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Oxidative Stress Marker Malondialdehyde and Gen Expression Level SOD in Hypertension Patients

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ABSTRACT

The development of technology in all areas of life has effects on people's behavior patterns and habits. Which results in hypertension gaining increasing prevalence and resulting in various complications. This study aims to provide an overview of plasma MDA levels and antioxidant gene expression SOD1 and SOD2 in hypertension patients: This study is an analytical observational with a case-control research design approach in the Angke sub-district, Tambora district, West Jakarta. A total of 60 respondents participated in this study. The data studied included subject characteristics (age, sex, disease history, anthropometric data, body mass index, blood pressure), MDA levels and SOD antioxidant gene expression. The research instruments used were questionnaires and laboratory tests of fasting blood glucose, total cholesterol, MDA levels, and antioxidant gene expression using PCR. Results: MDA levels in normotension patients were higher than in hypertension patients. SOD1 gene expression levels were relatively higher in normotension patients, with $p < 0.05$. Conclusion: SOD1 gene expression differs in meaning between hypertension and normotension groups.

1. Introduction

Hypertension is a medical condition with a high prevalence in the world and is a major risk factor for various cardiovascular diseases, including heart disease and stroke. It is estimated that more than one billion people worldwide suffer from hypertension, and its prevalence continues to increase. The pathogenesis of hypertension involves a few complex factors, including an imbalance between genetic and environmental factors, activation of the renin-angiotensin-aldosterone system, impaired regulation of fluid volume and blood pressure by the kidneys, and vascular resistance to vasoactive hormones [1], [2]. Hypertension causes direct damage to blood vessels, increases the heart's workload, and damages target organs such as the brain, heart, kidneys, and eyes. In addition, hypertension is also a risk factor for the development of

atherosclerosis and endothelial dysfunction, both of which are mechanisms underlying many cardiovascular diseases [3], [4].

Oxidative stress is a condition in which the production of free radicals and oxygen reactive species (ROS) exceeds the capacity of the cellular antioxidant system to neutralize them. It is thought that in hypertension patients, increased oxidative stress often occurs because of activation of the renin-angiotensin-aldosterone system, ROS production by vascular cells and the immune system, as well as various hypertension-related complications such as endothelial dysfunction and atherosclerosis. One of the end products of ROS-induced lipid peroxidation is malondialdehyde (MDA), which is often used as an indicator of oxidative damage at the molecular level. It would appear that there is evidence to suggest that high levels of MDA have been observed in plasma and tissue from patients with hypertension, which could indicate increased oxidative stress and associated lipid damage [3], [5].

It seems that cells include effective antioxidant defence mechanisms, including the enzymes superoxide dismutase (SOD), glutathione peroxidase, and catalase, in order to fight oxidative stress. In the context of hypertension, there is often a suggestion that the expression of the SOD gene, which catalyses the conversion of superoxide to hydrogen peroxide, plays a significant role. Previous research has indicated that there is a possibility that SOD activity or expression may be reduced in patients with hypertension, which could potentially leave cells more susceptible to oxidative stress and associated oxidative damage. It may therefore be beneficial to gain a deeper understanding of the regulation of SOD gene expression in the context of hypertension. This could potentially provide valuable insights into the role of antioxidant systems in the pathogenesis of this disease and the potential for the development of new therapies aimed at enhancing cellular antioxidant responses [6- 8].

Previous research concluded a correlation between MDA levels and SOD antioxidant gene expression in hypertension. Some research supports an inverse relationship between MDA levels and SOD gene expression, suggesting that increased oxidative stress, reflected in increased MDA, may produce an upregulatory response in SOD gene expression as a compensatory mechanism to reduce oxidative damage. However, other findings point to a lack of correlation or even a positive correlation between the two factors, raising questions about the complexity of the genetic regulation of antioxidants in the context of hypertension. Previous research has not fully uncovered the mechanism underlying the link between MDA and SOD gene expression in hypertension conditions. Therefore, the main aim of this study was to explore the correlation between MDA and SOD gene expression, to highlight the potential clinical implications of our findings in the management of hypertension.

2. METHOD

This study is an analytical observational study, using a case-control design, each group consists of 30 subjects, a minimal sample for statistical analysis. The inclusion criteria for this study were men and women aged ≥ 40 years, willing to participate in the study and sign *informed consent*. Exclusion criteria: have a history of malignancy, kidney failure, chronic liver disease, and a history of autoimmune diseases. Data collection was carried out using questionnaires for patient characteristic data, physical examination of blood pressure, weight, height, body mass index, laboratory examination of fasting blood glucose, and total cholesterol. In addition, MDA examination and SOD antioxidant gene expression were also carried out.

MDA

MDA assay was analyzed in plasma using a spectrophotometer by thiobarbituric acid method.11 Two hundred microliters of Trichloroacetic acid (TCA) were added to the sample and centrifuged at 5000 rpm

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for 10 minutes, the pellet was discarded and 0,4 mL of thiobarbituric acid (TBA) reagent was added. The solution was incubated in a boiling water bath for 10 min to produce a pink color. After cooling at room temperature, samples were read at 532 nm using a spectrophotometer.

RNA isolation

RNA was isolated from 2 ml of venous blood using the Zymoresearch Quick RNA Miniprep Kit. Blood samples were homogenized with RNA buffer and Proteinase K, and then incubated for 30 minutes. After that, isopropanolol was added and the samples were transferred to a Zymo-spin column for centrifugation. The sample was diluted with RNA until total RNA was produced. DNAse and DNA digestion buffer were added to the column and then incubated for 15 minutes. The sample was then centrifuged and washed with RNA Wash Buffer several times. Finally, the samples were resuspended with RNAse-free water, and the RNA concentration was measured using a spectrophotometer at a wavelength of 260 nm. The isolated RNA was then stored at -80oC.

Quantitative real-time PCR

In this study, a PCR primer search was conducted for the primer bank. Purified RNA was converted into cDNA using the SensiFAST cDNA Synthesis kit by adding buffer, reverse transcriptase enzyme, free water RNA, and 200 ng RNA. The denaturation process was carried out at 95 C for 15 seconds, followed by 60 oC for 60 seconds for 40 cycles. Quantitative measurement of SOD1, SOD2, CAT, and GPX gene targets was performed using the GADPH gene household. cDNA was added with SensiFAST SYBR Green No-ROX markers, forward and reverse primer genes, and free water RNA. Primer annealing temperatures can be seen in the primer table. Expression levels were measured using the RotorGene 6000, Q-Series machine software from Qiagen. The measurement results are given as the Ct value of the target gene. The calculation of the expression yield using the Livak method is the average of 2-ΔCt.

Blood pressure measurement

Blood pressure measurement is carried out using an automatic sphygmomanometer device brand Omron, and before the examination, the patient is prepared. The patient's preparation steps are as follows: The patient sits leaning on a chair with both feet on the floor. The patient remains silent for 3–5 minutes before the first measurement. Patients are advised not to consume caffeine, exercise, and smoke at least 30 minutes before measurement, and not to hold urination. Before the examination, remove the shirt in the upper arm area.

3. RESULTS

Table 1. Subject characteristic data

	Normotension (n=30)		Hypertension (n=30)	
Variable	mean±SD	n (%)	mean±SD	n(%)
Age	50.57 ± 8.89		57.67± 9.98	
Blood pressure				
Sistole	121.97±10.63		154.77±13.30	
Diastole	79.30±7.33		86.70±11.16	
Weight	61.15±11.22		69.54±13.79	
Height	1.59±0.08		1.56±0.11	
Body Mass Index	23.96±4.30		28.28±4.68	
Fasting blood glucose	93.27±23.65		102.57±50.43	

Total cholesterol	192.48±39.45	182.9±30.45
MDA plasma	1.16±0.55	0.79±0.27
Antioxidant supplements		
No	20 (66.67)	9 (30)
Yes	10 (33.33)	21 (70)
Sport		
No	15 (50)	13 (43.33)
Yes	15 (50)	17 (56.67)

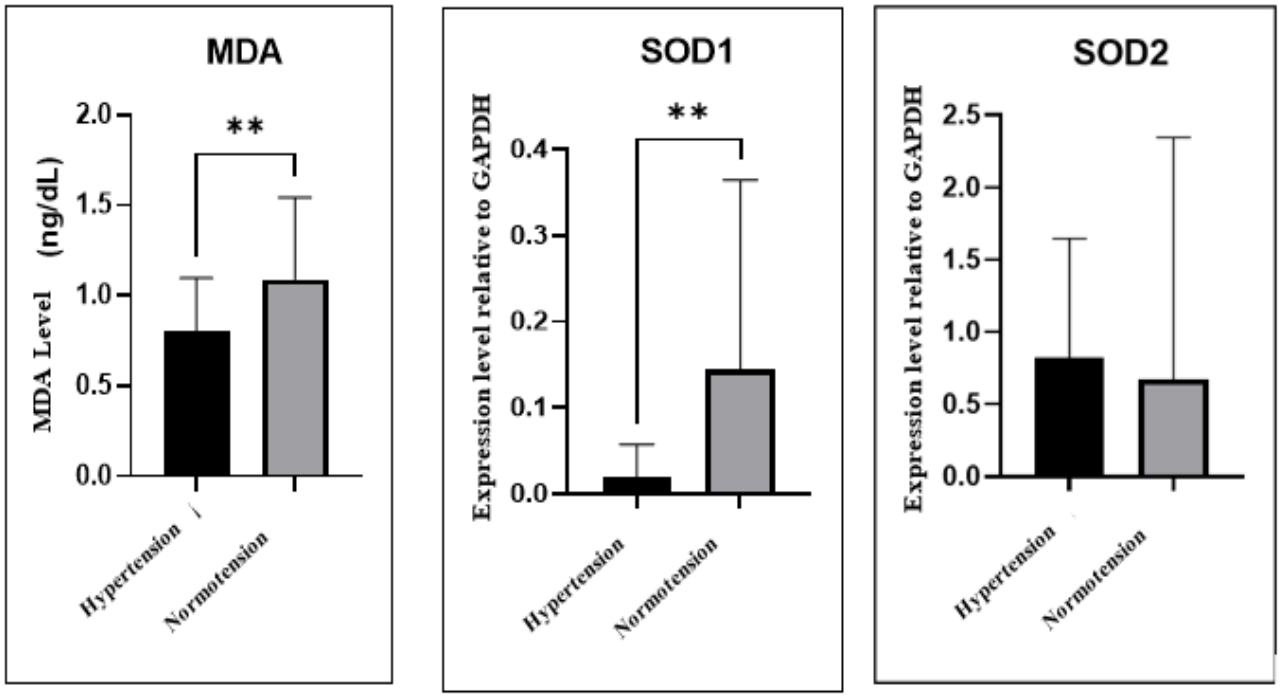

Figure 1. MDA Level, Expression Level Relative SOD1 and SOD 2 to GADPH

4. DISCUSSION

Hypertension is a medical condition that is frequently associated with an increase in blood pressure inside the body. The risk factor oxidative stress is associated with pathophysiological processes in hypertension that lead to inflammation, fibrosis, and end-organ damage [9]. Stress on the body occurs when the production of radical bebas reduces the body's ability to fight off oxidative stress. Antioksidan, such as SOD 1 and SOD 2, is an essential enzyme in antioksidan synthesis, which is involved in converting radikal superoksida into hidrogen peroksida and oksigen. Malondialdehyde (MDA) is one biomarker that is used to lower serum oxidative threshold since it is the result of lipid degradation following a basal redox reaction [10]. This study used MDA as an indicator because MDA levels have been widely used as an indicator to measure oxidative stress in unsaturated fats and is an indicator of the presence of free radicals. MDA is an end product of lipid peroxidation in the body and can be used as a parameter to measure the body's oxidative stress condition. MDA concentration indicates an oxidation process that occurs in the body's cell membranes due to metabolic processes. Excess production of reactive oxygen species (ROS) will cause the aging process. ROS is produced through the process of cellular respiration in mitochondria and the process of phagocytosis from the elimination of foreign bodies. ROS influences vascular function, aldosterone/mineralocorticoid actions, and immunoinflammation. These are all important processes that contribute to the development of hypertension [3], [11]. To maintain a stable amount of ROS, the body can use endogenous and exogenous antioxidants. Disruption of the balance between ROS and antioxidants can cause irreversible damage to cell organelle components up to the DNA level [12], [13].

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In the present study, MDA levels tended to be lower in hypertension patients compared to those without hypertension. This raises questions about the possible relationship between SOD 1 and 2 gene expression and MDA levels in the context of hypertension. In general, increased expression of SOD 1 and 2 would be expected to reduce oxidative stress and therefore lower MDA levels. However, the finding that hypertension patients have lower MDA levels suggests that there may be other factors that influence oxidative stress in this population. MDA levels are strongly influenced by many factors such as age, disease suffered, stress, various pollutants, food consumption, antioxidant supplements as well as excessive physical activity and exercise, and others. Naturally, the body will have defense mechanisms to overcome oxidative stress both by enzymatic and non-enzymatic reactions. Various stressors in addition to triggering the production of ROS, also trigger the production of enzymatic antioxidants such as catalase (CAT), hydroperoxidase (HPx), and superoxide dismutase (SOD) Antioxidants can prevent oxidative stress by inhibiting molecular oxidation Our body cells provide enzymatic and nonenzymatic antioxidants to neutralize ROS [14].

Our study showed MDA level of nonhypertension subjects was significantly higher than hypertension subjects ($p<0,05$). The finding that hypertension patients had lower MDA levels could be interpreted as a sign that their body's antioxidant system may be more active or effective in neutralizing free radicals. This is consistent with the theory that increased activity of antioxidant enzymes such as SOD may protect against oxidative stress, which is exacerbated by hypertension. Previous studies have shown that increased SOD expression can reduce oxidative damage to body tissues, including in patients with hypertension [11], [15]. As other parameters such as antioxidant enzyme activity and total antioxidant capacity also need to be assessed, MDA alone may not be a complete indicator of overall oxidative stress. Changes in complex oxidative metabolism are often associated with hypertension, which may affect different parts of the antioxidant system. It is therefore possible that the results of the study could be influenced by compensatory or adaptive mechanisms that are not yet known. Other studies found higher levels of MDA in elderly who lived in rural areas contributed to high-risk stroke. The elderly who live in rural areas have unhealthy diets such as high fat and sugar, smoking, working overtime, and less exercise [16].

The main defense mechanism against oxidative stress is comprised of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD), heat shock protein enzymes called heme oxygenases (HOs) that have vital antioxidant capacity for numerous tissues, including cells in the cardiovascular system [17], [18] and nonenzymatic substances like glutathione, metallothionein, α-tocopherol, and ascorbate. $O2\cdot-$ to hydrogen peroxide ($H2O2$) is catalyzed by SOD. Under normal conditions, catalase or glutathione peroxidase removes $H2O2$, which is a comparatively less harmful molecule. But $H2O2$ and $O2\cdot-$ can produce extremely hazardous and reactive hydroxyl radicals ($OH\cdot$). The quick conversion of mitochondrial $O2\cdot-$ to $H2O2$, which is less reactive and more readily crosses cell membranes, is carried out by the manganese-containing isoenzyme manganese superoxide dismutase (MnSOD), which is found inside the mitochondrial matrix. The identical reaction is catalyzed by a cytosolic enzyme known as copper/zinc-containing SOD (CuZnSOD). Superoxide dismutation is mostly mediated by this enzyme, while it also happens in a nonenzymatic manner [17], [18].

The enzyme superoxide dismutase (SOD) is an endogenous antioxidant that works by suppressing ROS production Thus, the role of molecules that have activity as antioxidants is needed to counteract oxidative stress. Various antioxidants play an important role in the maintenance of cell survival, DNA replication, protein synthesis, enzyme catalysis, membrane transport transduction, receptor action, intermediate metabolization, and cell maturation. Antioxidants are synthesized by gene sequences that are genetically expressed by gene sequences that make up enzyme proteins. When gene expression changes, antioxidant

synthesis is disrupted. Due to low antioxidants will cause increased oxidative stress, the onset of various diseases, and will increase the severity of the disease [19], [20].

Superoxide dismutase (SOD) is a metalloenzyme found in eukaryotic organisms and some prokaryotes. This enzyme can be found in the cytosol, mitochondria, and extracellular matrix of cells. SOD catalyzes the superoxide anion (.O2-) into hydrogen peroxide (H2O2) and molecular oxygen (O2). Hydrogen peroxide will then be converted into oxygen and water by the enzymes catalase and glutathione peroxidase. H2O2 will also form ROS in the form of (.HO) hydroxide ions via the Fenton reaction [8]. SOD has three isoform forms, namely SOD1, SOD2, and SOD3. SOD1, SOD2, and SOD3 have different catalytic ions, namely SOD1 with Cu/Zn, SOD2 with Mn/Fe, and SOD3 with Ni [21].

Several complex factors related to the pathophysiology and genetic regulation of hypertension are responsible for the reduced SOD gene expression in hypertension patients. The body's redox homeostasis is often altered by hypertension, which may alter the cellular response to oxidative stress. In chronic hypertension, high oxidative stress may activate signaling pathways that regulate the expression of antioxidant genes, including SOD1. SOD1 is a 32kDa homodimer encoded by a gene located on chromosome 21q22. SOD1 is expressed in the cytoplasm of the cell. SOD1 has cyanide sensitivity that can distinguish it from SOD2 [22].

However, in pathological conditions, it is possible that the antioxidant system may be fatigued or malfunctioning, which could result in a reduction in SOD1 expression as part of the body's adaptive response to prolonged oxidative stress. It is also worth noting that the expression of SOD1 is also regulated by genes.

Variations of gene affects the level of SOD1 expression in some people. Some types of genetic polymorphisms are linked to reduced SOD1 activities, which causes susceptibility to oxidative stress. This leads to cases of hypertension. For example, it is possible to have increased risk of hypertension due to a case of polymorphism within the promoter of SOD1. This may have impact on the binding of transcription factors that affects gene expression, a reduction in this factor may lead to failure of SOD1 productions in optimal amounts [23], [24].

Other than the previously stated factors, such as oxidative stress and genetic factors, environment and lifestyle of each people also influence the level of SOD1 production. Some factors that can affect SOD1 production and regulation are physical activity, contact with pollutants, and diet. Eating unhealthy foods, such as those with excessive sodium or oil are often associated with cases of hypertension, as this is linked with the amount of gene expression [25], [26].

This study finds that some complex biological factors are linked with an increase of SOD2 expression. This antioxidant enzyme is often found within mitochondria, and is responsible for mitochondrial production of superoxides. These superoxides affects blood pressure by increasing oxidative stress. The expression of SOD2 may be increased under high oxidative stress, which patients of chronic hypertension fall into, in order to protect the mitochondia from damage, and protect normal cell functions [27]. Certain signaling pathways are activated in response to long term oxidative stress, this leads to increased SOD2 levels on hypertension patients. This leads to the association between hypertension with mitochondrial dysfunction and increased productions of free radicals.

The previous factors lead to some transcriptional signalling pathways, for example Nrf2, or Nuclear factor-

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erythroid-2 related factor 2, or other factors that controls the regulation of antioxidant genes, such as SOD2. Activation of Nrf2 may lead to SOD2 transcription as a response to protect the body from oxidative stress and mitochondrial damage. Genetic factors also influence SOD2 production in hypertension patients. It is possible that genetic polymorphisms in the regulatory regions of SOD 2 or pathways related to oxidative stress and antioxidants may affect their response to hypertension conditions as well as the regulation of SOD 2 expression. Some studies have suggested that certain genetic variations may increase SOD2 expression and reduce the risk of mitochondrial damage and cardiovascular disease [28], [29].

These findings may have important clinical implications. Since the expression of SOD 1 and 2 has been shown to contribute to the reduction of MDA levels in hypertension patients, therapeutic strategies aimed at increasing the activity or expression of these enzymes could potentially reduce the oxidative stress associated with the complications of hypertension. However, further studies are needed to better understand the mechanisms involved and to validate these results in a larger, heterogeneous population.

5. CONCLUSION

Plasma MDA levels in this study were higher in the normotension group. The expression of the antioxidant gene SOD1 was relatively higher in the normotension group than in the hypertension group.

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AUTHORS' CONTRIBUTIONS

Conceptualization: M, Y, MDH. Data curation: Y, MDH. Formal analysis: M, EM, N. Methodology: M, MDH. Writing of original draft: M. Writing of review and editing: Y, MDH.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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