mRNA RELATIVE EXPRESSION CATALASE IN HYPERTENSION

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mRNA RELATIVE EXPRESSION CATALASE IN HYPERTENSION

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ABSTRACT: Hypertension is number one worldwide disease which lead to death. Initially hypertension is caused by changes in the walls of blood vessels. These vascular changes are due to the accumulation of ROS (Reactive Oxygen Species). ROS is the result of the balance between antioxidants and oxidants, but uncontrolled conditions will cause vascular damage. One of ROS product from metabolism which could played role in downregulation antioxidant gene expression is hydrogen peroxide. Hydrogen peroxide could be neutralized by catalase enzyme. Studies about catalase expression in hypertension subject is still limited. This research aims to determine mRNA relative expression catalase in hypertension. In this case control study, thrity subject hypertension and normotension subject were recruited within the age of 50-60 years. Hypertension subject was chosen according to JNC 8. Two millilitres vein blood was isolated into RNA. mRNA expression was detected by qRT PCR with 2 steps. Relative expression was determined using livak method. The data was analyzed using Mann Withney test with GraphPad software. Research results show that (1) catalase mRNA relative expression was 0.6 fold in hypertension; (2) catalase relative expression was not significantly lower in hypertension; (3) other antioxidants might have other mechanism to detoxify ROS and prevent hypertension.
Keywords: hypertension, mRNA, catalase, expression

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INTRODUCTION

Hypertension is an aging disease which characterized by an increasing blood pressure above 120/80 mmHg. Uncontrolled blood pressure will be one of the risk factors for cardiovascular disease (Krentz & Chilton, 2023).The prevalence of hypertension is increased with age around 22% (18-39 years), 54,5% (40-49 years) and 74,5% (over 60 years). Prevalence of hypertension in men (31,9%) is higher than women (30,1%). Increased blood pressure might develop the risk of disability and death (Mills et al., 2021).According to Riskesdas 2018, the prevalence of hypertension in Indonesia, based on the age of 44-54 years about 45.32%. The etiology of hypertension is still controversial but some evidence showed oxidative stress could be the one of it. Oxidative stress in hypertension plays a role in vascular changes such as endothelial, arterial fibrosis, renal disfunction and stimulation simpatic nerve (Touyz et al., 2020).

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Oxidative stress is a state of balance between reactive oxygen species (ROS) and antioxidant enzymes. ROS is a product from metabolic reaction of the respiratory chain. In cardiac, ROS product came from respiratory chain, xanthine oxidase, NAPDH oxidase and NO synthase. They are superoxide anions, hydroxyl, hydroperoxyl and hydrogen peroxide (Mills et al., 2021). This imbalance ROS production and antioxidant capacity will lead to cellular pathological conditions such as protein, lipid and nucleic acid damage including vascular changes (Baradaran et al., 2014). One form of ROS that has a role in the pathogenesis of hypertension is hydrogen peroxide (H_2O_2). H_2O_2 is one of the molecules that has a role in various cellular signalling such as vasodilator on the endothelium, protein modification, vascular remodelling, enhancing cell phosphorylation on cell migration and angiogenesis (Griendling et al., 2021).

Accumulation hydrogen peroxide induced endothel disfunction, vascular damage, inflammation and organ failure. It could impact vascular ability to dilate even as well as its effect on blood pressure. Vascular endothel released inflammation factor that influence vasoconstrictor molecule and made worst hypertension condition.The enzymes that act to neutralize H_2O_2 are catalase, gluthatione peroxidase and other peroxidase (Chrissobolis et al., 2008). Catalase is very common enzyme found in eukaryotic cells. It presents in peroxisome which has function to convert H_2O_2 to H_2O and O_2. It depends on hydrogen peroxide concentration. When H_2O_2 level detected higher then catalytic agent would be stimulate to detoxify however when H_2O_2 level detected lower, it would stimulate peroxidase agent to act (Nandi et al., 2019). Catalase could be found in hepatocyte, kidney cell, erythrocyte and many other cells. Catalase which located in cytosol has a role to protect erythrocyte membrane from degradation. Catalase expression in mitochondria liver was influenced by fatty acid level which led to uprise catalytic activity. Decreasing catalase activity caused H_2O_2 accumulation that led to increase incidence oxidative stress and triggers potential for inflammation associated with hypertension 29/09/2024 07:44:00.

Recently there is study about catalase and gluthation peroxidase activity enzyme in schizophrenic patient. The study showed the presence of oxidative stress in first-year patients who had symptoms characteristic of schizophrenia. Erythrocyte catalase and gluthatione peroxidase activity were significantly lower in schizophrenic patients than healthy person. It conclude that clinical manifestation patients depends on the duration of the illness, the episode of symptoms and the intake of antipsychotic drugs (Djordjević et al., 2022). Other studies found mRNA expression superoxide dismutase-1 (SOD1) was significantly lower in hypertension than normotension subjects. SOD1 was another antioxidant enzyme which could combat ROS. This evidence showed ROS could be higher in hypertension (Meiyanti et al., 2024). Conversely, catalase expression was higher when induced with oxidant in short term. This research was conducted on rat which was observing the changes in hepatocyte and astrocytes. Evidence suggests that catalase expression depends on oxidant concentration, exposure time and antioxidant enzyme capacity (Glorieux et al., 2015). Antioxidant expression has been widely studied in animal model but the research about catalase mRNA expression in

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hypertension subjects 50-60 years old is still limited. Therefore, the aim of this study is to analyze mRNA relative expression of catalase in hypertension.

METHOD

The research was approved by the ethics committee of the Faculty of Medicine of the University of Trisakti under the number 001/KER/FK/2024. This case control study used 60 subjects age 50-60 years were selected and classified into two groups based on blood pressure according to JNC VIII. This research in located in Kelurahan Angke, West Jakarta at September, 2023. The inclusion criteria are those who are willing to follow the research and are 50-60 years of age. The exclusion criteria is those who have liver disease, cancer, diabetes mellitus and autoimmune. Venous blood samples of 2 ml were taken with a tube given EDTA and an immediate RNA isolation was performed.

RNA was extracted from 2 ml of venous blood with Quick RNA Miniprep kit (*Zymoresearch*). Briefly blood samples was added to the cell lysis buffer then added Proteinase K. Sampel incubated for 30 minutes at a temperature of 20-30 degrees. Later isopropanolol is added 50% (v/v) and homogenized. After that sample was transferred to spin column and centrifugated at a speed of 16,000 g for 30 seconds, washed with RNA wash buffer and centrifuged 15,000 g over 30 seconds. The result was removed and added DNAse buffer and mixed until homogeneous. The process continued with incubation at room temperature 20-30 ^{o}C for 15 minutes. After incubating, the sample was added RNA buffer and centrifuged. The result was removed and added another RNA wash buffer and centrifuge. After the centrifuge, added other RNA wash buffer and centrifuged for 1 minute. At the end, RNAse free water and centrifuged for 1 minutes. Amount total RNA purification were measured for concentration and purity by spectrophotometer with wavelength of 260 nm. The sample is kept at -80^{o}C (Hardiany et al., 2022).

Relative expression analysis of mRNA performed with 2 step qRT-PCR (Rotorgene 6000). cDNA synthesis used SensiFAST cDNA Synthesis kit and amplified by PCR machine with a 95 ^{o}C for denaturation about 15 seconds, annealing 60 ^{o}C for 60 seconds for 40 cycles. qPCR was completed by adding SensiFAST SYBR Green No-ROX marker, forward and reverse primer into cDNA. (Artika et al., 2022) CAT sequence primer is GTG CGG AGA TTC AAC ACT GCC A (forward) and CGG CAA TGT TCT CAC ACA GAC G (reverse) with product size 109 bp while GAPDH as a housekeeping gene which sequence is GTC TCC TCT GAC TTC AAC AGC G (forward) and ACC ACC CTG TTG CTG TAG CCA A (reverse). mRNA level expression was measured by Ct value of the target gene. Calculation of expression results using Livak method $2^{-\Delta\Delta Ct}$ ratio (Livak & Schmittgen, 2001).\

Data distribution was not well distributed with the Shapiro wilk test. Mann Withney test was used to assess the difference between the two groups with a significance p<0,05. The results are presented in a median form with 95% CI.

RESULT AND DISCUSSION

Sixty subjects was measured and baseline characteristic was presented in Table 1.

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Table 1. Baseline of Characteristics Subject (n=60)

Characteristics	Normotension N=30	Hypertension N=30	
Gender			
Female	20 (66,67%)	22 (73,33%)	p=0.573
Male	8 (26,67%)	10 (33,33%)	
Mean of Age (years)	48,53	56,30	

Female subject diagnosed with hypertension was higher than male subject. In total sixty subject female was 66,67% with normotension and 73,33% diagnosed with hypertension. In male subject, there are 33,33% diagnosed with hypertension and 26,67% with normotension. Mean of age subject diagnosed with hypertension 56,3 years while mean of age with normotension 48,53 years. There was not statistically significantly gender with mRNA catalase expression (p=0,573).

Comparison catalase relative expression level toward GAPDH in hypertension and normotension subject, namely mRNA catalase relative expression in hypertension subject was 0,03 and normotension was 0,05. The comparison is presented in Figure 1.

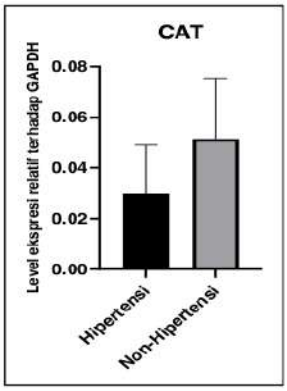

Figure 1. mRNA Relative Expression Catalase in Hypertension

The purpose our study was to analyze mRNA relative expression in hypertension. Our study discovered catalase expression was 0.6 fold in hypertension as shown in figure 1. This study also revealed the number of female subjects found was higher than that of males. In table 1 aligned with Novi et.al which found a higher number of female subject with hypertension than men at the age of 60 years (Kirana et al., 2020). Furthermore, a lot of women diagnosed with hypertension and to be treated with antihypertension drugs more than men (Defianna et al., 2021). Another finding was the average age subjects which diagnosed with hypertension was 56,3 years. This is leveled with Riskedas 2018 which disclosed subject with range of 54-65 years as the third number cases of hypertension in Indonesia. According to Suvila et.al, if someone was diagnosed with hipertension at the age of ≤ 55 years, then the incidence of cardiovascular disease and risk of death might increase. On the same study, subject who was doing

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medical check-up in Japan at the age of < 30 years, herediter factor was found (Suvila, 2020).

Catalase is an antioxidant enzyme found in aerobic organisms. It transformed hydrogen peroxide into oxygen and water. Catalase deficiency might caused various diseases such as diabetes mellitus, hypertension, schizophrenia, bipolar and skin disorders. (Griendling et al., 2021). In this study, the relative expression of mRNA catalase was found lower in hypertensive patients compared to normotension (Tain & Hsu, 2022). Our findings was in accordance with the results found by Azer B et.al, which demonstrates decreasing activity of superoxide dismutase and glutathione peroxidase enzyme in subject with early diagnosed hypertension. Hydrogen peroxide is found to be increasing in the early diagnosed hypertension (Griendling et al., 2021). In conclusion, antioxidant plays an important role in vascular damage by detoxifying ROS. Along with Vladimir et. al. showed that a decrease in enzyme catalase and glutathione peroxidase activity in patients with schizophrenia would lead to a reduction in H_2O_2 degradation into oxygen and water (Djordjević et al., 2022).

In physiology condition, ROS level plays a role in the celular level such as gene expression, growth, deferencial cell, and apoptosis. When H_2O_2 level detected higher, catalase would respond and transform it to become less harmfull. Previous study using rat as model for oxidative stress illustration in heart tissue. Higher ROS level might give consequence on blood pressure, atherosclosis, endotel disfunction, hyperthrophy cardiomyocyte, decreasing antioxidant enzyme, decreasing mitochondria function and avtivation protein kinase such as JNKs, Erk1/2, p38 MAPK, CAMK which lead to apoptosis cells (Dubois-Deruy et al., 2020). Further, relative expression superoxide dismutase SOD1 and SOD2 in hypertension was lower than normotension. Malondialdehyde (MDA) was also detected higher in normotension than hypertension. Overproduction ROS might caused cardiovascular disease and disrupt respiratory chain in mitochondria (Meiyanti et al., 2024).

CONCLUSION

Based on the result of this study, it can be concluded that expression of mRNA catalase is lower in hypertension. Hypertension might be the outcome from oxidative stress. Catalase relative expression was not significantly lower because there is various other mechanism to detoxify ROS. The degree of antioxidant activity is depended on the type of tissue. Limitation our research are limited blood sample subject and we didn't measure protein level. Our suggestion is that further research is needed on the addition of antioxidants as a supplement to hypertension to see changes in expression in the levels of gene and proteins.

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The research was approved by the ethics committee of the Faculty of Medicine of the University of Trisakti under the number 001/KER/FK/2024. This case control study used 60 subjects age 50-60 years were selected and classified into two groups based on blood pressure according to JNC VIII. This research in located in Kelurahan Angke, West Jakarta at September, 2023. The inclusion criteria are those who are willing to follow the research and are 50-60 years of age. The exclusion criteria is those who have liver disease, cancer, diabetes mellitus and autoimmune. Venous blood samples of 2 ml were taken with a tube given EDTA and an immediate RNA isolation was performed.

RNA was extracted from 2 ml of venous blood with Quick RNA Miniprep kit (*Zymoresearch*). Briefly blood samples was added to the cell lysis buffer then added Proteinase K. Sampel incubated for 30 minutes at a temperature of 20-30 degrees. Later isopropanolol is added 50% (v/v) and homogenized. After that sample was transferred to spin column and centrifugated at a speed of 16,000 g for 30 seconds, washed with RNA wash buffer and centrifuged 15,000 g over 30 seconds. The result was removed and added DNAse buffer and mixed until homogeneous. The process continued with incubation at room temperature 20-30 ^{o}C for 15 minutes. After incubating, the sample was added RNA buffer and centrifuged. The result was removed and added another RNA wash buffer and centrifuge. After the centrifuge, added other RNA wash buffer and centrifuged for 1 minute. At the end, RNAse free water and centrifuged for 1 minutes. Amount total RNA purification were measured for concentration and purity by spectrophotometer with wavelength of 260 nm. The sample is kept at -80^{o}C (Hardiany et al., 2022).

Relative expression analysis of mRNA performed with 2 step qRT-PCR (Rotorgene 6000). cDNA synthesis used SensiFAST cDNA Synthesis kit and amplified by PCR machine with a 95 ^{o}C for denaturation about 15 seconds, annealing 60 ^{o}C for 60 seconds for 40 cycles. qPCR was completed by adding SensiFAST SYBR Green No-ROX marker, forward and reverse primer into cDNA. (Artika et al., 2022) CAT sequence primer is GTG CGG AGA TTC AAC ACT GCC A (forward) and CGG CAA TGT TCT CAC ACA GAC G (reverse) with product size 109 bp while GAPDH as a housekeeping gene which sequence is GTC TCC TCT GAC TTC AAC AGC G (forward) and ACC ACC CTG TTG CTG TAG CCA A (reverse). mRNA level expression was measured by Ct value of the target gene. Calculation of expression results using Livak method $2^{-\Delta\Delta Ct}$ ratio (Livak & Schmittgen, 2001).\

Data distribution was not well distributed with the Shapiro wilk test. Mann Withney test was used to assess the difference between the two groups with a significance $p<0,05$. The results are presented in a median form with 95% CI.

RESULT AND DISCUSSION

Sixty subjects was measured and baseline characteristic was presented in Table 1.

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Table 1. Baseline of Characteristics Subject (n=60)

Characteristics	Normotension N=30	Hypertension N=30	
Gender			
Female	20 (66,67%)	22 (73,33%)	p=0.573
Male	8 (26,67%)	10 (33,33%)	
Mean of Age (years)	48,53	56,30	

Female subject diagnosed with hypertension was higher than male subject. In total sixty subject female was 66,67% with normotension and 73,33% diagnosed with hypertension. In male subject, there are 33,33% diagnosed with hypertension and 26,67% with normotension. Mean of age subject diagnosed with hypertension 56,3 years while mean of age with normotension 48,53 years. There was not statistically significantly gender with mRNA catalase expression (p=0,573).

Comparison catalase relative expression level toward GAPDH in hypertension and normotension subject, namely mRNA catalase relative expression in hypertension subject was 0,03 and normotension was 0,05. The comparison is presented in Figure 1.

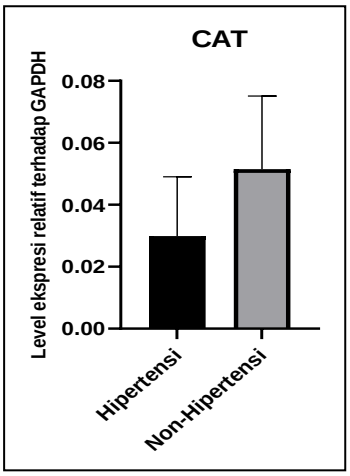

Figure 1. mRNA Relative Expression Catalase in Hypertension

The purpose our study was to analyze mRNA relative expression in hypertension. Our study discovered catalase expression was 0.6 fold in hypertension as shown in figure 1. This study also revealed the number of female subjects found was higher than that of males. In table 1 aligned with Novi et.al which found a higher number of female subject with hypertension than men at the age of 60 years (Kirana et al., 2020). Furthermore, a lot of women diagnosed with hypertension and to be treated with antihypertension drugs more than men (Defianna et al., 2021). Another finding was the average age subjects which diagnosed with hypertension was 56,3 years. This is leveled with Riskedas 2018 which disclosed subject with range of 54-65 years as the third number cases of hypertension in Indonesia. According to Suvila et.al, if someone was diagnosed with hipertension at the age of ≤ 55 years, then the incidence of cardiovascular disease and risk of death might increase. On the same study, subject who was doing

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medical check-up in Japan at the age of < 30 years, herediter factor was found (Suvila, 2020).

Catalase is an antioxidant enzyme found in aerobic organisms. It transformed hydrogen peroxide into oxygen and water. Catalase deficiency might caused various diseases such as diabetes mellitus, hypertension, schizophrenia, bipolar and skin disorders. (Griendling et al., 2021). In this study, the relative expression of mRNA catalase was found lower in hypertensive patients compared to normotension (Tain & Hsu, 2022). Our findings was in accordance with the results found by Azer B et.al, which demonstrates decreasing activity of superoxide dismutase and glutathione peroxidase enzyme in subject with early diagnosed hypertension. Hydrogen peroxide is found to be increasing in the early diagnosed hypertension (Griendling et al., 2021). In conclusion, antioxidant plays an important role in vascular damage by detoxifying ROS. Along with Vladimir et. al. showed that a decrease in enzyme catalase and glutathione peroxidase activity in patients with schizophrenia would lead to a reduction in H_2O_2 degradation into oxygen and water (Djordjević et al., 2022).

In physiology condition, ROS level plays a role in the celular level such as gene expression, growth, deferencial cell, and apoptosis. When H_2O_2 level detected higher, catalase would respond and transform it to become less harmfull. Previous study using rat as model for oxidative stress illustration in heart tissue. Higher ROS level might give consequence on blood pressure, atherosclosis, endotel disfunction, hyperthrophy cardiomyocyte, decreasing antioxidant enzyme, decreasing mitochondria function and avtivation protein kinase such as JNKs, Erk1/2, p38 MAPK, CAMK which lead to apoptosis cells (Dubois-Deruy et al., 2020). Further, relative expression superoxide dismutase SOD1 and SOD2 in hypertension was lower than normotension. Malondialdehyde (MDA) was also detected higher in normotension than hypertension. Overproduction ROS might caused cardiovascular disease and disrupt respiratory chain in mitochondria (Meiyanti et al., 2024).

CONCLUSION

Based on the result of this study, it can be concluded that expression of mRNA catalase is lower in hypertension. Hypertension might be the outcome from oxidative stress. Catalase relative expression was not significantly lower because there is various other mechanism to detoxify ROS. The degree of antioxidant activity is depended on the type of tissue. Limitation our research are limited blood sample subject and we didn't measure protein level. Our suggestion is that further research is needed on the addition of antioxidants as a supplement to hypertension to see changes in expression in the levels of gene and proteins.

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