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The Relationship between Hemoglobin Levels and Erythrocyte Morphology on the Third Day of Menstruation for Students in The Faculty of Health

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Abstract

Menstruation is the process of releasing the endometrial lining of the uterus due to the release of the hormones estrogen and progesterone, causing bleeding after ovulation from the uterus for approximately fourteen days at regular intervals. The first three days of menstruation is a condition where there is heavy bleeding, so there is iron loss, which can cause a decrease in hemoglobin levels and there is a risk of causing anemia. The occurrence of anemia gives an illustration of morphological changes of erythrocytes. The objective of this study was to determine the relationship between hemoglobin levels and erythrocyte morphology on the third day of menstruation. This type of research is analytic observational with a cross-sectional approach. The sample in this study were 30 students from the Health Faculty of Nahdlatul Ulama University Surabaya who experienced menstruation on the third day. The hemoglobin level was analysed using the Hematology Analyzer Xp-300 method. A blood smear test with Giemsa stain were used for abnormalities erythrocytes morphology observation. The results of the statistical analysis used the chi-square test showed there was no relationship between hemoglobin levels and erythrocyte size (p-value = 0.836), erythrocyte shape (p-value = 0.416). However, there was a relationship between hemoglobin levels and erythrocyte colour ($p<0.05$). Based on the study result, it can be concluded that there is no relationship between hemoglobin levels and erythrocyte morphology on the third day of menstruation.

Keywords

Erythrocyte Morphology, Hemoglobin Level, Menstruation.

INTRODUCTION

Puberty is a condition marked by the functioning of reproductive organs such as menstruation that arises due to physiological changes. Menstruation releases blood from the uterine wall gradually to the vagina. The menstrual cycle generally ranges from 21 to 35 days, while the length of menstruation ranges from 3 to 7 days (1). In chronic blood loss, women often have difficulty absorbing large amounts of iron from the small intestine to form hemoglobin proportional to the blood loss. The new created red blood cells contain only a small amount of hemoglobin (2).

Most menstrual blood flow occurs on the second and third day of menstruation based on research on daily menstrual blood loss and quality of life in women with heavy menstrual bleeding (3). The highest MBL (Menstrual Blood Loss) day occurs on the second and third day of the menstrual cycle, causing limited physical activity. Each menstrual phase, the quantity of blood that comes out is an average of 50 ml; when the blood loss is more than 80 ml, it is suspected to be pathological. Iron in the blood will experience a reduction of 12.5-15 mg/month or approximately 0.4-0.5 mg/day. The condition of reduced iron will trigger a decrease in hemoglobin levels which appears as a form of iron deficiency anemia (4).

Hemoglobin is an essential protein in carrying oxygen from the lungs to the peripheral tissues and carrying carbon dioxide from the peripheral tissues to the lungs. Hemoglobin is part of erythrocytes, a biomolecule that seeks to bind oxygen (5). Erythrocytes are biconcave, do not have a nucleus, do not move, have red color because they contain hemoglobin. Erythrocytes are 7 to 8 in diameter and 2.0 thick (6). The importance of hemoglobin causes hemoglobin examination to have an essential role in diagnosing anemia and is one of the routine examinations in the laboratory.

The prevalence of anemia globally, according to World Health Organization (WHO), is estimated at around 40-88%. The majority of anemia cases in women ranged from 13.4 to 87.5% (7). Those numbers show the relatively high prevalence of anemia in adolescent women (8). When the blood hemoglobin level is low, it does not necessarily mean that a person is anemic. The emergence of iron deficiency anemia or anemia due to morphological causes (normochromic normocytic anemia, hypochromic microcytic anemia, and hyperchromic macrocytic anemia) will provide an overview of the erythrocyte morphological changes. So, that other investigations can be added, such as observing the morphology of erythrocytes on a peripheral blood smear to confirm the presence of erythrocyte abnormalities.

The results of the previous study showed that 60% of menstruating women had hemoglobin levels of less than 12.0 g/dL which was below the normal limit and 40% of menstruating women had normal hemoglobin levels. Based on 20 research subjects, it was found that there were deformities of erythrocytes, ovalocytes, target cells, elliptocytes, spherocytes, lacrimacytes, centrocytes, burr cells, sickle cells, helmets, stomatocytes, and crenated cells. The color of the erythrocytes obtained was hypochromic and normochromic. The size of erythrocytes obtained anisocytosis and normocytic results (9).

The condition of female students in the health faculty who have a lot of student activities plus menstruation can cause iron loss, decreased hemoglobin levels and anemia. If anaemia treatment is not carried out properly, it will result in fatigue that interferes with productivity and cannot perform activities perfectly. Anaemia can be overcome by taking iron supplements, vitamin B12, folic acid, and iron-rich foods, such as brown rice, green vegetables, and beans that help produce haemoglobin formation. This reason triggered researchers to find out whether there is a relationship between haemoglobin levels and erythrocyte morphology on the third day of menstruation.

MATERIALS AND METHODS

This type of research is an analytical observational study with a cross-sectional approach to determine the relationship between hemoglobin levels and erythrocyte morphology on the third day of menstruation. The population in this study were female students of the Health Faculty at Universitas Nahdlatul Ulama Surabaya.

The sample in this study were 30 female students who were having their third day of menstruation. The inclusion criteria in this study were female students of the Faculty of Health who were willing to be respondents, aged 15-24 years, had normal menstrual cycles and were menstruating on the third day. The respondent's blood sample was taken intravenously as much as three ccs, then blood was stored in an EDTA vacuum blood collection tube and at a temperature of 4-8°C. Determination of respondents was carried out by purposive sampling and based on the permission of the ethics committee at Universitas Nahdlatul Ulama Surabaya. Purposive sampling technique is the selection of respondents independently by researchers who will use research-based on subjective and practitioner considerations (10).

The hemoglobin level was tested using the impedance Sysmex XP-300™ Automated Hematology Analyzer method, while the erythrocyte morphology examination was carried out by making a peripheral blood smear using Giemsa 1:19 dye for 20-30 minutes. The normal value of haemoglobin is 12-15 g/dL for adult women (11). The reference value for erythrocyte morphology is considered normal, as long as the peripheral blood smear does not change the morphology of the erythrocytes in terms of shape, colour, or size.

Data analysis in this study used the chi-square test performed with IBM SPSS version 21.0. The chi-square test is a non-parametric test statistical analysis. Therefore, the application of the chi-square test was used for categorical data. The chi-square test can be used in research to determine the relationship between variables located in rows and columns.

RESULTS

Based on the research, there were 30 respondents presented by the number of respondents who have standard, high, and low hemoglobin levels (Figure 1).

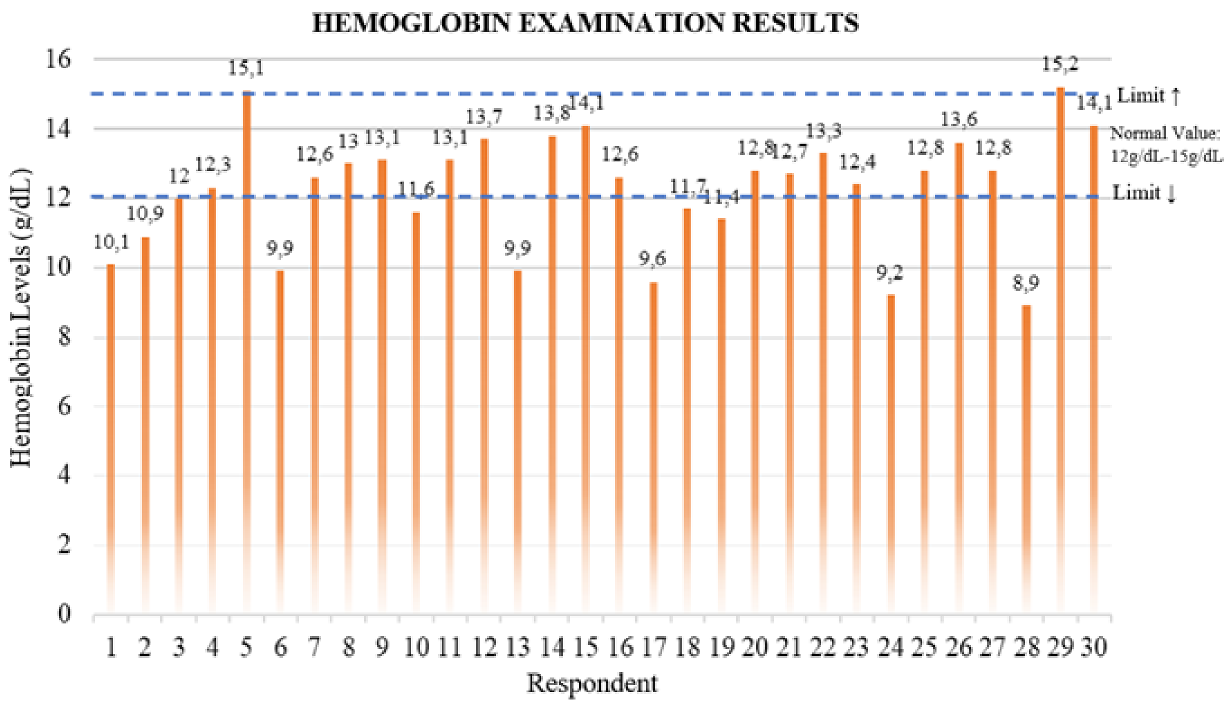

Figure 1. Graph showing student's hemoglobin level

Based on Figure 1, the lowest hemoglobin level of the respondent is 8.9 g/dL, the highest hemoglobin level is 15.2 g/dL with an average hemoglobin level of 12–15 g/dL.

Table 1. Distribution of the frequency of inspection results hemoglobin levels

Reference Value	Frequency (F)	Percentage (%)
<12 g/dL	10	33%
12 – 15 g/dL	18	60%
>15 g/dL	2	7%
Total	**30**	**100%**

Observations of erythrocyte morphology under a microscope were reported in Table 2. Those results obtained based on normocytic erythrocyte size and anisopoikilocytosis (Figure 2). Several erythrocyte deformities found, including ovalocytes, target cells, schistocytes, stomatocytes, sickle cells, cigar cells, spherocytes, lacrimacyte, and acantocyte. Based on the color, normochromic and hypochromic erythrocyte colors were found.

Table 2. Observation results of erythrocyte morphology

Sample Code	Observation results of erythrocyte morphology		
	Size	**Form**	**Color**
01	Anisopoikilocytosis	Ovalocytes, cigar cell, tear drop, target cell, scistocyt	Hypochrome
02	Anisopoikilocytosis	Acantocyte, tear drop, ovalocytes, target cell, scistocyt	Normochrome
03	Anisopoikilocytosis	Ovalocytes, tear drop, acantocyte, scistocyt	Normochrome
04	Anisopoikilocytosis	Ovalocytes, scistocyt, tear drop	Normochrome
05	Anisopoikilocytosis	Ovalocytes, tear drop, acantocyte, scistocyt, targett cell	Normochrome
06	Anisopoikilocytosis	Acantocyte, tear drop	Normochrome
07	Anisopoikilocytosis	Tear drop, scistocyt, sferosit, ovalocytes	Normochrome
08	Anisopoikilocytosis	Ovalocytes, cigar cell, stomatocyte	Normochrome
09	Anisopoikilocytosis	Acantocyte, scistocyt, ovalocytes, target cell	Normochrome
10	Anisopoikilocytosis	Ovalocytes, scistocyt, tear drop, acantocyte	Normochrome
11	Anisopoikilocytosis	Ovalocytes, acantocyte, sickle cell	Normochrome
12	Normocytic	Normocytic	Normochrome
13	Anisopoikilocytosis	Tear drop, ovalocytes, sickle cell	Hypochrome
14	Anisopoikilocytosis	Tear drop, ovalocytes, scistocyt, stomatocyte	Normochrome
15	Anisopoikilocytosis	Tear drop, ovalocytes, scistocyt, sickle cell	Normochrome
16	Anisopoikilocytosis	Tear drop, ovalocytes, scistocyt	Normochrome
17	Anisopoikilocytosis	Ovalocytes, tear drop, stomatocyte, scistocyt	Hypochrome
18	Normocytic	Normocytic	Normochrome
19	Anisopoikilocytosis	Target cell, stomatocyte, ovalocytes, sferosit cigar cell	Hypochrome
20	Anisopoikilocytosis	Ovalocytes, tear drop, stomatocyte	Normochrome
21	Anisopoikilocytosis	Stomatocyte, ovalocytes, scistocyt, cigar cell	Normochrome
22	Anisopoikilocytosis	Tear drop, ovalocytes	Normochrome
23	Anisopoikilocytosis	Ovalocytes, sickle cell, stomatocyte, tear drop	Normochrome
24	Anisopoikilocytosis	Ovalocytes, sickle cell, stomatocyte, sferosit, target cell, tear drop	Hypochrome
25	Anisopoikilocytosis	Ovalocytes, target cell	Normochrome
26	Anisopoikilocytosis	Ovalocytes, stomatocyte, scistocyt	Normochrome
27	Anisopoikilocytosis	Ovalocytes, scistocyt, sickle cell, stomatocyte, target cell, cigar cell	Normochrome
28	Anisopoikilocytosis	Ovalocytes, stomatocyte, tear drop, cigar cell	Hypochrome
29	Anisopoikilocytosis	Ovalocytes, tear drop, scistocyt	Normochrome
30	Anisopoikilocytosis	Scistocyt, stomatocyte	Normochrome

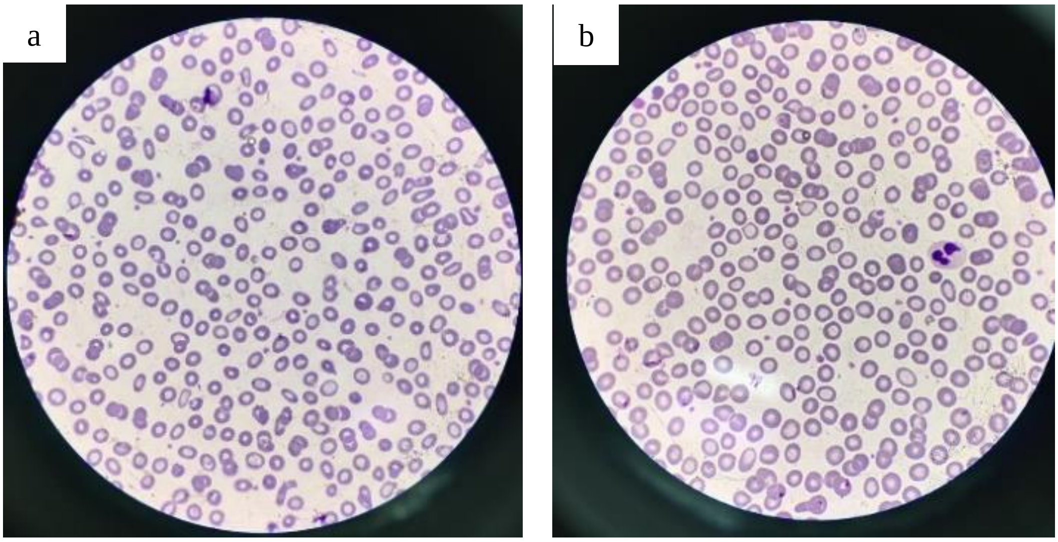

Figure 2. Microscopic observation results oferythrocyte morphology.
(a) Anisopoikilocytosis, (b) Hypochrome

Based on the data analysis using the chi-square test is known that the p-value of hemoglobin levels in erythrocyte size is 0.836, where p-value > 0.05, which indicates that there is no relationship between hemoglobin level and erythrocyte size (Table 3). The p-value of hemoglobin levels in the form of erythrocytes is 0.419, where the p-value >0.05 indicates that there is no relationship between hemoglobin levels and the condition of erythrocytes. The p-value of hemoglobin level in erythrocyte color is 0.001, where the p-value <0.05 indicates an association between hemoglobin level and erythrocyte color.

Tabel 3. Chi-square test results

Category	Pearson Chi-Square P-Value*
Hemoglobin Level with Erythrocyte Size	0.836
Hemoglobin Level with Erythrocyte Form	0.419
Hemoglobin Level with Erythrocyte Color	0.001

*Significance p-value <0,05

DISCUSSION

In this study, the research subjects used were adolescents aged 15-24 years. These age ranges are at risk of developing anaemia. One of the risk factors for anaemia is bleeding or blood loss during menstruation, so the blood will lose iron. Reduced iron will have an impact on hemoglobin levels. Anaemia interferes with the development of women in the decreased ability to concentrate on learning, decreased physical ability, and pale face (12).

All respondents have regular menstrual cycles, as well as the body mass index (BMI) values obtained tend to be in line with expectations. BMI values can affect the menstrual cycle, which can be seen through the role of the hormone estrogen. With the increase in calories in the body, therefore an increase in body weight contributes to an increased level of estrogen in the blood. This condition occurs when the patient experiences gained body fat, androgens in the body also elevate. Androgens convert into estrogens through the aromatization process in granulosa cells and fat tissue. Increased estrogen in the blood will impair GnRH secretion (13).

In this study, a sample of 18 respondents (60%) showed normal hemoglobin levels. This result is in line with Kirana's research (2011), which stated that a woman who experiences menstruation is not necessarily anemic. It means that the respondent's body has received nutritional intake from food sources that contain a lot of iron (Fe) so that it can increase the production of hemoglobin in the blood such as meat, fish, seafood, fruit, vegetable and fruit juice drinks containing vitamin C (ascorbic acid) (14).

Iron is an essential micronutrient for the production of haemoglobin, which functions to synthesize iron-containing enzymes to use oxygen during the cellular energy production process (15). Adequate iron in the body is the minimal amount of iron from food to avoid iron deficiency anemia (16).

Based on this study result, ten respondents (33%) had haemoglobin levels <12 g/dL. A study by Irianti (2014) showed that the more blood loss during menstruation, the higher risk of developing anaemia. One of the triggering factors for anaemia is a lack of nutrients that play a role in the formation of haemoglobin due to impaired absorption. Nutrients that catalyze heme in the haemoglobin molecule include iron, protein, and pyridoxine (vitamin B6) (17).

The first three days of menstruation are days that tend to bleed a lot. Decreased hemoglobin levels can make the body's metabolic disorder and nerve cells do not process optimally, resulting in an acceleration of reduced nerve impulses and disrupting the work of dopamine receptors (2). If the haemoglobin level is too low, it can also affect blood viscosity. This process will disrupt the body because it has low oxygen levels (hypoxia) and reduced oxygen supply to the organs, especially vital organs such as the brain and heart (18).

The results of the observation of erythrocyte morphology in this study are in line with research conducted in Mataram stated that respondents experience bleeding due to menstruation, it will trigger anemia to provide an overview of erythrocyte morphology changes that occur in abnormalities in erythrocyte size, erythrocyte shape, and erythrocyte color (4). The occurrence of pale erythrocyte coloration is due to the inhibition of the hemoglobin formation process due to interference with iron-binding. Erythrocytes formed with small cytoplasm indicate that the cell is immature and does not contain hemoglobin. Low iron content causes failure to form nucleated erythrocyte cytoplasm (19). The color of the erythrocytes is uneven, but the center is paler because it is thinner than the less colored periphery, which is called hypochromic. Under normal circumstances, the center of the size does not exceed 1/3 of its diameter, the cell is called normochromic.

The results of this study for the erythrocyte size obtained were abnormal. It means that on the third day of menstruation, a person experiences blood loss resulting in anemia due to decreased production of iron (Fe) in the blood so that erythrocyte synthesis continues and produces smaller cells (microcytic). An insufficient amount of iron causes a decrease in hemoglobin in each cell, resulting in hypothermia. Erythrocytes that have a larger size (macrocytic) will resemble reticulocyte cells due to the effectiveness of erythropoiesis by the bone marrow..

The standard erythrocyte shape on a peripheral blood smear is a biconcave disc with a red border compared to the centre part. This study found an abnormality in changes in the shape of erythrocytes. The occurrence of an abnormal shape of erythrocytes describes a chemical or physical change in the cell membrane or cytoplasm. Recent research in cell biology has contributed to increasing knowledge of these mechanisms. This condition occurs due to specific hematological or non-hematological abnormalities whenever an increase in the number of poikilocytosis is found (20).

Based on the results of data analysis, this study showed there was no relationship between hemoglobin levels and erythrocyte size on the third day of menstruation (p-value = 0.836). It was also showed that there was no relationship between hemoglobin levels and the shape of erythrocytes on the third day of menstruation (p-value = 0.419). In contrast, this study found a relationship between hemoglobin levels and erythrocyte colour on the third day of menstruation ($p < 0.05$), the direction of this relationship can indicate that if the subject has a more normal hemoglobin level, the result of erythrocyte staining is also more normochromic and vice versa. Overall, it can be concluded that there is no relationship between hemoglobin levels and erythrocyte morphology on the third day of menstruation.

Based on research conducted by Zhong et al., (2021) hemoglobin concentration can affect changes in erythrocyte morphology. The activity of the Na-K pump will be disrupted if there is insufficient ATP in the red blood cell membrane during storage, resulting in abnormal erythrocytes (21). According to research conducted by Hadijah et al., (9) it was found that there was an effect of the menstrual period on hemoglobin levels and morphology of erythrocytes. This study aims to determine hemoglobin levels and morphology of erythrocytes in menstruating women. The difference from the research of Hadijah et al., (9) the sample studied was the sample of the sixth day of menstruation. Bleeding during menstruation will cause iron levels to decrease. The longer the menstrual period, the more loss of iron deposits and a decrease in hemoglobin levels. The longer the menstruation duration, the more influential on anemia. During menstruating, it is necessary to maintain a balanced diet so the nutritional intake is adequate and hemoglobin levels remain normal and the morphology of erythrocytes does not experience many abnormalities (9).

CONCLUSIONS

Based on the study results, it can be concluded that during menstruation on the third day of menstruation, the average hemoglobin level is 12.3 g/dL. Menstruation is a condition that is quite vulnerable because it can cause iron loss, decrease hemoglobin levels and cause anemia. So that the emergence of risk factors for experiencing fatigue and disrupting productivity. On the third day of menstruation, women experience changes in erythrocyte morphology, with abnormalities in erythrocyte size (anisocytosis), erythrocyte shape (poikilocytosis), and erythrocyte colour (normochromic, hypochromic). There was no relationship between hemoglobin level and erythrocyte morphology on the third day of menstruation, such as erythrocyte size and shape. However, there is a relationship between hemoglobin levels and erythrocyte colour on the third day of menstruation.

AUTHOR CONTRIBUTIONS

Azzaubadilluah: conceptualization, formal analysis, investigation, writing - original draft, writing - review & editing. Andreas Putro Ragil Santoso: supervision, conceptualization, methodology, writing - original draf, formal analysis, validation, reviewing.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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