



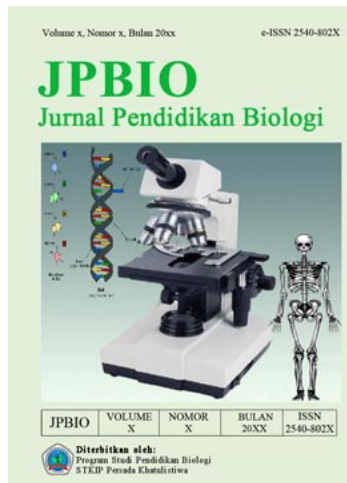


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Gene expression of sirtuin-1 in adult with hypertension

Yohana Yohana, Meutia Atika Faradilla, Endrico Xavieees Tungka, Kurniasari Kurniasari

Abstract

SIRT1 is a key member of the sirtuin family, exerts a unique protective effect on endothelial cells by modulating various proteins, and has a role as an anti-aging biomarker. Hypertension is pathology chronic pathological condition that disrupts ROS and antioxidants. Little evidence showed that sirtuin has a role in chronic oxidative stress. Therefore, the objective of this study was to analyze the relative expression of Sirtuin-1 in hypertension. This is a case-control study with 30 subjects, adults 50-60 years old in each group. JNC 8 was used to determine blood pressure. Quantitative Real Time PCR was used to calculate the level of Sirt-1. The Livak method was used for relative expression. JASP software was used for data analysis. Our study showed Sirtuin-1 mRNA expression was significantly lower in the hypertension group than the normotension group. It was 0.52 fold lower in hypertension. Sirtuin-1 stimulates antioxidants such as superoxide dismutase and catalase through FOXO-dependent signaling. It has a similar role to antioxidants for eliminating ROS. Sirtuin-1 expression is significantly reduced in individuals with hypertension, suggesting its potential role in oxidative stress regulation and its value as a biomarker for vascular aging and hypertension-related endothelial dysfunction.

Keyword: Sirtuin-1, hypertension, expression, mRNA

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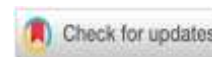
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Gene expression of sirtuin-I in adult with hypertension



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ABSTRACT

SIRT1 is a key member of the sirtuin family, exerts a unique protective effect on endothelial cells by modulating various proteins, and has a role as an anti-aging biomarker. Hypertension is pathology chronic pathological condition that disrupts ROS and antioxidants. Little evidence showed that sirtuin has a role in chronic oxidative stress. Therefore, the objective of this study was to analyze the relative expression of Sirtuin-I in hypertension. This is a case-control study with 30 subjects, adults 50-60 years old in each group. JNC 8 was used to determine blood pressure. Quantitative Real Time PCR was used to calculate the level of Sirt-I. The Livak method was used for relative expression. JASP software was used for data analysis. Our study showed Sirtuin-I mRNA expression was significantly lower in the hypertension group than the normotension group. It was 0.52 fold lower in hypertension. Sirtuin-I stimulates antioxidants such as superoxide dismutase and catalase through FOXO-dependent signaling. It has a similar role to antioxidants for eliminating ROS. Sirtuin-I expression is significantly reduced in individuals with hypertension, suggesting its potential role in oxidative stress regulation and its value as a biomarker for vascular aging and hypertension-related endothelial dysfunction.

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INTRODUCTION

Aging is one of the risk factors for hypertension. Increasing systolic blood pressure by 20 mmHg could have a twofold risk of death by stroke. It could trigger death suddenly because of a lack of symptoms. Global prevalence of hypertension is predicted to reach 60% of adults by 2025. In Indonesia, based on Indonesian Research in 2018, the prevalence of hypertension increased from 25% to 34%. It affected 55.28% of individuals aged 54-65 years old. Globally, hypertension poses a significant threat to human health, increasing the risk of premature mortality and disability. Moreover, its pathological mechanisms can disrupt the nervous, endocrine, and immune systems.



Asymptomatic nature leads to unavoidable complications, premature death, and disability. Systolic and diastolic blood pressure triggers chronic systemic inflammation, such as vascular stiffness due to reduced production of elastin and collagen in the arterial tunica media. Repetition of this process led to endothelial dysfunction of arteries. It reduced nitric oxide (NO) production and caused vasoconstriction (Manolis et al., 2020).

Few studies showed that degenerative disease has a higher level of reactive oxygen species (ROS). Recently, our study showed a significant negative correlation between ROS level and physical activity, while a positive correlation has also been found between ROS level and body mass index of people over 40 years old (Meiyanti et al, 2023). Other studies showed mRNA Catalase expression was lower in people over 50 years old. It demonstrates that ROS could be eliminated with antioxidant properties (Yohana et al, 2024). Another enzyme that is involved with oxidative stress and antioxidant properties is Sirtuin-I. It reduced ROS production and uncoupled eNOS (Wu et al, 2022).

Sirtuin is a NAD⁺-dependent deacetylase, regulates No. adaptation, vascular homeostasis, cellular signaling, immunity, inflammation, and oxidative stress protection (Teixeira et al., 2020). SIRT1 protects the cardiovascular and vascular systems through deacetylase activity, suppressing ROS production. Recent studies indicate ROS and inflammation contribute to blood pressure elevation (Griendling, K, et al, 2021). SIRT1 inhibits pro-inflammatory gene expression, mitigating inflammatory responses. Animal studies demonstrate Sirtuin's role in reducing blood pressure and cardiac hypertrophy via SirtI/NF κ B/MAPK signaling (Yang et al., 2022).

Sirtuin activation could reduce endothelial dysfunction, atherosclerosis, and blood pressure through SirtI-AMPK-eNos signaling through grape seed proanthocyanidin extract (Chen et al., 2020). Sirtuin-I is involved in cardiovascular protection. However, sirtuin-I expression in hypertension is still unclear. This study is among the few clinical investigations that quantitatively assess the relative mRNA expression of SIRT1 in adult human subjects with hypertension. Unlike previous studies that mainly rely on experimental or animal models, our findings provide direct clinical evidence that SIRT1 expression is significantly downregulated in hypertensive patients, supporting its role as a potential biomarker of oxidative stress and endothelial dysfunction in human hypertension. Therefore, the objective of this research is to analyze the relative expression of Sirtuin-I in hypertension among individuals over 50 years old.

RESEARCH METHODS

Research Design

This research was approved by the Ethics Committee Faculty of Medicine Univeristas Trisakti, with number 006/KER/FK/10/2024. This is a case-control study with 60 respondents divided into the hypertension and normotension groups according to JNC VIII.

Population and Samples

The method used for selecting the sample for this study was the purposive sampling method. A total of 60 subjects (30 per group) were selected based on statistical and practical considerations. This sample size provides adequate power for detecting significant differences in SIRT1 gene expression using quantitative PCR, while also considering feasibility, resource limitations, and the biological variability of the target age group (50–60 years). The age range was chosen to focus on individuals with a higher risk of hypertension and endothelial dysfunction. Stored biological material whose sample maintenance is carried out in the Biomolecular Laboratory, Medical Faculty, Universitas Trisakti. Subjects are participants who are invited to take part in the study in Angke Village. Selected subjects must meet the following inclusion and exclusion criteria.

This study was conducted by respondents who were 50-60 years old, with inclusion and exclusion criteria. Inclusion criteria were agreed to participate in research, whereas exclusion criteria

were respondents with liver disease, autoimmune disease, and cancer. After agreeing to the informed consent, 2 ml of blood was taken from the respondent.

Instruments

The research instruments used were a nanophotometer, a Polymerase Chain Reaction (PCR) machine Labcycler (SENSOQUEST), and a quantitative real-time PCR (qPCR) machine QiAquant 96 5 plex. The nanophotometer was used to assess the concentration and purity of nucleic acids (DNA and RNA) by measuring absorbance at specific wavelengths (e.g., 260 nm and 280 nm), which is essential before downstream molecular biology applications. The Labcycler (SENSOQUEST) is a conventional thermal cycler used to perform standard PCR, which amplifies specific DNA sequences through repeated cycles of denaturation, annealing, and extension. This instrument is crucial for generating sufficient quantities of DNA for further analysis. The QiAquant 96 5 plex is a high-performance real-time PCR system capable of detecting and quantifying nucleic acids in real time using fluorescent dyes or probes. The term "5 plex" refers to its ability to detect up to five different targets simultaneously in a single reaction, allowing multiplexing. This system provides precise quantification of gene expression levels, pathogen load, or genetic mutations through amplification curve analysis and Ct (cycle threshold) values.

Procedures

1. RNA extraction

Two millilitres of blood were extracted and transformed into total RNA by the Quick RNA Miniprep kit (Zymoresearch). According to the kit procedure, briefly, blood was combined with cell lysis buffer and Proteinase K, then incubated for 30 minutes at 20-30 degrees Celsius. Isopropanolol was added 50% (v/v). After the sample was homogenized, it was transferred into the spin column and centrifuged at 16.000 g for 30 seconds. The sample was washed with RNA wash buffer and centrifuge it 15.000 g for 30 seconds. Yield was eliminated and do addition of DNA-se buffer. Sample mixed until homogenized, then incubated at 20-30 degrees Celsius for 15 minutes. After 15 minutes, the sample was washed with RNA buffer and centrifuged for 1 minute. At the terminal point, the Sample wash added RNA-free water and centrifuged for 1 minute. The yield was measured for concentration and purity by a nanophotometer wavelength of 260/280 nm. The sample was kept in -80 degrees Celsius. The sample was managed by the Biomolecular Laboratory of the Faculty of Medicine at Trisakti University. At first total RNA needed to be checked for concentration and purity by a nanophotometer. The sample concentration that can be used is more than 20 ng/μL. Purity results fall within the range of 1.7 – 2.0, they can be categorized as pure isolation results.

2. Specimen Examination: Measurement of mRNA expression SIRT I

a. cDNA Synthesis Procedure

Copy DNA was synthesized using SensiFAST cDNA Synthesis kit and amplified using a PCR machine (SENSOQUEST) with 95 degrees Celsius for denaturation for 15 seconds, annealing at 60 degrees Celsius for 40 cycles. Minimum RNA sample for the reaction was 200 ng, and 20μl master mix. The product was diluted 1:10 and stored at -20°C for quantitative real-time PCR.

b. qRT PCR procedure (quantitative Reverse Transcriptase Polymerase Chain Reaction)

Relative expression was analyzed using 2-step qRT PCR (QiAquant 96 5 plex). 4 ul cDNA was added into the PCR tube along with SensiFAST SYBR Green No.-ROX marker, forward and reverse primers, and Nucleus Free Water up to 20 ul. The total PCR reaction was 50 cycles. Sirtuin-I Sequence primer was CTATACCCAGAACATAGACACG (forward),



ACAAATCAGGCAAGATGC (reverse). GADPH sequence primer was GTC TCC TCT GAC TTC AAC AGC G (forward), ACC ACC CTG TTG CTG No. CCA A (reverse). Both primers have an annealing temperature of 56,5 degrees Celsius. The result was presented in the cycle threshold (Ct). Ct value was measured to calculate expression in the hypertension and normotension groups. Relative expression was determined using the Livak method.

Data Analysis

CT value was well distributed within the two groups, with the Wilk test. Student t-test was used to figure out differences between the 2 groups with a significance $p < 0.05$. Analysis statistics used JASP software 0.19.2. JASP (Jeffreys's Amazing Statistics Program) is an open-source software designed to facilitate both classical and Bayesian statistical analysis with an intuitive and user-friendly interface, as well as producing publication-ready output in the form of tables and graphs.

RESULTS

The primary objective of this study was to investigate the relative mRNA expression of Sirtuin-I (SIRT1) in individuals diagnosed with hypertension, specifically in a population over 50 years old. Sirtuin-I is a gene associated with cellular stress resistance, metabolic regulation, and vascular health. This research aims to provide molecular evidence by quantifying and comparing the expression levels of SIRT1 mRNA between hypertensive and normotensive individuals, thus helping to understand its potential role in the pathophysiology of hypertension.

Figure 1 illustrates the relative mRNA expression levels of SIRT1 in two groups: Hypertension and Normotension. The y-axis represents the relative expression level of SIRT1 mRNA, while the x-axis distinguishes between the two groups. The normotensive group shows a significantly higher level of SIRT1 expression, averaging around 6-fold relative expression. The hypertensive group shows a much lower average expression, around 3-fold, with a wider range as indicated by the error bar. Error bars indicate the standard deviation or standard error (depending on the analysis), showing variability within each group. The difference between groups was found to be statistically significant ($p < 0.05$), indicating a true difference in expression rather than one due to random chance. The results demonstrate that SIRT1 mRNA expression is significantly reduced in hypertensive individuals compared to normotensive controls.

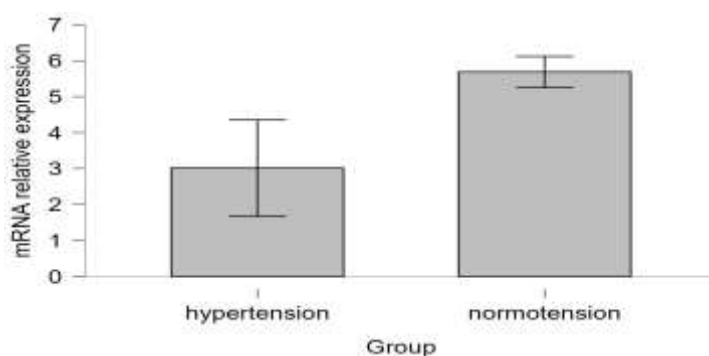


Figure 1. mRNA relative expression Sirtuin-I ($p < 0.05$)*

DISCUSSION

This study found that the average age of the hypertension group was 48 years old. This result showed people aged over 40 years have considerably higher hypertension than the younger age (Astutik et al, 2020). A similar result was found in other research held in rural areas, demonstrating that older age could potentially have a significant risk for hypertension, cause vascular wall might have modified.(Mulia et al, 2020).



Hypertension is a complex degenerative disease characterized by elevated blood pressure, leading to numerous complications. The underlying pathophysiological processes, including inflammation and fibrosis, are linked to oxidative stress. Oxidative stress arises from an imbalance between oxidants and antioxidants, leading to impaired cellular signaling and oxidative damage in hypertension (Touyz, et al, 2020). The production of reactive oxygen species (ROS) is increased in hypertension, leading to oxidative stress. However, ROS also play important physiological roles, particularly in low concentrations, where they contribute to redox regulation, maintaining endothelial integrity, and vascular function. The interaction between flowing blood and endothelial cells induces mechanical forces, such as shear stress, which influence the formation and release of nitric oxide (No.) and ROS, as well as the activation of signal transduction pathways and gene and protein expression. These mechanisms play critical roles in maintaining vascular homeostasis. Notably, laminar flow increases endothelial nitric oxide synthase (eNOS) expression, activity, and No. production, whereas oscillatory flow during hypertension leads to increased ROS formation and subsequent oxidative damage (Amponsah-Offeh et al, 2022).

Although cells have many defensive mechanisms to maintain a fine balance between antioxidant and oxidant systems, improper biochemical reactions within the cell as well as certain external factors, can lead the cell into a state of oxidative stress. An imbalance between oxidants and antioxidants in favor of excessive oxidants, leading to a disruption of redox signaling and control, and/or molecular damage (Sun H, et al, 2024). Research has implicated sirtuins in the regulation of antioxidant defenses and redox signaling pathways, highlighting their crucial role in maintaining redox homeostasis and preventing disease pathogenesis (Alam F, et al, 2021).

SIRT1 is a key member of the sirtuin family, exerts a unique protective effect on endothelial cells by modulating various proteins, including eNOS, LKBI, p53, NFκB, FOXO1, Notch, and p66Shc (Sazdova et al, 2024). Consequently, SIRT1 prevents endothelial senescence, promotes angiogenesis and migration, enhances endothelium-dependent vasodilation, and suppresses inflammation and foam cell formation (Begum MK et al, 2021). Notably, another study demonstrated that endothelial SIRT1 maintains vasodilator responses by upregulating sGC in smooth muscle cells, independently of eNOS and No., providing an alternative therapeutic pathway to mitigate vascular aging and associated diseases, including hypertension (Ren C, et al, 2022).

SIRT1 is an evolutionarily conserved enzyme that deacetylates multiple intracellular targets important for a variety of cellular functions such as DNA damage repair, cell cycle regulation, apoptosis, senescence, and remodeling of large arteries contributes to the elevation of blood pressure and increased risk of cardiovascular diseases (Shahgald S et al, 2021). In this present study, we investigate the relative expression of the SirtI gene in hypertension and normotension among individuals over 50 years old. Figure 1 showed that SIRT1 expression was relatively 0.52-fold lower in the hypertension group compared to the normotension group. This result is consistent with our previous study result that the antioxidant was lower in hypertension than normotension (Yohana et al, 2024). This study is supported by another study with the elderly who have hypertension. It revealed that superoxide dismutase was significantly lower in the elderly with hypertension than in controls. Carbonyl as ROS marker was significantly higher in the hypertension group. There was a correlation between superoxide dismutase and systolic blood pressure. (Penantian et al, 2023).

SIRT1 attenuates oxidative stress and inflammation to regulate vascular endothelial functions through several important signal mediators, such as AMPK, NOXs, eNOs, and FOXOs (Kong et al, 2019). There is a complex crosstalk network between AMPK and SIRT1. Studies showed that SIRT1 can stimulate AMPK via the modulation of upstream AMPK kinase such as liver kinase B1(LKBI), suppressing the production of ROS and inflammation response in

HUVECs, while AMPK influences SIRT1 deacetylation activity by increasing cellular NAD⁺ levels or directly phosphorylating SIRT1 (Maiese K, 2021). Furthermore, increased activity of NOX (NADPH oxidase) may also enhance NAD⁺ content to elevate SIRT1 levels in endothelial cells (Wan X et al, 2021). In addition, SIRT1 deacetylates FOXOs and stimulates FOXO-dependent antioxidant [such as catalase (CAT), manganese superoxide dismutase (MnSOD) and thioredoxin] expression to eliminate ROS in endothelial cells, and prevent endothelial dysfunction (Huang et al, 2019). The activation of SIRT1 stimulates the expression of c-Myc by promoting the degradation of FOXO1 to prevent endothelial cell dysfunction and angiogenesis induced by hyperglycemia. eNOs, a member of NOS families, is expressed in vascular smooth muscle (Negre-Salvayre, et al, 2022).

Interestingly, Sirtuin expression is not just only depressed in hypertension, Sirtuin expression could be found to be increased. Research revealed that Sirtuin-I (Sirt-I) is overexpressed in spontaneously hypertensive rats (SHR), contributing to hypertension. This study investigated Sirt-I's role in hypertension and underlying mechanisms. Results showed that Sirt-I inhibitor EX-527 reduced blood pressure by 76 mmHg, inhibited heart rate, and attenuated oxidative stress (Husein, Y, et al, 2022). These findings suggest Sirt-I inhibitors may be effective in treating hypertension-related cardiovascular complications. Another study showed that overexpression of SIRT1 in endothelial cells attenuated the augmented blood pressure and adverse arterial remodeling. Mechanistically, SIRT1 inhibited LKB1 protein binding to the promoter of transforming growth factor beta 1 (TGFβ1), a potent modulator of arterial remodeling, thus preventing the activation and proliferation of smooth muscle cells (Arifen et al, 2022).

Inhibition of Sirt-I also attenuated the enhanced levels of superoxide anion, NADPH oxidase activity, and the overexpression of NADPH oxidase subunits; Nox2, Nox4 and P47phox proteins in VSMC isolated from EX-527-treated SHR. Furthermore, the decreased levels of endothelial nitric oxide synthase (eNOS) and nitric oxide (No) and increased levels of peroxynitrite (ONOO-) in VSMC from SHR were also restored to control levels by Sirt-I inhibitor (Lie Y, et al. 2021). These results suggest that the inhibition of overexpression of Sirt-I through decreasing the enhanced levels of Giα proteins and nitro-oxidative stress attenuates the high BP in SHR. It may thus be suggested that inhibitors of Sirt-I may have the potential to be used as therapeutic agents in the treatment of cardiovascular complications associated with hypertension (Zhang et al, 2017)

It has been reported that SirtI directly impacts the endothelial function of arteries by deacetylating endothelial No. synthase (eNOS), which in turn is activated and preserves vascular homeostasis through No. production. Consistently, the inhibition of SirtI in the endothelium of arteries inhibits endothelium-dependent vasodilation and decreases bioavailable No. An elegant study performed by Bai et al. demonstrated that the overexpression of human SirtI in the endothelium in eNOS-deficient mice is protective against hypertension and counteracts adverse arterial remodeling occurring in aging vessels (Campagna et al, 2024).

CONCLUSION

Sirtuin-I relative expression was 0.52 fold lower in hypertension. Sirtuin-I has a preserved effect on cardiovascular and vascular wall through balancing another protein level in the mediation of oxidative stress. A limitation of this research was protein level was not measured. Further research suggestions are to explore the relationship between a diet containing Sirtuin and its expression at the protein level.

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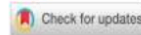
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Gene expression of sirtuin-I in adult with hypertension



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ABSTRACT

SIRT1 is a key member of the sirtuin family, exerts a unique protective effect on endothelial cells by modulating various proteins, and has a role as an anti-aging biomarker. Hypertension is pathology chronic pathological condition that disrupts ROS and antioxidants. Little evidence showed that sirtuin has a role in chronic oxidative stress. Therefore, the objective of this study was to analyze the relative expression of Sirtuin-I in hypertension. This is a case-control study with 30 subjects, adults 50-60 years old in each group. JNC 8 was used to determine blood pressure. Quantitative Real Time PCR was used to calculate the level of Sirt-I. The Livak method was used for relative expression. JASP software was used for data analysis. Our study showed Sirtuin-I mRNA expression was significantly lower in the hypertension group than the normotension group. It was 0.52 fold lower in hypertension. Sirtuin-I stimulates antioxidants such as superoxide dismutase and catalase through FOXO-dependent signaling. It has a similar role to antioxidants for eliminating ROS. Sirtuin-I expression is significantly reduced in individuals with hypertension, suggesting its potential role in oxidative stress regulation and its value as a biomarker for vascular aging and hypertension-related endothelial dysfunction.

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INTRODUCTION

Aging is one of the risk factors for hypertension. Increasing systolic blood pressure by 20 mmHg could have a twofold risk of death by stroke. It could trigger death suddenly because of a lack of symptoms. Global prevalence of hypertension is predicted to reach 60% of adults by 2025. In Indonesia, based on Indonesian Research in 2018, the prevalence of hypertension increased from 25% to 34%. It affected 55.28% of individuals aged 54-65 years old. Globally, hypertension poses a significant threat to human health, increasing the risk of premature mortality and disability. Moreover, its pathological mechanisms can disrupt the nervous, endocrine, and immune systems.



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Asymptomatic nature leads to unavoidable complications, premature death, and disability. Systolic and diastolic blood pressure triggers chronic systemic inflammation, such as vascular stiffness due to reduced production of elastin and collagen in the arterial tunica media. Repetition of this process led to endothelial dysfunction of arteries. It reduced nitric oxide (NO) production and caused vasoconstriction (Manolis et al., 2020).

Few studies showed that degenerative disease has a higher level of reactive oxygen species (ROS). Recently, our study showed a significant negative correlation between ROS level and physical activity, while a positive correlation has also been found between ROS level and body mass index of people over 40 years old (Meiyanti et al, 2023). Other studies showed mRNA Catalase expression was lower in people over 50 years old. It demonstrates that ROS could be eliminated with antioxidant properties (Yohana et al, 2024). Another enzyme that is involved with oxidative stress and antioxidant properties is Sirtuin-I. It reduced ROS production and uncoupled eNOS (Wu et al, 2022).

Sirtuin is a NAD⁺-dependent deacetylase, regulates No. adaptation, vascular homeostasis, cellular signaling, immunity, inflammation, and oxidative stress protection (Teixeira et al., 2020). SIRT1 protects the cardiovascular and vascular systems through deacetylase activity, suppressing ROS production. Recent studies indicate ROS and inflammation contribute to blood pressure elevation (Griendling, K, et al, 2021). SIRT1 inhibits pro-inflammatory gene expression, mitigating inflammatory responses. Animal studies demonstrate Sirtuin's role in reducing blood pressure and cardiac hypertrophy via SirtI/NF κB/MAPK signaling (Yang et al., 2022).

Sirtuin activation could reduce endothelial dysfunction, atherosclerosis, and blood pressure through SirtI-AMPK-eNos signaling through grape seed proanthocyanidin extract (Chen et al, 2020). Sirtuin-I is involved in cardiovascular protection. However, sirtuin-I expression in hypertension is still unclear. This study is among the few clinical investigations that quantitatively assess the relative mRNA expression of SIRT1 in adult human subjects with hypertension. Unlike previous studies that mainly rely on experimental or animal models, our findings provide direct clinical evidence that SIRT1 expression is significantly downregulated in hypertensive patients, supporting its role as a potential biomarker of oxidative stress and endothelial dysfunction in human hypertension. Therefore, the objective of this research is to analyze the relative expression of Sirtuin-I in hypertension among individuals over 50 years old.

RESEARCH METHODS

Research Design

This research was approved by the Ethics Committee Faculty of Medicine Univeristas Trisakti, with number 006/KER/FK/10/2024. This is a case-control study with 60 respondents divided into the hypertension and normotension groups according to JNC VIII.

Population and Samples

The method used for selecting the sample for this study was the purposive sampling method. A total of 60 subjects (30 per group) were selected based on statistical and practical considerations. This sample size provides adequate power for detecting significant differences in SIRT1 gene expression using quantitative PCR, while also considering feasibility, resource limitations, and the biological variability of the target age group (50–60 years). The age range was chosen to focus on individuals with a higher risk of hypertension and endothelial dysfunction. Stored biological material whose sample maintenance is carried out in the Biomolecular Laboratory, Medical Faculty, Universitas Trisakti. Subjects are participants who are invited to take part in the study in Angke Village. Selected subjects must meet the following inclusion and exclusion criteria.

This study was conducted by respondents who were 50-60 years old, with inclusion and exclusion criteria. Inclusion criteria were agreed to participate in research, whereas exclusion criteria



were respondents with liver disease, autoimmune disease, and cancer. After agreeing to the informed consent, 2 ml of blood was taken from the respondent.

Instruments

The research instruments used were a nanophotometer, a Polymerase Chain Reaction (PCR) machine Labcycler (SENSOQUEST), and a quantitative real-time PCR (qPCR) machine QiAquant 96 5 plex. The nanophotometer was used to assess the concentration and purity of nucleic acids (DNA and RNA) by measuring absorbance at specific wavelengths (e.g., 260 nm and 280 nm), which is essential before downstream molecular biology applications. The Labcycler (SENSOQUEST) is a conventional thermal cycler used to perform standard PCR, which amplifies specific DNA sequences through repeated cycles of denaturation, annealing, and extension. This instrument is crucial for generating sufficient quantities of DNA for further analysis. The QiAquant 96 5 plex is a high-performance real-time PCR system capable of detecting and quantifying nucleic acids in real time using fluorescent dyes or probes. The term "5 plex" refers to its ability to detect up to five different targets simultaneously in a single reaction, allowing multiplexing. This system provides precise quantification of gene expression levels, pathogen load, or genetic mutations through amplification curve analysis and Ct (cycle threshold) values.

Procedures

I. RNA extraction

Two millilitres of blood were extracted and transformed into total RNA by the Quick RNA Miniprep kit (Zymoresearch). According to the kit procedure, briefly, blood was combined with cell lysis buffer and Proteinase K, then incubated for 30 minutes at 20-30 degrees Celsius. Isopropanolol was added 50% (v/v). After the sample was homogenized, it was transferred into the spin column and centrifuged at 16,000 g for 30 seconds. The sample was washed with RNA wash buffer and centrifuge it 15,000 g for 30 seconds. Yield was eliminated and do addition of DNA-se buffer. Sample mixed until homogenized, then incubated at 20-30 degrees Celsius for 15 minutes. After 15 minutes, the sample was washed with RNA buffer and centrifuged for 1 minute. At the terminal point, the Sample wash added RNA-free water and centrifuged for 1 minute. The yield was measured for concentration and purity by a nanophotometer wavelength of 260/280 nm. The sample was kept in -80 degrees Celsius. The sample was managed by the Biomolecular Laboratory of the Faculty of Medicine at Trisakti University. At first total RNA needed to be checked for concentration and purity by a nanophotometer. The sample concentration that can be used is more than 20 ng/μL. Purity results fall within the range of 1.7 – 2.0, they can be categorized as pure isolation results.

2. Specimen Examination: Measurement of mRNA expression SIRT1

a. cDNA Synthesis Procedure

Copy DNA was synthesized using SensiFAST cDNA Synthesis kit and amplified using a PCR machine (SENSOQUEST) with 95 degrees Celsius for denaturation for 15 seconds, annealing at 60 degrees Celsius for 40 cycles. Minimum RNA sample for the reaction was 200 ng, and 20μl master mix. The product was diluted 1:10 and stored at -20°C for quantitative real-time PCR.

b. qRT PCR procedure (quantitative Reverse Transcriptase Polymerase Chain Reaction)

Relative expression was analyzed using 2-step qRT PCR (QiAquant 96 5 plex). 4 ul cDNA was added into the PCR tube along with SensiFAST SYBR Green No.-ROX marker, forward and reverse primers, and Nucleus Free Water up to 20 ul. The total PCR reaction was 50 cycles. Sirtuin-1 Sequence primer was CTATACCCAGAACATAGACACG (forward),



ACAAATCAGGCAAGATGC (reverse). GAPDH sequence primer was GTC TCC TCT GAC TTC AAC AGC G (forward), ACC ACC CTG TTG CTG No. CCA A (reverse). Both primers have an annealing temperature of 56,5 degrees Celsius. The result was presented in the cycle threshold (Ct). Ct value was measured to calculate expression in the hypertension and normotension groups. Relative expression was determined using the Livak method.

Data Analysis

CT value was well distributed within the two groups, with the Wilk test. Student t-test was used to figure out differences between the 2 groups with a significance $p < 0.05$. Analysis statistics used JASP software 0.19.2. JASP (Jeffreys's Amazing Statistics Program) is an open-source software designed to facilitate both classical and Bayesian statistical analysis with an intuitive and user-friendly interface, as well as producing publication-ready output in the form of tables and graphs.

RESULTS

The primary objective of this study was to investigate the relative mRNA expression of Sirtuin-I (SIRT1) in individuals diagnosed with hypertension, specifically in a population over 50 years old. Sirtuin-I is a gene associated with cellular stress resistance, metabolic regulation, and vascular health. This research aims to provide molecular evidence by quantifying and comparing the expression levels of SIRT1 mRNA between hypertensive and normotensive individuals, thus helping to understand its potential role in the pathophysiology of hypertension.

Figure 1 illustrates the relative mRNA expression levels of SIRT1 in two groups: Hypertension and Normotension. The y-axis represents the relative expression level of SIRT1 mRNA, while the x-axis distinguishes between the two groups. The normotensive group shows a significantly higher level of SIRT1 expression, averaging around 6-fold relative expression. The hypertensive group shows a much lower average expression, around 3-fold, with a wider range as indicated by the error bar. Error bars indicate the standard deviation or standard error (depending on the analysis), showing variability within each group. The difference between groups was found to be statistically significant ($p < 0.05$), indicating a true difference in expression rather than one due to random chance. The results demonstrate that SIRT1 mRNA expression is significantly reduced in hypertensive individuals compared to normotensive controls.

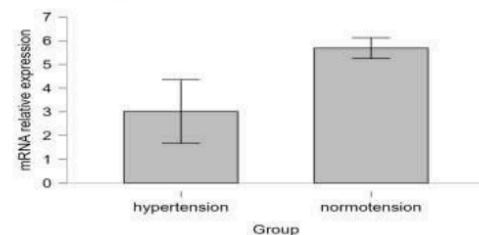


Figure 1. mRNA relative expression Sirtuin-I ($p < 0.05$)*

DISCUSSION

This study found that the average age of the hypertension group was 48 years old. This result showed people aged over 40 years have considerably higher hypertension than the younger age (Astutik et al, 2020). A similar result was found in other research held in rural areas, demonstrating that older age could potentially have a significant risk for hypertension, cause vascular wall might have modified.(Mulia et al, 2020).



Hypertension is a complex degenerative disease characterized by elevated blood pressure, leading to numerous complications. The underlying pathophysiological processes, including inflammation and fibrosis, are linked to oxidative stress. Oxidative stress arises from an imbalance between oxidants and antioxidants, leading to impaired cellular signaling and oxidative damage in hypertension (Touyz, et al, 2020). The production of reactive oxygen species (ROS) is increased in hypertension, leading to oxidative stress. However, ROS also play important physiological roles, particularly in low concentrations, where they contribute to redox regulation, maintaining endothelial integrity, and vascular function. The interaction between flowing blood and endothelial cells induces mechanical forces, such as shear stress, which influence the formation and release of nitric oxide (No.) and ROS, as well as the activation of signal transduction pathways and gene and protein expression. These mechanisms play critical roles in maintaining vascular homeostasis. Notably, laminar flow increases endothelial nitric oxide synthase (eNOS) expression, activity, and No. production, whereas oscillatory flow during hypertension leads to increased ROS formation and subsequent oxidative damage (Amponsah-Offeh et al, 2022).

Although cells have many defensive mechanisms to maintain a fine balance between antioxidant and oxidant systems, improper biochemical reactions within the cell as well as certain external factors, can lead the cell into a state of oxidative stress. An imbalance between oxidants and antioxidants in favor of excessive oxidants, leading to a disruption of redox signaling and control, and/or molecular damage (Sun H, et al, 2024). Research has implicated sirtuins in the regulation of antioxidant defenses and redox signaling pathways, highlighting their crucial role in maintaining redox homeostasis and preventing disease pathogenesis (Alam F, et al, 2021).

SIRT1 is a key member of the sirtuin family, exerts a unique protective effect on endothelial cells by modulating various proteins, including eNOS, LKBI, p53, NFkB, FOXO1, Notch, and p66Shc (Sazdova et al, 2024). Consequently, SIRT1 prevents endothelial senescence, promotes angiogenesis and migration, enhances endothelium-dependent vasodilation, and suppresses inflammation and foam cell formation (Begum MK eal, 2021). Notably, another study demonstrated that endothelial SIRT1 maintains vasodilator responses by upregulating sGC in smooth muscle cells, independently of eNOS and No., providing an alternative therapeutic pathway to mitigate vascular aging and associated diseases, including hypertension (Ren C, et al, 2022).

SIRT1 is an evolutionarily conserved enzyme that deacetylates multiple intracellular targets 54 tis54 important for a variety of cellular functions such as DNA damage repair, cell cycle regulation, apoptosis, senescence, and remodeling of large arteries contributes to the elevation of blood pressure and increased risk of cardiovascular diseases (Shahgald S et al, 2021). In this present study, we investigate the relative expression of the SirtI gene in hypertension and normotension among individuals over 50 years old. Figure I showed that SIRT1 expression was relatively 0.52-fold lower in the hypertension group compared to the normotension group. This result is consistent with our previous study result that the antioxidant was lower in hypertension than normotension (Yohana et al, 2024). This study is supported by another study with the elderly who have hypertension. It revealed that superoxide dismutase was significantly lower in the elderly with hypertension than in controls. Cabonyl as ROS marker was significantly higher in the hypertension group. There was a correlation between superoxide dismutase and systolic blood pressure. (Penantian et al, 2023).

SIRT1 attenuates oxidative stress and inflammation to regulate vascular endothelial functions through several important signal mediators, such as AMPK, NOXs, eNOs, and FOXOs (Kong et al, 2019). There is a complex crosstalk network between AMPK and SIRT1. Studies showed that SIRT1 can stimulate AMPK via the modulation of upstream AMPK kinase such as liver kinase BI(LKBI), suppressing the production of ROS and inflammation response in



HUVECs, while AMPK influences SIRT1 deacetylation activity by increasing cellular NAD⁺ levels or directly phosphorylating SIRT1 (Maiese K, 2021). Furthermore, increased activity of NOX (NADPH oxidase) may also enhance NAD⁺ content to elevate SIRT1 levels in endothelial cells (Wan X et al, 2021). In addition, SIRT1 deacetylates FOXOs and stimulates FOXO-dependent antioxidant [such as catalase (CAT), manganese superoxide dismutase (MnSOD) and thioredoxin] expression to eliminate ROS in endothelial cells, and prevent endothelial dysfunction (Huang et al, 2019). The activation of SIRT1 stimulates the expression of c-Myc by promoting the degradation of FOXO1 to prevent endothelial cell dysfunction and angiogenesis induced by hyperglycemia. eNOS, a member of NOS families, is expressed in vascular smooth muscle (Negre-Salvayre, et al, 2022).

Interestingly, Sirtuin expression is not just only depressed in hypertension, Sirtuin expression could be found to be increased. Research revealed that Sirtuin-I (Sirt-I) is overexpressed in spontaneously hypertensive rats (SHR), contributing to hypertension. This study investigated Sirt-I's role in hypertension and underlying mechanisms. Results showed that Sirt-I inhibitor EX-527 reduced blood pressure by 76 mmHg, inhibited heart rate, and attenuated oxidative stress (Husein, Y, et al, 2022). These findings suggest Sirt-I inhibitors may be effective in treating hypertension-related cardiovascular complications. Another study showed that overexpression of SIRT1 in endothelial cells attenuated the augmented blood pressure and adverse arterial remodeling. Mechanistically, SIRT1 inhibited LKB1 protein binding to the promoter of transforming growth factor beta 1 (TGFβ1), a potent modulator of arterial remodeling, thus preventing the activation and proliferation of smooth muscle cells (Arifen et al, 2022).

Inhibition of Sirt-I also attenuated the enhanced levels of superoxide anion, NADPH oxidase activity, and the overexpression of NADPH oxidase subunits; Nox2, Nox4 and P47phox proteins in VSMC isolated from EX-527-treated SHR. Furthermore, the decreased levels of endothelial nitric oxide synthase (eNOS) and nitric oxide (No) and increased levels of peroxynitrite (ONOO⁻) in VSMC from SHR were also restored to control levels by Sirt-I inhibitor (Lie Y, et al. 2021). These results suggest that the inhibition of overexpression of Sirt-I through decreasing the enhanced levels of Giα proteins and nitro-oxidative stress attenuates the high BP in SHR. It may thus be suggested that inhibitors of Sirt-I may have the potential to be used as therapeutic agents in the treatment of cardiovascular complications associated with hypertension (Zhang et al, 2017).

It has been reported that SirtI directly impacts the endothelial function of arteries by deacetylating endothelial No. synthase (eNOS), which in turn is activated and preserves vascular homeostasis through No. production. Consistently, the inhibition of SirtI in the endothelium of arteries inhibits endothelium-dependent vasodilation and decreases bioavailable No. An elegant study performed by Bai et al. demonstrated that the overexpression of human SirtI in the endothelium in eNOS-deficient mice is protective against hypertension and counteracts adverse arterial remodeling occurring in aging vessels (Campagna et al, 2024).

CONCLUSION

Sirtuin-I relative expression was 0.52 fold lower in hypertension. Sirtuin-I has a preserved effect on cardiovascular and vascular wall through balancing another protein level in the mediation of oxidative stress. A limitation of this research was protein level was not measured. Further research suggestions are to explore the relationship between a diet containing Sirtuin and its expression at the protein level.

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