THE RELATIONSHIP BETWEEN ABSOLUTE NEUTROPHIL COUNT AND ERYTHROCYTE INDEX IN HYPERURICEMIA PATIENTS

Meri Meri[1], Noval Pahrul Nurhidayat [2]
[1] Bakti Tunas Husada Tasikmalaya College of Health Sciences

*Corresponding author. Email: meri@stikes-bth.ac.id

ABSTRACT

Hyperuricemia is a condition of a person with uric acid levels in the blood that exceed normal values. Increased uric acid can be detrimental because it is considered as damage-associated molecular pattern molecules (DAMPs), causing an immune response and detrimental to health. However, uric acid can act as an antioxidant in the body. Uric acid can protect the quality of erythrocytes. However, erythrocytes can suffer damage due to oxidative stress as a result of the inflammatory process. An increase in the absolute neutrophil count is an indication of inflammation. The aim of this study is to determine the relationship between absolute neutrophil count and erythrocyte index in hyperuricemia. Sampling was done by purposive sampling with the criteria of males aged 18-65 years, not obese, hyperuricemia, and fasting. The sample used was venous blood, then examined using a haematology analyzer at Jasa Kartini Hospital, Tasikmalaya. The results of the study of 30 respondents showed that the average uric acid was 8.863 mg / dl. Based on statistical tests using the Spearman correlation test, it showed that there was no significant relationship between the absolute number of neutrophils and the erythrocyte index, which was indicated by P> 0.05. The conclusion is that hyperuricemia with a mean of 8.863 mg / dl did not show any relationship between the absolute neutrophil count and the erythrocyte index.

Keywords: Hyperuricemia, absolute neutrophil count, erythrocyte index.

INTRODUCTION

A uric acid level above normal is known as hyperuricemia. Hyperuricemia levels for men> 7 mg / dl, for women> 6 mg / dl [1]. Hyperuricemia occurs due to several things, such as excess production of uric acid (high purine food intake), a lack of the ability of the kidneys to excrete uric acid, and can be both[2].

Hyperuricemia can be both beneficial and detrimental. Uric acid levels in the blood can be beneficial because of its role as an antioxidant in the blood, which protects erythrocytes from the effects of oxidative stress damage. Protected erythrocytes will have normal morphology, while damaged erythrocytes certainly have abnormal morphology. This can occur due to the influence of reactive oxygen species (ROS), one of which is released by neutrophil cells during inflammation [3][4].

Erythrocyte quality can also be influenced by the erythropoietin enzyme, which is predominantly synthesized by the kidneys [5]. If the kidneys are impaired, the kidneys are unable to produce erythropoietin properly so that it can affect the quality of erythrocytes. One of the quality of erythrocytes can be seen from the erythrocyte index parameter. In addition, erythrocyte morphology examination on peripheral blood smears can be performed. MCV, MCH and MCHC are some parameters of erythrocyte index that can assess the type of anemia. Anemia based on the erythrocyte index is classified into microcytic hypochromic anemia, normocytic normochromic anemia and macrocytic hypochromic anemia. Anemia associated with abnormal

erythrocyte size. Erythrocyte size can be measured by looking at the level of MCV in the blood. Chronic kidney disorders can usually cause anemia because the kidneys begin to decrease their ability to produce erythropoietin [6].

Hyperuricemia can harm the body because uric acid is considered as damage-associated molecular pattern molecules DAMPs[7] by the immune system and can cause various diseases such as kidney failure, cardiovascular disorders [4], hypertension[8], diabetes mellitus and others [9]. Hyperuricemia can lead to an inflammatory response if the levels increase and become saturated and form a urate monosodum. Monosodium urate that is formed can settle in organs such as kidneys and mostly in joint tissue ([10]. In the kidneys, kidney disorders can be detected if the cystatin-C level increases in the blood ([11]. This cystatin-C parameter is a biomarker of abnormal kidney function that is superior to creatinine ([12].

Based on the explanation above, the relationship between the absolute neutrophil count and the erythrocyte index in hyperuricemia patients cannot be explained and provides information on whether there is a relationship. Thus, this study contributes to the review of laboratory examination results related to the absolute neutrophil count and erythrocyte index in hyperuricemia patients.

MATERIAL AND METHODS

This study used a cross sectional study design. The sampling technique was carried out by purposive sampling with inclusion criteria: Men aged 18-65 years who experienced hyperuricemia, could fast 8-12 hours. Exclusion criteria: being infected, obese, and unwilling to contribute to the study. Drop out criteria: illness or death during the study period. The sample size is the total population of the male sample according to the criteria as many as 30 people. The research was conducted at Jasa Kartini Hospital, Tasikmalaya and the laboratory of the D3 Technology Study Program, Medical Laboratory of STIKes Bakti Tunas Husada, Tasikmalaya.

The tools and materials include tourniquet, syringe, macrocentrifugation, alcohol cotton, tape, blood sample tube, photometer, tube rack, test tube, and hematology analyzer.

Preparation: respondents were given a form of willingness to be part of the research and informed consent of the study. If the respondent is willing, continue to check the uric acid level and count the number of abolute neutrophils and the erythrocyte index.

Digital Uric Acid Screening

Hyperuricemia screening uses a digital instrument with the principle that the blood sample touches the target area of the sample from the strip, which is automatically attracted to the inside of the reaction area of the strip. The test results will be displayed on the screen after 150 seconds. The stage begins by inserting the battery into the tool and then turning it on. Set the time, date and year. Look at the strip expiration date. Check the tool by inserting the chip. If the screen appears "ERROR" it means the device is damaged. If the screen appears "OK" it means the device is ready to use. On the screen the code will appear according to the bottle strip. Next, a flickering image of blood drops appears, indicating that the sample is ready to be examined. The blood is touched on the side edge of the EasyTouch blood test strip. The blood will soak up to the end of the strip and sound beeps. Results appear on the screen after 150 seconds. The strip is pulled out and thrown away. The chips are stored again in the bottle after use, then close tightly (Easy Touch)

Blood Collection

Prepare tools and materials. Respondents had blood drawn from the veins by tilting the arms upward. Check the veins that are clearly visible. Insert the blood collection needle at a 45o angle. Take 3 ml of blood.

1.5 ml of blood is entered into the tube containing the anticoagulant for testing the absolute neutrophil count and erythrocyte index. The remaining blood (1.5 ml) was put into a tube that did not contain an anticoagulant for uric acid examination using a photometer [13].

Uric Acid Examination

The uric acid level is checked using a photometer to make it more accurate. Examination begins with a serum and standard control examination. If the control and standard values are in accordance with the value range, then the participant sample examination can be carried out. The method is 20 µl of control serum and 20 µl of standard, put into the test tube. Add uric acid reagent (mixture R1 + R2) 1000 µl. Incubation for 5 minutes at 37oC. Read at a wavelength of 546 nm. Put the participant's serum sample put 20 ul into another test tube. Add 1000 ul of uric acid reagent (mixture R1 + R2). Incubation for 5 minutes at 37oC. Read at a wavelength of 546 nm. Interpretation of the results is to compare with normal values in men between 3.4- 7.0 mg / dl, while women between 2.4 - 5.7 mg / dl (PT Accurate Intan Madya)

Examination of Absolute Neutrophil Count and Erythrocyte Index.

Blood was checked with a hematology analyzer MINDRAY BC 5300 after Quality Control (QC). The results are seen on the print out of the tool. The normal value of absolute neutrophils is 2.5-7.0 x 103 / ul (Jasa Kartini Hospital), while the normal value of the erythrocyte index is MCV 80-100 fl; MCH 27-34 pg; MCHC 32-36g / dl (Jasa Kartini Hospital).

Data analysis

The data that has been collected is then analyzed using SPSS 25 for Windows software. Normality test using the Kolmogorov-Smirnov. If it is normally distributed, it will be followed by a relationship test, namely the Pearson Test. If not in normal distribution use the Spearman.

RESULTS

Respondents who took part in this study were 30 men, were fasting, not obese and had characteristics that can be seen in table 1.

Table 1. Characteristics of participants

variable	Age (Year)	Height (cm)	Weight (cm)	Body Mass Index (BMI)	Uric Acid (mg/dl)	Hemoglobin (g/dl)	MCV (fL)	MCH (pg)	MCHC (g/dl)	Neutrophil Absolut Count (sel×10^3/µL)
Number of Sample	30	30	30	30	30	30	30	30	30	30
Mean ±SD	42,60 ±8,920	167,27 ±5,583	66,87 ± 10,0301	23,83 ±2,972	8,863 ±1,0486	14,93 ±1,94	85,6300 ±6,59614	29,40±2, 68063	34,3800± 1,01418	4,9143± 1,73561

Table 2. Test of Spearman's Relationship between the Number of Neutrophils and the Erythrocyte Index

Variable	N	p	α=0,05
MCV (fl)	30	0,406	P>0,05
MCH (pg)	30	0,631	P>0,05
MCHC (g/dl)	30	0,730	P>0,05

DISCUSSION

The results of Kolmogorov Smirnov was obtained P = 0.031, so the data was not normally distributed due to P <0.05. The next test is using the Spearman test. The erythrocyte index (MCV, MCH and MCHC) had a P value> 0.05which means that the absolute neutrophil count and erythrocyte index in hyperuricemia had no relationship.

The results showed that the participants' uric acid levels increased by an average of 8.863 ± 1.0486 mg / dl or 0.5270 ± 0.0624 mmol / L. The measurement results of the absolute neutrophil count were normal with an average of 4.9143 ± 1.73561 cells x 10^3 / µL. This number does not indicate inflammation. Neutrophils did not experience this average increase in uric acid levels.

Respondents' MCV levels were normal with an average of 85.6300 ± 6.59614 fl. MCV levels do not indicate an abnormality in erythrocyte size. Erythrocyte size does not change. As stated in Song's research. et.al, 2019, increased uric acid levels can protect erythrocytes, especially on the surface of erythrocyte membranes. The participants' MCV values were within the normal range so that it could be stated that the participants had normal erythrocyte size (normal value = 80-100 fl) or normocytic. If the size is less than the normal value, it is called a microcytic, whereas if it is more than the normal value, it is called a macrocytic[14].

The MCH level was normal with an average of 29.40 ± 2,68063 pg. The MCH level does not indicate an abnormality in the mean amount of hemoglobin in erythrocytes. This value is still within the normal range of 27-34 pg (Jasa Kartini Hosptal). If the value is still normal, it is called = normochrome; less than normal value = hypochromic; more than normal value = hyperchrome[15].

MCHC levels were normal with a mean of 34.3800 ± 1.01418 g / dl. The MCHC level did not show any abnormality from the calculated value of the hemoglobin molecule density in erythrocytes. Participants still had normal MCHC levels, no significant abnormalities in the hyperuricemia state.

The results of the research by Song et al., 2019 illustrate that uric acid is very effective in protecting erythrocytes from oxidative stress damage. Uric acid can maintain the surface of the erythrocyte membrane thereby preventing the formation of echinocytes and spheres. Low uric acid levels showed that the number of erythrocyte speroekinocytes was higher than participants with high uric acid levels[3].

On the other hand, high uric acid levels or in a saturated state will form monosodium urate crystals which can trigger an immune response, especially neutrophil cells. Neutrophil cells will respond by phagocytic monosodium urate and induce the release of various cytokines as shown in Figure 1. However, the uric acid levels of the participants had a mean of 8.863 ± 1.0486 mg/dl which did not give an indication of an increase in the absolute neutrophil count. There is no systemic inflammation. Neutrophil cells do not secrete cytokines or other substances that

can damage erythrocytes so that the quality of erythrocytes is still normal, which can be seen on the erythrocyte index values (MCV, MCH and MCHC) which are still normal range.

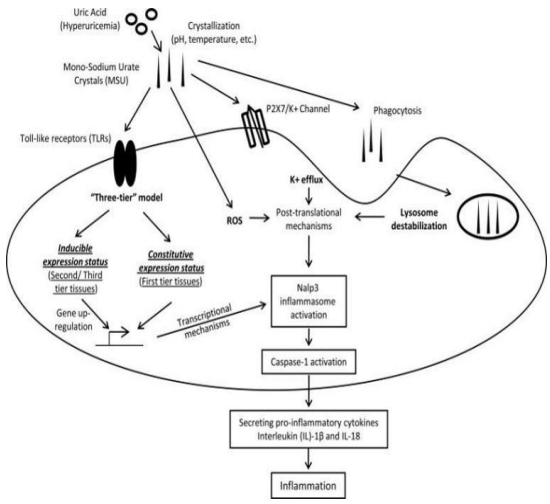

Figure 1. Inflammation Process by Uric Acid [16].

In adults, the kidneys are organs that produce erythropoietin> 90%. Some of it is in the heart and brain. Thus, kidney disease can cause anemia, because the kidneys are inadequate in producing erythropoietin (a hormone that plays a role in the process of forming red blood cells). The oxygen sensor in the kidneys will detect a lack of oxygen so that the kidneys will regulate the release of the amount of erythropoietin released into the blood (Figure 2). This hormone acts on erythrocyte precursor cells in the bone marrow to stimulate proliferation, maturation and increase the number of red blood cells in the peripheral circulation ([17].

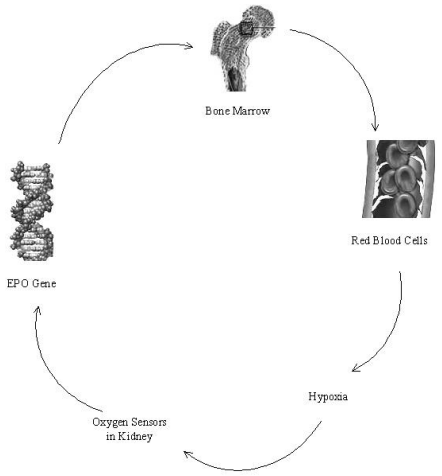

Figure 2. The Relationship between Kidney and Bone Marrow on Erythrocyte Production [17].

In kidney failure can cause normochromic anemia, but occurs within a few weeks. Meanwhile, in chronic renal failure anemia can occur with a hemoglobin level below 6 g / dl. Anemia can occur due to a lack of erythropoietin synthesis which plays a role in the erythropoiesis regulatory process[15]. The participants did not show any abnormalities of anemia, which could be seen at normal hemoglobin levels (table 1); MCV and MCH levels are normal, which indicates that the erythrocyte cells are normal (normochromic normocytic). If the hemoglobin level is below normal values, it can be called normochromic normositary anemia. Normochromic anemia can occur in someone with progressive chronic kidney disease [18].

Thus, erythrocyte indexes such as MCV, MCH and MCHC, were still in normal condition in hyperuricemia condition with a mean of 8.863 ± 1.0486 mg / dl. The absolute neutrophil count with erythrocyte index did not have a statistically significant relationship in hyperuricemia patients.

CONCLUSION

In hyperuricemia patients with a mean of 8.863 ± 1.0486 mg / dl, the absolute neutrophil count and erythrocyte

index were not statistically significant (P> 0.05).

REFERENCES

P. Su, L. Hong, Y. Zhao, H. Sun, and L. Li, 2016 ,"The Association Between Hyperuricemia and Hematological Indicators in a Chinese Adult Population," *Medicine (Baltimore).*, vol. 95, no. 7, pp. 1–6.

Y. Di, J. Wang, Y. Chen, N. Sun, L. Wu, and X. Dai, 2017 , "Elevated Interleukin 1 β and Interleukin 6 Levels in the Serum of Children With Hyperuricemia," *J. Clin. Rhematology*, vol. 00, no. 00, pp. 1–5.

Y. Song *et al.*, 2019 , "Uric Acid Provides Protective Role in Red Blood Cells by Antioxidant Defense : A Hypothetical Analysis," vol. 2019.

M. Murea, 2012 . "Advanced Kidney Failure and Hyperuricemia," *Adv. Chronic Kidney Dis.*, vol. 19, no. 6, pp. 419–424.

D. Bolignano, G. D'Arrigo, A. Pisano, and G. Coppolino,2015, "Pentoxifylline for anemia in chronic kidney disease: A systematic review and meta-analysis," *PLoS One*, vol. 10, no. 8, pp. 1–16.

T. L. T. Ho, N. T. T. Hoang, J. Lee, J. H. Park, and B. Kim, 2018, "Determining MEan Corpuscular Volume and Red Blood Cell Count Using Electrochemical Collsion Events," *Biosens. Bioelectron*.

A. Ramadan, W. G. Land, and S. Paczesny,2017, "Editorial: Danger signals triggering immune response and inflammation," *Front. Immunol.*, vol. 8, no. AUG, pp. 1–3.

D. J. Stewart, V. Langlois, and D. Noone, 2019 , "Hyperuricemia and Hypertension : Links and Risks," *Integr. Blood Press. Control*, vol. 12, pp. 43–62.

A. K. Mandal and D. B. Mount,2015, "The Molecular Physiology of Uric Acid Homeostasis," *Annu. Rev. Physiol.*, vol. 77, no. 1, pp. 323–345.

M. A. Martillo, L. Nazzal, and D. B. Crittenden, 2014 , "The Crystallization of Monosodium Urate," *Curr Rheumatol Rep*, vol. 16, no. 2, pp. 1–13.

Verdiansah, 2016 , "Pemeriksaan Fungsi Ginjal," *CDK-237*, vol. 43, no. 2, pp. 148–154.

S. Kar, S. Paglialunga, and R. Islam, 2018 , "Cystatin C Is a More Reliable Biomarker for Determining eGFR to Support Drug Cystatin C for Staging Chronic Kidney," no. January,.

G. Soebrata, 1984, *Penuntun Laboratorium Klinik*. Jakarta: Dian Rakyat.

D. Provan *et al.*, 2007, *ABC of Clinical Haematology*, Third. London: Blackwell.

H. Theml, H. Diem, and T. Haferlach, 2004, *Color Atlas Of Hematology-Practical Microscopic and Clinical Diagnosis*, 2nd revise. New York: Thieme.

M. Jin *et al.*, 2012 , "Uric Acid, Hyperuricemia, and Vascular Disease," *Front Bosci*, vol. 17, no. 2, pp. 656–669.

G. Molineux, M. A. Foote, and S. G. Elliott, 2006, *Erythropoietins and Erythropoiesis-Molecular, Cellular, Preclinical and Clinical Biology*. Switzerland: Birkhauser Verlag.

V. Cernaro *et al.*, 2018 , "Erythropoiesis and chronic kidney disease – related anemia : From physiology to new therapeutic advancements," *Med Res Rev*, no. June, pp. 1–34.