TURNITIN_Correlation_between_Ferritin

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Correlation between Ferritin Levels with Malondialdehyde and Neutrophil Lymphocyte Ratio on Iron Overload

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ABSTRACT

Thalassemia major is one of the types of thalassemia that need a routine blood transfusion. If not treated immediately, the patient will only last for 1-8 months. Blood transfusions performed at least or more than 10 times causes iron overload. Excess levels of Fe ions in the body will be stored in the form of ferritin. If the ferritin level is high, cell damage will occur in the presence of a fat peroxidation reaction or Malondialdehyde (MDA). Cell damage can trigger proinflammation, which increases neutrophil counts and decreases lymphocyte counts. The Neutrophil-Lymphocyte Ratio (NLR), which measures the ratio between Absolute Neutrophil Count (ANC) divided by Absolute Lymphocyte Count (ALC) with a manual peripheral blood picture. This study aimed to determine the correlation of ferritin levels with MDA and NLR in iron overload. This study used an analytical observational design with a cross-sectional approach, with samples were thalassemia patients who received repeated blood transfusions at the General Hospital Dr. R Soetrasno, Rembang City and Regional General Hospital Dr. R Soedjati, Grobogan Purwodadi. Inclusion criteria were age 10-18 years, transfusion 10-20 times, normal body temperature. Exclusion criteria were Fe therapy orally, leukocytosis, chronic kidney disease. In the MDA levels, there was no significant difference where p=0.25 by Spearman test. In the NLR there was no significant difference where p=0.91 by Spearman test. There is no correlation between ferritin levels with MDA and NLR in iron overload.

Keywords: Ferritin, malondialdehyde, neutrophil-lymphocyte ratio, iron overload

INTRODUCTION

Thalassemia is a blood disorder characterized by fragile red blood cells or shorter than normal blood cells age (less than 120 days) hence patients will experience a deficiency of red blood cells. There are has several types of thalassemia, based on genotypes (alpha and beta) and or without genotypes (hydrops fetal thalassemia, thalassemia major, thalassemia intermediate, and thalassemia minor).[1] The incidence of thalassemia patients in Indonesia in 2013 was 1.5% and 1,653 people were suffering from thalassemia major.[2]

Under normal body metabolism, Fe is bound to transferrin, and the body stores it in the form of ferritin to maintain Fe levels in the body.[3,4] Ferritin can accommodate a maximum of 4500 $Fe3^+$ ions in a single ferritin molecule, but usually, ferritin only contains less than 2000 $Fe3^+$ ions.[5] If the transferrin binding capacity and ferritin threshold are exceeded, the free Fe ions will increase. In the case of chronic iron overload, Fe deposits will increase in the form of ferritin.[6] High levels of ferritin can produce Fe ions. Fe ions can act as free radicals and also stimulate the breakdown of intracellular hemoglobin into heme and globin, which can damage cells.[7]

The impact of the process of solving heme and intracellular globin due to high ferritin levels is that the cell will produce Reactive Oxygen Species (ROS). When ROS reacts with the fatty acid component in the cell membrane, Malondialdehyde (MDA) is formed. Malondialdehyde will cause the breakdown of fatty acid chains into various toxic compounds and cause damage to cell membranes.[8] Reactive oxygen species also cause mitochondrial damage, caspase activation, and apoptosis accompanied by the release of proinflammatory cytokines such as Interleukin1 (IL-1), Interleukin 6 (IL-6), and Tumor Necrotizing Factor-α (TNF-α) through transcription factors (NF-kB).[7] One of the physiological responses of the immune system to the presence of information is an increase in the number of neutrophils and a decrease in the number of lymphocytes. Toxic metabolism released by activated neutrophils and inflammatory cytokines will damage tissues and cause organ dysfunction. Inflammation that occurs due to iron overload can also be measured using the Ratio of Neutrophil

Lymphocytes (NLR). The NLR is the ratio between Absolute Neutrophil Count (ANC) divided by Absolute Lymphocyte Count (ALC), where ANC and ALC are obtained from a hematology analyzer, which is reviewed with a manual peripheral blood picture.[9] In this study, researchers aimed to examine whether there was a relationship between ferritin levels with MDA and NLR levels in iron overload.

METHODS

This study used an analytical observational design with a cross-sectional approach. The study population was thalassemia major patients receiving repeated blood transfusions. The affordable population of this study is patients with blood transfusion services at the Dr. R Soetrasno General Hospital, Rembang and Dr. R Soedjati General Hospital, Grobogan Purwodadi. The samples of this study were thalassemia patients who received repeated blood transfusions at the Dr. R Soetrasno General Hospital, Rembang and Dr. R Soedjati, Grobogan Purwodadi who met the inclusion and exclusion criteria.

The research data were taken from interviews, physical examinations, and laboratory examinations of patients. Information about kidney disease was obtained from the number of urination frequencies and urine volume. Liver disease is excluded by looking at the physical condition of the patient such as jaundice and interviewing. EDTA samples were examined directly on the same day using a hematology analyzer XN-1000, while for frozen samples, blood was centrifuged and serum was taken to be stored at -20^{0}C, before MDA examinations were carried out at the biochemistry laboratory of Diponegoro University Faculty of Medicine (FK UNDIP) and ferritin in the chemistry analyzer BIOLABO in the laboratory due to iodine deficiency disorders (IDD) from FK UNDIP.

Data analysis using statistical software programs with computerization. The data normality test used the Shapiro-Wilk test. If the data distribution was normal then the relationship test used the Pearson test. If the data distribution was not normal, then the data transformation would be carried out until the data was normal. If the data remained abnormal then the relationship test used the Spearman test. Sig. value was considered significant if $p < 0.05$. A significant and strongly correlated relationship test would be followed by a correlation test using a linear regression test to determine the approximate formula for MDA and NLR levels based on ferritin levels.

This study was approved by the Ethics Committee of Dr. Telogorejo, Semarang with ethical clearance number 13262/TU.710/KEPK/K/2020.

RESULTS AND DISCUSSIONS

The population of the research sample was 28 people consisting of 12 male samples and 16 females. The age range of the overall study sample was 6 to 16 years; with an average age of the study sample of 10.21±2.63 years. The frequency of the amount of blood transfusion ever obtained by a sample of patients in this study was more than ten times. Three samples in this study had never received chelation therapy, while 25 samples had received chelation therapy to reduce iron overload status (Table 1).

Ferritin levels in the study sample had an average of 2765,14±1816,86 ng/mL with a median value of 2692,50 ng/mL and a minimum value of 504ng/mL and a maximum value of 9521 ng/mL. All samples had higher ferritin levels than the normal value of > 400 ng/mL. Ferritin data normality test results obtained abnormal data distribution ($p < 0.05$) with the Shapiro-Wilk test. Data transformation has been carried out on the ferritin parameter but still obtained an abnormal data distribution.

Malondialdehyde levels in the study sample had an average of 6.23±4.21 mmol/l with a median value of 4.64 mmol/l with a minimum value of 0.74mmol/l and a maximum value of 14.88 mmol/l. Data on MDA variables in this study had abnormal data distribution ($p < 0.05$) with the Shapiro-Wilk test. Data transformation has been done on MDA parameters but still obtained abnormal data distribution.

Neutrophil levels in the study sample had an average of 53.93±8.02% with a median value of 54.50% with a minimum value of 39% and a maximum value of 68%. Lymphocyte levels in the study sample had an average of 37.71% with a median value of 37%; a minimum value of 23%, a maximum value of 55%.

The ratio of neutrophils and lymphocytes was measured by dividing the absolute neutrophil levels with absolute lymphocyte levels. Neutrophil-lymphocyte ratio levels in this study had an average of 1.54±0.57 with a median value of 1.48; a minimum value of 0.73 and a maximum value of 2.74. The data on the NLR variable in this study had an abnormal data distribution $p < 0.05$ with the Shapiro-Wilk test. Data transformation had been done on the NLR parameters but still obtained an abnormal data distribution.

Table 1. Characteristics of research data

Characteristics	Mean±SD	Median (minimum-maximum)
Gender (n)		
Males 12		
Females 16		
Age (years)	10.21±2.63	10 (6–16)
Chelation (n)		
Not 3		
Yes 25		
Ferritin (ng/mL)	2765.14±1816.86	2692.50 (504–9521)
MDA (mmol/L)	6.23±4.21	4.64 (0.74–14.88)
Neutrophils (%)	53.93±8.02	54.50 (39–68)
Lymphocytes (%)	37.71	37 (23–55)
NLR	1.54±0.57	1.48 (0.73–2.74)

The relationship test was performed using the Spearman test because the data distribution was not normal between ferritin levels and MDA levels. From the results of the Spearman test, no significant relationship between ferritin levels and MDA levels with p=0.25 was found (Figure 1).

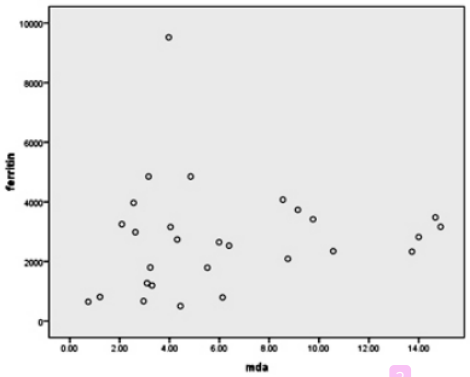

Figure 1. Box plot of the relationship between ferritin levels and MDA levels

The relationship test was performed using the Spearman test because the data distribution was not normal between ferritin levels and NLR levels. From the results of the Spearman test, no significant relationship between ferritin levels and NLR levels with p=0.91 was found (Figure 2).

The only treatment for thalassemia major is routine blood transfusions for life. If not given blood transfusion immediately, the patient will become weak, and then die.[4] However, blood transfusions with a frequency of at least or more than 10 times will cause iron overload (excess iron). This study used

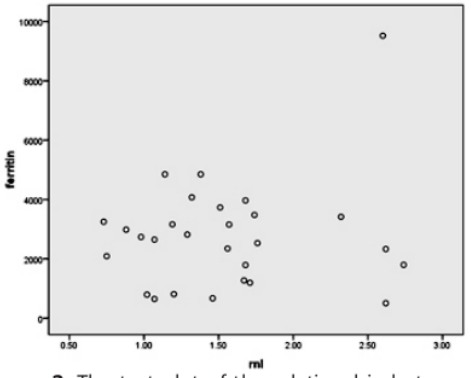

Figure 2. The test plot of the relationship between ferritin levels and NLR levels

samples with an average age of 10.21±2.63 years.[10] Late diagnosis of thalassemia major in Indonesia was due to lack of early examination in patients and did not see the initial symptoms in patients.

Malondialdehyde is oxidative stress and inflammation marker in cells through the lipid peroxidation pathway in the liver so that an increase in MDA levels is associated with excess iron in the liver. Lipid oxidation reaction in the liver can increase MDA and ferritin into circulation. In this study MDA, levels in the study samples had an average of 6.23±4.21 mmol/l. Previous research stated that normal MDA levels with populations from Jakarta in 2005 were 0.08±0.01 nmol/mL.[11] In the study of Sengsuk *et al.* it was found that MDA levels were significantly higher in patients with transfusion-dependent β-thalassemia than in normal patients ($p < 0.001$).[12] However, the study did not compare MDA levels in normal patients.

Walter *et al.* also explained that the amount of iron affects the reticuloendothelial system (bone marrow and spleen) and hepatocytes, heart, and endocrine glands. Under normal conditions, the liver has a large capacity to store more iron in the form of ferritin and when needed by the body it can be redistributed. Hepatocellular damage from $Fe2^{+}$ triggers peroxide damage from the lysosomal membrane of the lipids.[13-15]

This statement was not following the results of this study because there was no significant relationship between ferritin levels and MDA levels with p=0.25. This difference was caused by the presence of several variations of characteristics and age factors, the type of iron chelation drugs, and compliance with the consumption of iron chelation drugs in this study so that it could affect the results.

The NLR is part of the routine blood index that shows an inflammatory status. This parameter is a new, easy and suitable parameter for detecting inflammatory status in cardiovascular disease, pancreatitis, and trauma. However, research related to these parameters in the case of major transfusion-dependent thalassemia patients and their relationship to ferritin levels in these patients is still unknown.

The results of this study indicated that the average value of the ratio of NLR in patients with dependent transfusion-β thalassemia was 1.54±0.57. Emokpae *et al.* found an average NLR in sickle cell anemia patients of 1.31±0.02. The study also compared patients with hemoglobin abnormalities and found that patients with sickle cells had significantly increased NLR values compared to patients without hemoglobin abnormalities ($p < 0.001$). The increase in NLR in patients with sickle cell anemia was 1.28 compared to normal patients. This study did not compare NLR levels of thalassemia dependent transfusion patients with patients without hemoglobin abnormalities. So far, studies related to NLR in patients with thalassemia-dependent transfusion have not been found. Neutrophils act as active non-specific inflammatory mediators of cellular immunization while lymphocytes act as protective or adaptive responses to inflammation. Increased NLR can be used to determine patients who do not have resistance to inflammatory responses and poor survival.[15]

Neutrophil-lymphocyte ratio values according to Lee *et al.* have differences in males and females. The average NLR in South Korea of all ages was 1.65±0.79, while the NLR value in males was 1.63±0.76 and females 1.66±0.82. The research had not considered race and was only done in South Korea. The normal value of NLR does not yet have a standardized value and when viewed from studies from other countries such as Emokpae *et al.*, Valencia *et al.* showed enough variation so that it required further multicenter research and factors that could influence the results of the examination.[15]

Inflammatory and oxidative stress processes are related to one another because inflammation can trigger oxidative stress and vice versa. Based on a meta-analysis study, when the levels of oxidative mediators increased such as MDA and nitric oxide, the levels of antioxidants such as superoxide dismutase, catalase, and glutathione decreased but the level of lipid peroxidation in the blood circulation increased.[16]

Lipid peroxidation reactions in the liver added MDA and ferritin levels into the blood circulation so that the results of inflammatory parameters such as NLR were expected to increase. A study conducted by researchers explained that the comparison of the value of ferritin and NLR levels in patients with dependent transfusion-β thalassemia did not find a significant relationship with a p-value of = 0.91. However, Valencia *et al.* study showed a correlation between ferritin and NLR values with p=0.0001 in patients with recurrent blood transfusions as in hemodialysis patients. The Valencia *et al.* study sample did not have a hemoglobin abnormality so differences in characteristics between studies were able to be one of the factors influencing the results of this study.[14]

CONCLUSION AND SUGGESTIONS

In this study, there was no correlation between ferritin levels with MDA and NLR levels in iron overload. The Spearman test results also showed that there were no significant differences between ferritin levels with MDA and NLR levels in iron overload. Researchers suggestion to examine oxidant status that can affect the result of MDA and NLR.

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