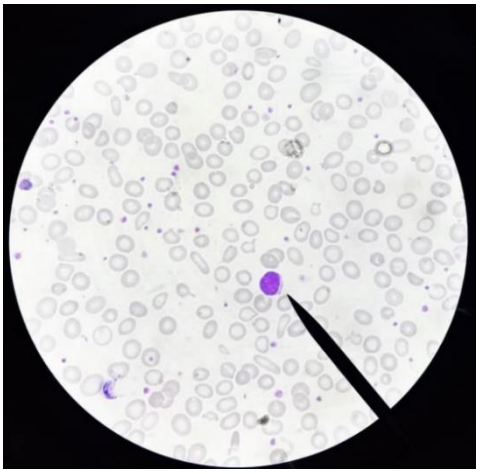

Figure 4. Hypochromic Microcytic Erythrocyte Morphology in Women of Reproductive Age with Anemia Giemsa Stain Method with 1000x Magnification

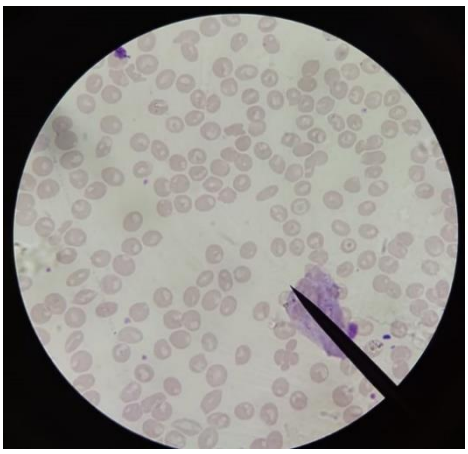

Figure 5. Poikilocytosis (teardrop cells, target cells, ellipstocytes, and stomatocytes)
Erythrocyte Morphology in Women of Reproductive Age with Anemia Giemsa Stain Method with 1000x Magnification

Poikilocytosis is an abnormality in erythrocyte cells, typified by various forms of erythrocyte cells in one peripheral blood smear. Erythrocyte deformity can be found in sickle cell anemia, bone marrow stimulation, and hemolysis. Poikilocytosis can also be caused by pre-analytic factors, such as mismatching of blood ratios with anticoagulants and delay in processing the preparation of Peripheral Blood Smear (PBS) (Ademola and Abiola, 2016). Apart from pre-analytic factors, target cells can also be found in cases of thalassemia. In folic acid deficiency and vitamin B12 deficiency anemia, erythrocyte deformities, such as target cells and teardrop cells, are common.

In this study, the morphology of red blood cells in women of reproductive age with anemia did not match the prevalence in Indonesia and even in the world, where the highest prevalence of anemia was microcytic hypochromic anemia or iron deficiency anemia. This study concluded that the highest prevalence was found in normocytic normochromic anemia, with as many as 38 samples (93%). This was attributed to bleeding and chronic infection. In women of reproductive age, bleeding can occur due to menstruation, in which the volume of blood is excessive during menstruation (menorrhagia). Moreover, anemia during menstruation can be worsened if women do not consume Fe tablets. In this study, data on menorrhagia and the use of Fe tablets during menstruation were not collected. The respondents in this study were unmarried women of reproductive age and did not have any experiences in delivering babies; and thus, the bleeding was not attributed to postpartum hemorrhage or anemia during pregnancy where the need for iron increases, while the intake is less, so the bleeding in these respondents was potentially due to menstruation. The drawback in this study deals with the population used, female students aged 19-24 years. The population did not cover women of reproductive age with all age ranges, namely 15-49 years so that it did not describe the entirety of women of reproductive age. Another contributing factor is that Indonesia is a developing country with many cases related to worm infections. According to Masrizal (2007), worm infection in Indonesia is a significant problem for anemia cases, because it is estimated that worms can suck 2,100 cc of blood every day.

Improper PBS preparation procedure can affect erythrocyte morphology. In making PBS preparation, difficulties were found that could affect the results of the morphology of erythrocytes. The POCT method of hemoglobin examination uses a capillary blood sample and if a low hemoglobin level is obtained (<12 g / dl), the respondent is declared to be anemic so that the step is followed by taking venous blood for samples of making a PBS. The collection of venous blood as a sample for making a PBS may face difficulty in the phlebotomy process. Incomplete phlebotomy results in prolonged use of the tourniquet, which can lead to hemoconcentration of the blood sample. In the phlebotomy process, attention must be given when using 70% alcohol swabs, because the use of wet alcohol can cause lysis of blood samples. After the venous blood is taken with a 3cc syringe, the sample is inserted into the EDTA vacuum tube covered in purple, so that the venous blood obtained must remain 3cc by ensuring the blood flows through the tube wall to avoid lysed blood sample. After the blood is successfully stored in the EDTA tube, homogenization is immediately carried out by turning the tube back and forth eight to 10 times. EDTA anticoagulant has the advantage of not destroying the morphology of erythrocytes so that it is used in making of PBS preparations. Improper comparison of blood with anticoagulants causes errors that can affect the morphology and lead to crenation of erythrocytes (Riswanto, 2013).

This study encountered difficulties in the phlebotomy process so that the obtained blood samples were often less than 3cc, contributing to the unsuitable ratio of blood and anticoagulant. EDTA blood samples are ideally stored in less than two hours after taken and must be affixed using absolute methanol. Changes in erythrocyte morphology, such as crenation, occur due to the limitations in blood sample examination, causing the non-uniformity in the storage and preparation of blood samples.

Crenation takes place because erythrocytes are hypertonic, so that the fluid in the erythrocytes comes out, causing the erythrocytes to shrink. According to Kiswari (2014), damage to erythrocyte morphology can happen due to room humidity and the delays in carrying out PBS preparation after the

blood has been collected. Erythrocyte stability at 18-25°C is two hours, so delaying the PBS preparation process can damage the morphology of blood cells. Also, the high room humidity can cause the smear to take a longer time to dry so that the erythrocyte cells will be damaged.

PBS preparation must also have good smear characteristics so that it can be read properly. Kiswari R (2014) mentions several good visual characteristics of a preparation. First, a preparation has graded thickness, which is thicker in the head area and thinner in the tail area. Also, the blood smear made does not go beyond or touch the edge of the object-glass, is not wavy or broken, and is not perforated, where the tail does not form a torn flag. Moreover, a good smear is approximately 2/3 the length of the glass slide.

In making a good preparation that meets the aforementioned characteristics, a medical laboratory technologist requires particular training and skills. Similar to the making of PBS preparation, staining also influences cell morphology observations. In this research, the peripheral blood smear was stained using Romanowsky's Giemsa staining. Before doing the staining, the researchers prepared the Giemsa solution. The solution was prepared on the day the staining was done because it was ideally new. Giemsa solution does not contain high levels of methanol, so before staining with Giemsa, it is necessary to fix it with absolute methanol and ensure that the absolute methanol is not polluted by air by storing methanol in a tightly closed glass bottle (Gandosoebrate, 2016). The preparation was too blue when observed under a microscope and this was caused by short staining duration and the highly acidic dye solution.

Kiswari (2014) also suggests that Giemsa staining takes 20 minutes and the pH of the dye solution is 6.4; thus, short staining duration and high alkaline pH cause the color of preparation to become too blue when observed under a microscope. In this study, the pH of the dye solution was not measured; however, the Giemsa quality test was performed before the solution was used because its quality can greatly affect preparation staining.

Riswanto (2013) points out that the Giemsa quality test can be carried out by placing a horizontal filter paper on a petri dish or glass beaker (the middle of the filter paper should not touch any object), dropping 1-3 beads of the solution on the filter paper, and then leaving it until permeates or expands. The formed circle is observed; if Giemsa is good, the outer edge of the circle will be red (eosin), after that the circle becomes purple (methylene azure), and the circle in the center is blue (methylene blue).

The dried preparations are then examined under a microscope with 1000x objective lenses using immersion oil. Observations are made by monitoring several fields of view in zone 5 and comparing the size of red blood cells with small lymphocyte nuclei. According to Kiswari (2014), in zone 5, erythrocytes are evenly distributed, do not overlap, and are not crowded so that the shape is still intact, which is the right reading zone for assessing erythrocytes.

CONCLUSION

The morphology of erythrocytes in women of reproductive age with anemia includes normocytic normochromic (38 samples of 93%), microcytic hypochromic (three samples or 7%), and poikilocytosis consisting of teardrop cells, target cells, ellipstocytes, and stomatocytes (five samples or 18%).

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