Correlation Between Absolute Neutrophil Counts with the Neutrophil Lymphocyte Ratio in Hyperuricemia

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Abstract—**Objectives: The purpose of this study was to investigate the correlation between absolut neutrophil counts with the Neutrophil Lymphocyte Ratio (NLR) in hyperuricemia. Method: Participants in this study were men 18-65 years old (n = 30). Living in Indonesia the city of Tasikmalaya. Participation selection is based on hyperuricemia, fasting and not obese . Results and Discussion: uric acid levels (mean= 8,863 mg/dl, SD=1.0486); Absolute Neutrophil Counts (x10^3/µl) (mean=4,9143 mg/dl, SD =1,73561) and NLR (mean=2,04, SD=1,252). Coefisien correlation (r) = 0.699, significant (ρ) = <0.05. This means that absolute neutrophil counts and NLR are strongly related to hyperuricemia with an average of 8.863 mg/dl and shows no systemic inflammation at these levels. Conclusion: Relationship of absolute neutrophil counts with NLR had a strong relationship level (r = 0.699, ρ <0.05). Both of these parameters in hyperuricemia (an average of 8.863 mg/dl), still in normal condition, do not indicate systemic inflammation.**
Keywords: absolute neutrophil counts, NLR, hyperuricemia

I. INTRODUCTION

Hyperuricemia is a condition characterized by an increase in uric acid levels in the blood. Hyperuricemia is a cause of gout, kidney dysfunction, hypertension, hyperlipidemia, obesity and diabetes[1]. This molecule becomes clinically important in the form of monosodium urate crystals (MSU), which will settle to the joints or other tissues [2], including in the kidneys[1].

MSU crystals will trigger an immune response because it is a danger signal. MSU triggers inflammatory pathways to produce proinflammatory cytokines such as interleukin 1β (IL-1β)[2], IL8[3], tumor necrosis factor (TNF) alpha [4] , monocyte chemoattractant protein-1 (MCP-1), high-sensitivity C reactive protein (hsCRP), IL-10, IL-18, and endothelin-1 [5].

IL8 is a cytokine that has an important role in neutrophil recruitment[3] . Neutrophils are part of the inate immune system that will be activated by the presence of MSU crystals. Toll Like Receptors (TLRs) present in neutrophils will recognize MSU crystals as danger-associated molecular patterns (DAMPs) [6]. When recognized by the inate immune system, an inflammatory response will occur which causes the release of various cytokines that cause neutrophil recruitment to the inflammatory part. Neutrophils will produce superoxide

which is the cause of tissue damage[4]. An increase in the number of neutrophils is a sign of ongoing inflammation [7].

NLR is a parameter that indicates inflammation. NLR is obtained from the calculation of the absolute neutrophil number divided by the number of lymphocytes. Increased NLR can be caused by various factors one of which is kidney disorders. Increased NLR indicates systemic inflammation. Likewise with neutrophils. Therefore, the relationship needs to be known so that the use of these parameters is only one of the systemic diagnoses of inflammation.

II. MATERIAL AND METHOD

A. Material and Apparatus

The tools and materials used to conduct research include torniquet, syringes, test tubes, cotton alcohol, plaster, blood sample tubes with anticoagulants K-3 EDTA, centronorm, aquabidest, end point uric acid reagents (PT. Akurat Intan Madya (AIM), micropipette, easy touch digital, uric acid sticks, fotometer TC 3300 and hematology analyzer MINDRAY BC5300.

B. Procedure

Respondents receive informed consent to be studied and understood. If agreed, then examined uric acid. For hyperuricemia, blood will be taken for examination of absolute neutrophils and lymphocytes.

How to examine uric acid quickly is as follows:

a) The first step is to insert the test strip by removing the strip from the bottle and immediately closing it again. Insert the test strip into the slot test, and the tool will turn on automatically. Then, the code number will appear briefly. Make sure the code number on the screen is the same as the code number on the test strip vial. If the code numbers do not match, then enter the code lock correctly.

b) The second step, which is taking a blood sample by dropping a drop of blood. When the instrument has displayed a blood symbol, then immediately drop a blood

sample on the intended area, from the test strip. The test strip will automatically absorb blood samples into the reaction site. The tool will then measure uric acid levels, by displaying the value on the digital device's screen [8].

How to examine uric acid with a photometer TC 3300 is as follows:
a) Prepare as many as 4 test tubes, according to table 1, namely for blanks, controls, standards and tests.
b) Enter as much as 1000 μl of uric acid reagent that has been mixed between R1 and R2, into each tube.
c) Enter as much as 20 μl for distilled water in the blank tube, 20 μl of control serum in the control tube, 20 μl of standard solution in the standard tube and 20 μl of serum in the test tube.
d) Homogenize and incubate for 5 minutes at 37 ° C incubator, then read using a photometer [8].

TABLE 1. URIC ACID EXAMINATION PROCEDURE

Materials	Blanko (μl)	Control Serum (μl)	Standart (μl)	Sample Test (μl)
R1+R2 (5:1)	1000	1000	1000	1000
Serum	-	-	-	20
Standard	-	-	20	-
Control	-	20	-	-
Aquabidest	20	-	-	-
Read the absorbance results after incubation for 5 minutes at $37^{o}C$ or 25 minutes at $25^{o}C$				

The procedure for examining absolute neutrophils is as follows :
a) The screen will display MAIN MENU.
b) Press "RUN" and select type "CBC and CBC + Diff".
c) Enter the patient's name-ID and Press "OK".
d) Wait for the Needle in the exit tool.
e) Insert blood samples into the needle.
f) Press "START", the tool will process the sample.
g) Click the Graph or Table menu to see the results.
h) Click print to print the results [8]
The interpretation of the results is to compare with the normal value of 2.5-7.0 $x10^3$/μl (Jasa Kartini Hospital) [8].

The procedure to get an NLR is the absolute number of neutrophils divided by the number of absolute lymphocytes [9].

C. Data Analysis

The research data were analyzed using the SPSS 25 statistical test. It was tested using Spearman Ranks. Research data are presented in tabular form.

III.   RESULTS

Respondents were male as many as 30 people with inclusion criteria namely hyperuricemia, fasting and not obese. Characteristics of respondents can be seen in Table 2..

TABLE 2. CLINICAL CHARACTERISTICS OF RESPONDENTS

Variabel	Mean	Std Deviasi	N
Age (tahun)	42,60	8,920	30
Height (cm)	167,27	5.583	30
Weight (kg)	66,87	10,301	30
Body Mass Index (BMI)	23,73	2,982	30
Uric acid (mg/dl)	8,863	1.0486	30
Absolute Neutrophil Counts ($x10^3$/μl)	4,9143	1,73561	30
NLR	2,04	1,252	30

TABLE 3. STATISTICAL RESULTS OF THE RELATIONSHIP OF ABSOLUTE NEUTROPHIL COUNTS WITH NLR IN HYPERURICEMIA

Component	Correlation coefficient	Sig. (2-Tailed)	$\alpha = 0,05$
Absolute Neutrophil counts with RNL	0,699	0,000	$\rho < 0,05$

IV.   DISCUSSION

Based on table 2. respondents have high uric acid levels (hyperuricemia) that is with an average of 8.9 mg/dl. Respondents were not obese with an average body mass index of 23.73. The result of the average absolute neutrophil is normal, which is 4.91 x 10^3/μl, and NLR has an average normal value of 2.04. The indication of inflammation in the RNL value is> 5, while the absolute neutrophil count is 2.5-7.0 $x10^3$ /μl (Jasa Kartini Hospital).

Based on table 3., the correlation coefficient on the relationship between absolute neutrophil counts and NLR is 0.699 and the relationship is strong, significant ($\rho <0.05$). The absolute neutrophil count is dominated by the normal neutrophil count, which is an average of 4.91 x 10^3/μl. This does not increase the absolute neutrophil count in the blood (normally is 2.5-7.0 x 10^3 / μl). The state of the number of neutrophils does not indicate inflammation. Inflammation is marked one of them by an increase in the number of neutrophils, which is above the 7.0x10^3/μl.

In hyperuricemia (mean 8.863 mg/dl), absolute neutrophil counts were still normal and indicated no systemic inflammation.
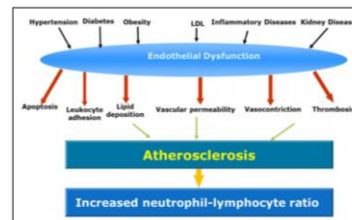

Figure 1. Causes Of An Increase In Neutrophil Lymphocyte Ratio (Rnl)[10]

In figure 1., kidney disorders can cause atherosclerosis due to endothelial dysfunction. One of the causes of kidney disorders is hyperuricemia. Previous studies have found hyperuricemia with an average of 8,928 mg/dl having cystatin C levels with an average of 1,080 mg/dl (normal cystatin C is 0.56 - 0.98

mg/dl)[8]. This indicates renal impairment because cystatin C levels have abnormal values. This cystatin C parameter is considered as a superior parameter compared to creatinine values in assessing kidney function.

Uric acid levels increased in this study, with an average of 8.863 mg / dl, indicating a normal NLR value (mean of 2.04) with a normal value of $\leqslant 5$. There is no systemic inflammation. Inflammation is likely to occur in the area of inflammation, still in organs such as the kidneys or joints so that it does not show an increase in NLR in the blood.

Hyperuricemia will cause damage to the kidneys due to the accumulation of uric acid in the kidneys. Damaged cells in the kidneys can cause an immune response. One of the immune cells that plays a role is neutrophil cells. Neutrophils will destroy cells that die through phagocytosis. The average absolute neutrophil count in this study was $4.91 \times 10^3/\mu l$ (normal value is $2.5\text{-}7.0 \times 10^3/\mu l$). This number has not increased, because there is no systemic inflammation.

Absolute neutrophil counts can still be normal due to uric acid in the kidneys or joints that have not activated the inflammatory NALP3, which causes the ASC and caspase-1 adapters to not activate through pyryine domain (PYD-PYD) and caspase-recruitment domain (CARD-CARD) activation so does not produce pro-IL-1β maturation into its active form, IL-1β. If IL-1β is not active then it will not activate an IL-1R complex in recruiting MyD88 through TIR-TIR homotypic relationships or interactions. This process can result in the inactivation of the nuclear factor-κB (NF-κB) molecule so that it cannot move chemokine transcription (S100, IL-8, or macrophage inflammatory protein 2 (MIP-2)) in recruiting neutrophils to the site of inflammation and the number of neutrophils will still in normal state [11].

The number of neutrophils will increase if uric acid has formed crystals and settles in various organs, especially in the kidney organs and joints. The crystal can be considered a DAMP which is a danger signal to the body, and will trigger a non-specific immune response. The immune response begins with the introduction of uric acid crystals by neutrophil cells and allows activating inflammatory NLRP3 which will then produce various cytokines[12].

Absolute neutrophil counts have a strong relationship with NLR because they do have similarities in terms of inflammatory assessment. Inflammation is characterized by an increasing number of neutrophils, as well as RNL. So that both continue to play a role in the assessment of inflammation which statistically has a strong relationship. Therefore, the assessment of inflammation can be represented by NLR or also by only the absolute neutrophil count. Absolute neutrophil counts are still normal and also NLR. This indicates that there is no systemic inflammation in hyperuricemia with an average uric acid level of 8,863 mg/dl.

V. CONCLUSION

It was concluded that the relationship of absolute neutrophil counts with RNL had a strong relationship level (r = 0.699, ρ <0.05). Both of these parameters in hyperuricemia (an average of 8.863 mg / dl), still in normal condition, do not indicate systemic inflammation.

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