INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND ANALYSIS

ISSN(print): 2643-9840, ISSN(online): 2643-9875

Volume 06 Issue 09 September 2023

DOI: 10.47191/ijmra/v6-i9-74, Impact Factor: 7.022

Page No. 4521- 4526

The Effect of Omega-3 Fatty Acids on Malondialdehyde and Superoxide Dismutase Levels (Experimental Studies in Dyslipidemic Rat Models)

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ABSTRACT: Dyslipidemia is a lipid profile abnormality characterized by the increase of low-density lipoprotein (LDL), total cholesterol, triglycerides (TG), and a decrease of high-density lipoproteins (HDL). Dyslipidemia causes oxidative stress which can be shown by increasing levels of malondialdehyde MDA and decreased levels of antioxidants superoxide dismutase (SOD). This study aims to determine the effect of omega-3 fatty acids on MDA and SOD levels in dyslipidemic rat models. This study is experimental research with a post-test-only control group design. The research subject was 24 male Wistar rats were randomly divided into four groups, namely K0 (control), K1, K2, and K3. Dyslipidemia was induced by giving quail egg yolk 4ml/kg BW/day for 14 days and then followed by giving an omega-3 fatty acids dose of 36 mg/kg BW/day to the K2 group and dose of 72mg/kg BW/day to the K3 group for 14 days. The MDA statistical analysis used the One Way Anova and Post-hoc test and the SOD data analysis used the One-Way Anova and Tamnhane test. The MDA rate was higher in K2 compared to K1, and K3 with a value of p>0.05. SOD levels in K2 increased compared to K1 and K3 with a p>0.05. The administration of omega-3 fatty acids at doses of 36 mg/kg BW/day and 72 mg/kg BW/day for 14 days did not affect reducing MDA levels in dyslipidemic rats but the administration of omega-3 fatty acids at a dose of 36 mg/kg BW/day for 14 days increased SOD levels insignificantly.

KEYWORDS: Omega-3 fatty acids, MDA levels, SOD levels, dyslipidemia

I. INTRODUCTION

Disturbances in the process of fat metabolism are characterized by changes in the lipid fraction in the blood known as dyslipidemia, which can be either increased or decreased.(1) Dyslipidemia refers to an abnormal condition in which there is an increase in blood levels of density of lipoprotein, total cholesterol, and triglycerides, while the level of high-density lipoprotein (HDL) decreases in the bloodstream. This condition is affected by the acceleration of oxidative reactions caused by stress, and vice versa can also accelerate the occurrence of oxidative *stress. Oxidative* stress arises due to excessive production of free radicals and lack of antioxidants in the bloodstream and has the potential to result in oxidation of lipids in cell membranes, impaired endothelial function, and increased inflammatory responses.(2) Stress-triggered oxidative responses trigger an increase in lipid peroxides which have an important role in promoting the development of atherosclerosis. Furthermore, this response also contributes to an increase in degenerative heart disease or stroke, accelerates the aging process, and supports the possibility of various disease mechanisms, including the risk of developing cancer.(3)

The National Health and Nutrition Examination Survey states that as much as 53% of the total American population, amounting to 105.3 million people, have abnormalities in lipid levels.(4) Dyslipidemia or abnormalities in lipid levels in the blood play a central role in the process of forming atherosclerosis in blood vessels, which triggers coronary heart disease and stroke.(5) Based on data from RISKESDAS, it was found that the incidence of coronary heart disease (CHD) reached 1.5%, and this rate increased with increasing age, especially in the 65-74 years age group which had the highest rate. Meanwhile, the number of individuals who have had a stroke in Indonesia is around 2.5%, or around 250 thousand people who have died, and the rest have experienced minor disabilities.(6)

The Effect of Omega-3 Fatty Acids on Malondialdehyde and Superoxide Dismutase Levels (Experimental Studies in Dyslipidemic Rat Models)

The need for natural supplements that act as antioxidants, such as omega-3 fatty acids in fish oil, is important to consider.(7) Omega-3 fatty acids can influence the formation of lipoproteins in the liver which are then channeled through the blood circulation to lower cholesterol levels.(8) Omega 3 can be called an enhancing factor in antioxidant defense against ROS.(9) Omega-3 fatty acids are obtained from food sources such as fish oil, which contain significant amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as from plant-derived alpha-linolenic acid(10). Research on the addition of omega-3 fatty acids to MDA and SOD levels in rats induced by dyslipidemia is relatively small. Therefore, it is important to conduct this research to explore the impact of giving omega-3 fatty acids on MDA (malondialdehyde) and SOD (superoxide dismutase) levels in dyslipidemic conditions. This study will include giving omega-3 doses at different levels, namely 36 mg/kg BW and 72 mg/kg BW, with a longer treatment period of 2 weeks.

II. MATERIAL AND METHOD

This research is an in vitro experimental research method using Test Only Control Group Design. It is a research design where the results are observed after the treatment. This study used male Wistar rats as research subjects. This study was divided into four groups, the control group (K0), the control group induced dyslipidemia (K1), the treatment group that was given omega-3 fatty acid therapy at a dose of 36 mg/kg BW (K2), and the treatment group that was given Omega-3 fatty acid therapy at dose of 72 mg/kg BW (K3).

III. RESULT

In this study, researchers found that omega-3 fatty acids were not able to reduce MDA levels but were able to increase SOD levels in male Wistar rats depending on the dose given (Table 1).

Table 1. Results of Average Analysis, Normality Test, Homogeneity Test on TNF-α and MCP-1 Expression

Variable	Group				
	K0 N=5	K1 N=5	K2 N=5	K3 N=5	Sig.(p)
MDA (ppm)					
Mean	0.989	1.030	1.324	1.069	
Std. deviation	0.193	0.095	0.223	0.157	
Shapiro Wilk	0.462*	0.763*	0.989*	0.398*	
Levene Test					0.382**
One-Way ANOVA					0.033***
SOD (ng/ml)					
Mean	2.825	2.128	2.762	1.637	
Std. deviation	0.185	0.510	0.627	0.283	
Shapiro Wilk	0.731*	0.419*	0.414*	0.222*	
Levene Test					0.019
One-way ANOVA					0.002***
Information: *Normal p>0.05 **Homogeneous p>0.05 ***Significant p<0.05					

The Effect of Omega-3 Fatty Acids on MDA Levels

Table 1 shows that the highest average levels of MDA were in the K2 group which was standard-fed and induced dyslipidemia and received omega 3 at a dose of 36/kg BW/day (1,324 ppm). Group K0 obtained the lowest MDA level (0.989 ppm) by given standard feed without being induced dyslipidemia, followed by group K1 by being standard fed and induced dyslipidemia but not given omega-3 fatty acid, then the K3 group was given standard feed and induced dyslipidemia and given omega-3 fatty acids at a dose of 72 mg/kg BW/day. One-way ANOVA test result showed significant differences in all groups with a p-value of 0.033 (p <0.05).

Table 2. Differences in MDA levels between the two groups

Group	*p-Value*
K0 vs K1	0.981
K0 vs K2	0.035*
K0 vs K3	0.884

K1 vs K2	0.073
K1 vs K3	0.984
K2 vs K3	0.138

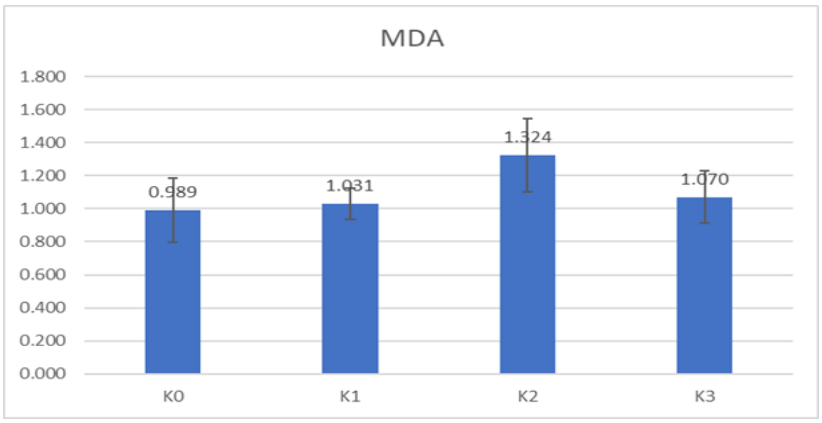

Figure 1. The Average of MDA levels between groups

Post Hoc test result (Table 2) shows that the MDA level in the K0 group was significantly different from the K2 group with a p-value of 0.035. While the K0 group did not differ significantly from the K1 group with a p-value of 0.981, the K0 group to the K3 group with a p-value of 0.884, the K1 group to the K2 group with a p-value of 0.073, the K1 group to the K3 group with a p-value 0.984, and group K2 to group K3 with a value of 0.138 (Figure 2). Based on the data above, it can be concluded that giving omega-3 fatty acids at doses of 36 mg/kg and 72 mg/kg has no effect on reducing MDA levels in male Wistar rats-induced dyslipidemia.

The Effect of Omega-3 Fatty Acids on SOD Levels

The highest mean SOD levels in Table 1 were in the K0 group which was given standard feed without being induced dyslipidemia (2,825 ng/ml). The K3 treatment group was given doses of omega-3 fatty acids72 mg/kg BW/day with induced dyslipidemia and obtained the lowest average SOD level (1,637 ng/ml), then followed successively by group K1 with standard feeding and induced dyslipidemia and group K2 with standard fed induced dyslipidemia and given omega-3 fatty acids at a dose of 36 mg/kg BW. One-way ANOVA test result showed significant differences in all groups with a p-value of 0.002 ($p < 0.05$).

Table 3. The differences in SOD levels between the two groups

Group	*p-Value*
K0 vs K1	0.190
K0 vs K2	1.000
K0 vs K3	0.001*
K1 vs K2	0.533
K1 vs K3	0.292
K2 vs K3	0.071

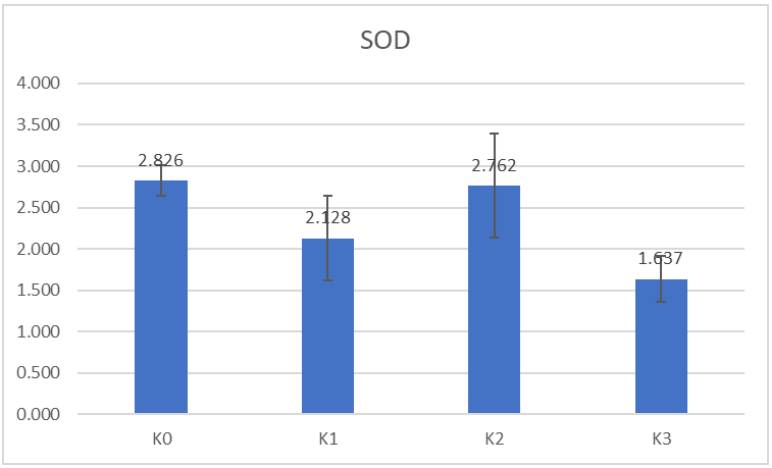

Figure 2. The average of SOD level between groups

Post hoc Tamhane test result (Table 3) shows there was no significant difference in SOD levels between K0 and K1 group with a p-value of 0.190 and 1.000, while there was a significant difference to group K3 with a p-value of 0.001 ($p < 0.05$). There was no significant difference between the K1 group and the K2 group with p-values of 0.533 and 0.492. There was no significant difference between the K2 and K3 groups with a p-value of $p=0.071$ ($p<0.05$). Based on the data above, it can be concluded that the administration of omega-3 fatty acids at a dose of 36 mg/kg/day has no significant effect on increasing SOD levels in male Wistar rats which was induced by dyslipidemia while the administration of omega-3 fatty acids at a dose of 72 mg/kg/day did not affect the increase in SOD levels.

V. DISCUSSION

Dyslipidemia conditions result in increased accumulation of lipids in the liver, thereby reducing the body's ability to reduce blood fat(11). Accumulation of cholesterol in endothelial cells, hepatocytes, leukocytes, erythrocytes, and platelets triggers its production of reactive oxygen species (ROS) and reduces antioxidant defense mechanisms(12). This condition causes oxidative stress and affects body changes(13). Consumption of foods that are high in cholesterol and high in carbohydrates causes changes in lipid profile, oxidative stress, and inflammation.(14) Groups K1, K2, and K3 showed abnormal lipid profiles as a result of being given a high-fat diet using quail egg yolks of 4ml/kg BW/day by sonde for 14 days.

The examination result of MDA levels in the K1 group which was induced by dyslipidemia without giving omega-3 fatty acids increased compared to the K0 group. However, the K3 and K2 groups that were given a dose of omega-3 fatty acids actually experienced an increase in MDA levels as shown in Table 1. Dyslipidemia conditions will trigger lipid peroxidation. Lipid peroxidation is a reaction between free radicals and polyunsaturated fatty acids (polyunsaturated fatty acid, PUFA) which are present in cell membranes and LDL. Polyunsaturated fatty acids undergo peroxidation and then form products that are toxic to the body, Malondialdehyde (MDA).(15)

The MDA level of the group induced by dyslipidemia and receiving omega 3 fatty acids at doses of 36mg/kg BW and 72mg/kg BW actually increased as shown in Table 1. The effect of omega 3 on plasma lipids and lipoproteins can generally reduce triglyceride levels by 20-50% in healthy people, people with hypertriglyceridemia and diabetes. The PUFA class of fatty acids contained in fish oil are omega-3 fatty acids, especially EPA and DHA, which also play a good role in microsomal metabolism and act as antioxidants.(16) In this study, MDA did not experience a decrease in the possibility associated with the formation of MDA. MDA is formed through enzymatic and non-enzymatic pathways. The non-enzymatic pathway is a pathway through lipid peroxidation so that it is related to lipid levels. If the lipid profile can be improved, the MDA formed through the lipid peroxidation pathway will decrease. In addition to this, MDA is formed through an enzymatic pathway, namely the arachidonic acid pathway, which is strongly influenced by stress factors.(17,18) In research, the possibility of the formation of MDA through this enzymatic pathway cannot be controlled and inherited. In this study, the omega-3 fatty acids used were derived from fish oil. There is research that states that the consumption of fish oil increases the status of oxidative stress.(19) Another study stated that anchovy which is a group of fish oil has a high content of omega-3 fatty acids, namely 14 mg per gram with a composition of 5 mg of EPA and 9 mg of DHA. However, showing its use as an alternative source of omega-3 fatty acids is still rarely used.(19)

The results of an examination of SOD levels in the K2 group induced dyslipidemia by giving omega 3 fatty acids at a dose of 36 mg/kg BW/day experienced a significant increase compared to the control group (K0), the group that was fed without dyslipidemia induced (K1) and those given fatty acids omega 3 dose of 72 mg/kg BW/day (K3) as shown in Table 1. Feeding fat can induce ROS which will have an impact on lipid metabolism. An increase in the size and amount of adipose tissue leads to the production of pro-inflammatory cytokines, one of which is IL-6. This is caused by oxidative stress which will activate Bax in mitochondria so that release occurs cytochrome-c and decreased SOD production.(20) Omega-3 fatty acids play an important role in PPAR gene expression as an antioxidant.(19)

The SOD level in the group induced by dyslipidemia and the administration of omega 3 fatty acids at a dose of 36 mg/kg BW/day increased more than the dose of 72 mg/kg BW/day as shown in Table 1. Giving omeg-3 fatty acids can increase gen nuclear factor erythroid 2-related factor 2 (NRF2) which plays a role in increasing the synthesis of endogenous antioxidants by increasing antioxidant enzymes such as SOD, glutathione peroxidase, and catalase.(21) Meanwhile, giving DHA that is too high from fish oil will cause inflammation and oxidative stress.(17) This could be related to the low SOD level in the K3 group who received 72 mg/kg BW/day of omega-3 fatty acids. The PUFA class of fatty acids contained in fish oil are primarily omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) which have health effects on the body. EPA and DHA have 20 carbon atoms (C20) with 5 double bonds and 22 (C22) with 6 double bonds in their molecules. Omega-3 fatty acids are a group of polyunsaturated fatty acids (polyunsaturated fatty acid /PUFA) which has a double bond on the number 3 carbon atom. Dyslipidemia significantly increases ROS and reduces antioxidants such as SOD, and induces the secretion of proinflammatory

cytokines IL-1β, IL-6, and TNF which can increase hepatic parenchymal damage and induce apoptosis. The inflammatory response continues to increase with the release of proinflammatory cytokines, such as TNF, IL-6, and IL-1β, along with growth factors such as PDGF, CTGF, TGF-β and IL-13. SOD is an enzyme that can suppress ROS with the mechanism of producing molecular oxygen from the oxidation of hydrogen peroxide, which is called disproportionation. SOD enzyme activity depends on the structural conformation of three essential domains, the heme moiety on the active site, the reduced NADPH bonds in the NADPH binding domain, and the secondary structure of the complex formed by linking and interlocking of long peptide loops during tetramerization. There is research that states that the consumption of fish oil increases the status of oxidative stress.(19) There is research that states that omega-3 fatty acids can optimize their function in increasing antioxidants when combined with vitamin E.(22) Researchers only examined cholesterol, LDL, triglycerides, and HDL in several samples after the end of the treatment so it was hoped that lipid profiles could be examined for all study samples after treatment.

V. CONCLUSION

There was a significant difference in the mean MDA levels in the control group compared to the group of dyslipidemic rats which received omega3 fatty acids at a dose of 36mg/kgBW/day and there was a significant difference in the mean SOD levels in the control group compared to the group of dyslipidemic rats who received omega3 fatty acids at a dose of 72mg/kgBW/day

ACKNOWLEDGEMENT

The authors declare no conflict of interest and no involvement of sponsors or funding for this research.

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