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ANALYSIS OF MALONDIALDEHYDE LEVELS IN CHILDREN WITH BETA THALASSEMIA: A CROSS-SECTIONAL STUDY

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Abstract

Beta thalassemia is an autosomal recessive genetic condition affecting people worldwide, including in Indonesia. Malondialdehyde levels, a peroxidation product, can be used to see if there is an iron buildup in the body due to lifelong transfusions. This research is a novelty because it analyzes malondialdehyde levels in children with beta-thalassemia: a cross-section study. The study aims to analyze malondialdehyde levels in children with beta-thalassemia. Methods in this study, a cross-sectional investigation was carried out at Dr. Hospital Wahidin Sudirohusodo, Makassar. The study was carried out between April and August of 2022. The study sample consisted of participants diagnosed with beta-thalassemia and non-thalassemia (controls) eligible to participate. Patients with and without beta-thalassemia had their malondialdehyde levels measured. The study results showed that the 60 children, aged six months until 18 years, were separated into two groups, 30 of whom had beta-thalassemia and another 30 who did not. With a significant P-value of 0.000, it was determined that beta-thalassemia children had more substantial amounts of malondialdehyde than children without the condition. Children with beta-thalassemia major had significantly higher malondialdehyde levels than those with beta-thalassemia HbE (P-value = 0.000). The conclusion was that malondialdehyde levels were more significant in beta-thalassemia kids than non-thalassemia kids. Malondialdehyde values are more effective in kids with beta-thalassemia major than those with beta-HbE thalassemia.

Keywords: Malondialdehyde; Beta thalassemia; Transfusion; Children.

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INTRODUCTION

Thalassemia is a heterogeneous group of blood disorders that affect the hemoglobin gene and result in ineffective erythropoiesis. Decreased hemoglobin production causes anemia at an early age, and frequent blood transfusions are required to maintain hemoglobin levels (1)(2).

Patients with beta-thalassemia require regular blood transfusions throughout life to maintain hemoglobin levels above 9–10.5 g/dL to suppress ineffective erythropoiesis activity in the bone marrow and prevent growth disorders. However, repeated blood transfusions can result in iron overload because there is a continuous accumulation of iron, while the body's ability to excrete iron is minimal (3). Moreover, because erythropoiesis is ineffective, extra iron is absorbed into the gastrointestinal system, leading to the abundant iron that beta-thalassemia patients experience. Excessive iron harms structures and can cause cirrhosis, developmental disorders, heart failure, and endocrine abnormalities (4).

Malondialdehyde (MDA) may be able to detect an excess of iron. Because elevated ferritin levels are related to iron excess and inflammation When iron storage capacity is low, free iron in beta-thalassemia patients catalyzes the formation of harmful reactive oxygen species (ROS), such as the hydroxyl radicals (OH-) generated from hydrogen peroxide via the Fenton reaction. Oxidative stress happens when the antioxidants' capacity to resist ROS is exceeded. This will result in the production of malondialdehyde molecules (5).

Our study aims to analyze and explain the relationship between malondialdehyde levels in beta-thalassemia patients with iron overload conditions.

MATERIALS AND METHODS

Study Design and Participant

A single – center cross – sectional observational was performed at the infection center at Dr. Wahidin Sudirohusodo Hospital Makassar. Hospital, Makassar, Indonesia, from April to August 2022. The Wahidin Hospital enlisted beta-thalassemia patients between the ages of 6 months through 18 years who were recruited for this research. The samples used in this study were all populations that met the inclusion criteria. 1) All patients with beta-thalassemia major and HbE who received outpatient treatment at the hematology-oncology polyclinic or who received one-day care were eligible. 2) Non-thalassemia patients who received outpatient treatment at the growth and development clinic or were hospitalized with the acute disease but no comorbidities (as controls). 3) Willingness to participate in research, as evidenced by parental consent and signing an informed consent form. Children with liver disease, kidney disease, thalassemia patients who were bleeding and had severe pain that required

hospitalization, and patients with poor nutrition were excluded.

Subject allocation

Participating in this study was available to all subjects who fulfilled the inclusion and exclusion criteria. The research subjects who met the research criteria were grouped into three groups, namely a group of children with beta-thalassemia major, children with beta HbE thalassemia, and children with non-thalassemia (control), which consisted of the children with mild diseases. Then the research sample is taken.

Data Collection

Body weight and length measurements were taken on each subject to determine nutritional status. Then, 1-2 ml of blood was taken, and the sample was taken to the HUMRC laboratory to examine malondialdehyde levels.

Measurement Method

Nutritional status: Body weight was measured using a stepping and lying scales that had been calibrated with an accuracy of 50 grams. Before weighing, first check whether the instrument is in a balanced state (the needle shows zero). The child is weighed in a standing position without shoes with minimal clothing. Children aged < 2 years use a baby scale. Measurement of body length for age <2 years using an infantometer and measuring height for age 2 years using a microtoise at 0.1 cm of precision.

Malondiadehyde Levels

Within 30 minutes of obtaining the sample, 1-2 ml of blood were centrifuged after being put in a red blood sample tube. For 8 to 72 hours, all blood samples were kept at a temperature of 2 to 8^{o}C in a cooler box with an ice pack. For one to one and a half years, all samples are preserved in a freezer (80^{o}C). Prior to usage, the samples and The malondialdehyde test kit should be gradually warmed at room temperature for about 30 minutes. After being deposited on a plate, the sample was incubated with reagents and ELISA liquid for 60 minutes at 37°C. Wring the plate out five times. Substrates A and B are combined into one liquid. For 10 minutes or until the color changes, incubate at 37 °C. The study sample is prepared for analysis after a 10-minute plate. After drying the plate for 10 minutes, the research sample is ready for analysis.

Definition

Beta thalassemia A patient who has a point mutation in the beta chain that occurs at position 26. A patient who has impaired total loss of the beta-globin chain has beta thalassemia major. Both thalassemias were diagnosed from HB analysis in the patient's medical record.

Malondialdehyde levels are malondialdehyde levels in blood taken from venous blood and measured using the spectrophotometric thiobarbituric acid (TBA)

test method. Normal malondialdehyde values are 0.5-1.3 nmol/ml.

Nutritional status based on NCHS 2000 parameters for children aged five years (6), by assessing actual body weight against ideal body weight according to actual height multiplied by 100%: Nutritional obesity if it is above 120%; More nutrition if it is between 110-120%; Good nutrition if it lies at 90-100%; Malnutrition if it lies at 70-90%; Malnutrition if located at < 70%.

Nutritional status based on WHO parameters for children aged < 5 years, based on body weight for age (7): Obesity if > 3 SD; Overnutrition if between 2 SD to 3 SD; At risk of overnutrition if between 1 SD to 2 SD; Good nutrition if between -2 SD to 1 SD; deficit, if between -2 SD to -3 SD; Malnutrition if obtained < -3 SD.

Data Analysis

SPSS version 26 is used for data analysis. The frequency, mean value, percentage, and minimum and maximum values describe the characteristics of the subject. The chi-square test was used to assess differences between gender, and nutritional status, against the beta thalassemia group. An Independent t-test was used to assess differences in age, hemoglobin, MCV, MCH, RDW, Fe, and TIBC in the beta thalassemia group. Mann-Whitney was used to assess the difference between Mentzer index, ferritin, and transferrin saturation in the beta thalassemia group.

Ethical Clearance

In carrying out this research, every action will be carried out after providing information and obtaining the consent of the patient's parents. The Faculty of Medicine of Hasanuddin University Makassar's Ethical Approval for Health Sciences in Humans also approved this work with number 294/UN4.6.4.5.31/PP36/2022.

RESULTS AND DISCUSSION

Result

Our research consisted of a total of 60 children, 30 who either did not have thalassemia and also the other 30 did. The characteristics of the study participants are shown in Table 1. The non-thalassemia population exhibited a greater prevalence of excellent nutritional status as compared to the thalassemia group. There was a significant difference in nutritional status between the two groups ($p<0.05$). The two groups' average ages also varied considerably from each other (p 0.05). When contrasted with the non-thalassemia group, the beta-thalassemia group's mean age was older (10.6 years vs. 6.1 years).

The characteristics of the participant groups having beta thalassemia major and beta HbE are shown in Table 2. The two groups have distinct Mentzer indices. The average Mentzer index value for the beta HbE thalassemia group is substantially lower in this case (19.1) than for the beta thalassemia major group (25.9), with a value of $p=0.018$.

Table 3 shows the contrast between the beta-thalassemia group and the non-thalassemia group's malondialdehyde levels. Between the non-thalassemia group and the beta-thalassemia group, mean levels of malondialdehyde were 2.02 ng/ml and 14.65 ng/ml, respectively. The mean amounts of malondialdehyde between the beta-thalassemia and non-thalassemia groups were significantly different. With p = 0.000 ($p<0.05$), the mean level of malondialdehyde in the thalassemia group was substantially greater (14.65 ng/ml) than in the non-thalassemia group (2.02 ng/ml).

Table 4 compares the amounts of malondialdehyde in the beta thalassemia major group with the beta thalassemia HbE group. Beta thalassemia major and beta thalassemia HbE groups' respective mean values were 18.65 ng/ml and 6.67 ng/ml. The mean malondialdehyde levels in the beta thalassemia major group were considerably greater (18.65 ng/ml) than in the beta HbE thalassemia group (6.67 ng/ml), with a value of p = 0.000 ($p<0.05$).

Discussion

Congenital anemia is caused by a series of hematological illnesses known as thalassemia that is characterized by insufficient or abnormal synthesis of the hemoglobin globin chain, which leads to diminished development of functional hemoglobin and/or red blood cells (RBC) (8).

Iron overload is a major problem in both transfusion-dependent thalassemia (TDT) and non-transfusion-dependent (NTDT) thalassemia. It is known that excess iron is caused by oxidative stress caused by blood transfusions. However, blood transfusion is used as the main therapy for thalassemia. One of the markers of oxidative stress is malondialdehyde (MDA) (9). So, it can be said that MDA is oxidative stress and a marker of inflammation in cells through the lipid peroxidation pathway in the liver, so an increase in MDA levels is associated with iron overload in the liver. Lipid oxidation reactions in the liver can increase MDA and ferritin in circulation (10).

Our findings revealed that the thalassemia group's average malondialdehyde level (14.65 ng/ml) was significantly higher than the control group (2.02 ng/ml). The statistical test showed that there was a significant difference between the serum levels of malondialdehyde in the non-thalassemia group and the beta-thalassemia group with p = 0.000 ($p<0.05$). This is in line with Bhagat et al. (2012), who found a higher mean malondialdehyde level in the thalassemia population (2.38 ng/ml) compared to the control group (1.12 ng/ml), with p =0.001 (11). Participants with beta-thalassemia versus those without the condition had significantly different amounts of malondialdehyde (0.43 mol/L and 0.14 mol/L, respectively; p = 0.001) (12).

Showed that beta-thalassemia patients who experienced iron overload had an increase in MDA levels by an average of 6.69 0.9 nmol/mL. This is because beta thalassemia major patients experience iron accumulation in their bodies. Actually, the body already has a mechanism for storing iron through ferritin, which can be released back to be used as needed. This ferritin will bind to iron so that ionized iron (Fe2+) does not reach toxic levels in cells. When the iron storage capacity has been exhausted, free iron will catalyze the formation of high concentrations of hydroxyl radicals (OH-) from the hydrogen peroxide component through the Fenton reaction, which will cause membrane damage, protein denaturation, and damage DNA replication (13).

The reticuloendothelial system (bone marrow and spleen), hepatocytes, heart, and endocrine glands are all affected by the amount of iron in the body. Under normal conditions, the liver has a vast ability to store additional iron in the form of ferritin, which may then be redistributed as needed by the body. Fe2+-induced hepatocellular injury causes peroxide breakdown of lysosomal lipid membranes (10).

Elevated MDA levels in thalassemic patients support the hypothesis that free radicals play a significant role in the breakdown of red blood cells and hemolysis by reacting with labile polyunsaturated fatty acids in red blood cell membranes and probably other important organ membranes. The amount of lipid peroxidation reflects how much ROS is created that is not eliminated by the defense (LPO) (14). Repeated blood transfusions and increased gastrointestinal iron absorption cause iron excess in the body, resulting in a vicious cycle and chronic oxidative stress. Free radicals and peroxidative tissue injury accompany severe anemia (Hb range 2-7 gr/dl) and unavoidable consequences, which exacerbate multi-organ illnesses, particularly in iron-accumulating organs such as the liver, spleen, pancreas, heart, and kidneys, among others. Excess iron load causes congestive heart failure, which is the leading cause of death in beta-thalassemia patients (11).

Malondialdehyde concentrations were studied between the beta thalassemia major and beta thalassemia HbE groups. The mean value in the beta thalassemia major group (18.65 ng/ml) was greater than the mean value in the beta thalassemia HbE group (6.67 ng/ml). There was a statistically significant difference (p0.05). Individuals with major thalassemia who often got blood transfusions had considerably higher MDA levels than individuals without major thalassemia (15).

Ineffective erythropoiesis caused by disruptions in globin chain synthesis contributes significantly to the incidence of oxidative stress in thalassemia patients. Reduced globin chain synthesis causes an accumulation of unstable free chain globin,

which causes red blood cell death and the release of heme-form reactive iron. This causes excess iron to accumulate, especially in patients with thalassemia major who require frequent transfusions. Major thalassemia patients receiving frequent blood transfusions had higher MDA levels versus thalassemia intermedia patients supports this as well (12)(15).

Excess iron accumulation, both in blood plasma and intracellularly, results in the creation of ROS molecules that disturb the integrity of cell membranes, including erythrocytes, such as superoxide anions (O2-), hydrogen peroxide (H2O2), and particularly hydroxyl radicals (OH). ROS compound aggression, particularly hydroxyl radicals, causes the breakdown of polyunsaturated fatty acid (PUFA) chains found in cell membrane structures, resulting in the formation of lipid peroxidation chains in reactions that produce toxic compounds, one of which is MDA as the reaction's end product (12).

In this study, MDA levels increased in all beta-thalassemia groups, both beta-thalassemia major and beta-thalassemia HbE. This can be caused by the frequent transfusion frequency and when first diagnosed for a long time, resulting in an increase in oxidative stress and free radicals, even though all patients in this study had taken vitamin E since they were first diagnosed. Iron loading in beta-thalassemia children may potentially have an effect on MDA levels, and the administration of vitamin E as an antioxidant supplement can also affect MDA levels. This shows that more adequate antioxidants are needed to reduce the formation of free radicals so that they can reduce MDA levels in beta-thalassemia patients. MDA is the end product of lipid peroxidation that can be used as a biomarker of oxidative stress. This oxidative stress causes damage to the liver, heart, and endocrine glands and neurological complications in patients with beta-thalassemia (5).

CONCLUSION

The conclusion was that malondialdehyde levels were more significant in beta-thalassemia kids than non-thalassemia kids. Malondialdehyde values are more effective in kids with beta-thalassemia major than those with beta-HbE thalassemia.

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