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32-38

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48-54

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55-57

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58-67

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68-73

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74-78

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83-91

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92-99

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Madhur Yadav; Swati Mittal; Siddharth Vardhan Singh , Babita Kumar
126-136

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Naseem Akhtar Khan, Ghulamuddin Sofi, Md Najibur Rahman, Barkati Md Tarique, Talat Nahid
146-153

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Analyzing FOXO3A gene as a marker in adults with hypertension

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ABSTRACT

Hypertension remains a global health problem. It caused high morbidity and death. It occurs in people in low- and middle-income countries which aged 30 to 80 years old. Symptoms are often asymptomatic; it became unaware until reached an advanced stage. Vascular aging is suspected to be one of the causes of high blood pressure. Modification layers of blood vessels such as thick, stiff, and inelastic, are caused by oxidative stress. This development is influenced by the FOXO3A gene. FOXO3A plays a role in slowing aging which is a transcription factor that plays a role in signal modulation, cell death, oxidation-reduction reactions, cell metabolism and DNA damage. Previous research on FOXO3A gene expression in people over 70 years of age found significantly lower levels compared to those aged 50-60 years. Because of the limited knowledge regarding FOXO3A gene expression in hypertension, this study aims to analyzed FOXO3A gene expression in the elderly. Sixty subjects were divided into hypertension and normotension based on JNC 8 criteria. Quantitative Real Time PCR was used to calculate FOXO3A expression. The Livak formula was used to measure relative expression. FOXO3A relative expression was 7.95 fold lower in hypertension than normotension. FOXO3A expression was reduced in hypertension. Further research at the protein level is needed to confirm these findings.

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Research Article

Analyzing FOXO3A gene as a marker in adults with hypertension

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Abstract

Hypertension remains a global health problem. It caused high morbidity and death. It occurs in people in low- and middle-income countries which aged 30 to 80 years old. Symptoms are often asymptomatic; it became unaware until reached an advanced stage. Vascular aging is suspected to be one of the causes of high blood pressure. Modification layers of blood vessels such as thick, stiff, and inelastic, are caused by oxidative stress. This development is influenced by the FOXO3A gene. FOXO3A plays a role in slowing aging which is a transcription factor that plays a role in signal modulation, cell death, oxidation-reduction reactions, cell metabolism and DNA damage. Previous research on FOXO3A gene expression in people over 70 years of age found significantly lower levels compared to those aged 50-60 years. Because of the limited knowledge regarding FOXO3A gene expression in hypertension, this study aims to analyze FOXO3A gene expression in the elderly. Sixty subjects were divided into hypertension and normotension based on JNC 8 criteria. Quantitative Real Time PCR was used to calculate FOXO3A expression. The Livak formula was used to measure relative expression. FOXO3A relative expression was 7.95 fold lower in hypertension than normotension. FOXO3A expression was reduced in hypertension. Further research at the protein level is needed to confirm these findings.

Keywords: hypertension, expression, mRNA, FOXO3A

INTRODUCTION

Hypertension remains one of the major global health challenges in the category of non-communicable diseases, the incidence of hypertension continues to increase with age. Usually, they are not aware of the symptoms and signs of high blood pressure. It could cause death and disability in all age groups, especially the elderly.¹ Based on 2024 data, Indonesia ranks 4th in the world with the number of hypertension diseases. More than 70% of individuals aged over 60 years experienced this condition and is a major risk factor for various cardiovascular diseases such as coronary heart disease, heart failure, and stroke. Data from the World Health Organization (WHO) reports that hypertension affects around 1.28 billion adults worldwide, with the majority living in low- and middle-income countries. Based on data from the 2023 Indonesian Health Survey (SKI), the prevalence of hypertension in the population aged 18 years and over in Indonesia reached 30.8% - 34.11%.² This prevalence has increased significantly year by year and became a serious health threat with complications of heart disease and stroke. The burden of disease caused by hypertension is not only limited to individual morbidity and mortality, but also has a significant impact

on health systems and national economies through long-term care costs and lost productivity.³

One of the factors that cause hypertension was vascular aging. It plays a major role in the pathogenesis of hypertension in the elderly population. The aging process of the cardiovascular system is characterized by progressive structural and functional changes in blood vessels, including endothelial dysfunction, increased arterial stiffness, intima-media thickening, and chronic low-grade inflammation. Shear stress and blood viscosity factors also made endothelial damage inevitable. These changes collectively contribute to the increase in peripheral resistance and systolic blood pressure, which are characteristic of hypertension in the elderly. A study demonstrated that vascular aging is associated with increased oxidative DNA damage and decreased expression of DNA repair enzymes such as 8-Oxoguanine DNA Glycosylase (OGG1). These findings indicate that the accumulation of poorly repaired molecular damage is a fundamental mechanism driving vascular aging and its pathological consequences, including age-related hypertension. At the cellular level, the aging process involves the progressive accumulation of molecular damage, including oxidative DNA damage and

mitochondrial dysfunction.⁴ It reported that excessive mitochondrial reactive oxygen species (ROS) in aging endothelial cells not only exacerbate cellular oxidative damage but also trigger chronic inflammation in the arterial wall through the activation of pro-inflammatory pathways such as NF- κ B, ultimately accelerating atherogenesis and vascular dysfunction.⁵ Other regulator that has a significant impact on cellular response, inflammation, and vascular health is FOXO3A. It is a transcriptional factor involved in oxidative stress resistance, mitochondrial function, DNA repair, and cell survival. Superoxide dismutase and catalase are antioxidant enzymes also affected by it.⁶ In the cardiovascular system, FOXO3A has been shown to protect endothelial cells and vascular smooth muscle cells from various physiological and pathological stresses. Genetic association studies have identified FOXO3A variants, particularly rs2802292, as one of the most consistent genetic factors associated with human lifespan across populations, suggesting a protective role against age-related diseases including hypertension, cardiovascular disease, diabetes, and cancer.⁷ A distinct study showed relative mRNA expression of FOXO3A was higher in people between 60-70 years old than people over 70 years old. Older people have lower FOXO3A expression. Aligned with this study, Sirtuin-1 relative expression decreased in participants with hypertension over 50 years while catalase relative expression was not significantly lower in hypertension adults.⁸ Genetic and translational studies in human populations provide strong evidence for the role of FOXO3A in moderating cardiovascular risk. concluded that the FOXO3 genotype associated with longevity (rs2802292 variant) exerts a protective effect against coronary artery disease in men with hypertension. Interestingly, interaction analysis showed that the protective effect of the FOXO3A genotype was only seen in hypertensive subjects, not in normotensive subjects. Proteomic analysis in the same cohort revealed specific proteins associated with amelioration of chronic biological stress in FOXO3A longevity genotype carriers, involving innate immune response pathways, inflammatory responses, and signal transduction moderation.⁹ These findings suggest that FOXO3A may moderate the adverse effects of hypertension on the cardiovascular system through mechanisms that enhance cellular resilience to hemodynamic and metabolic stress.¹⁰ Yet, various studies about the expression FOXO3A gene contributing to patho-mechanism hypertension still remain unclear. Therefore, the purpose of this study is to analyse expression FOXO3A in adults with hypertension over 50 years old.

MATERIAL AND METHODS

This research was conducted with Ethical clearance 001/KER/FK/12/2025 from an ethical committee Faculty of Medicine Universitas Trisakti. Sixty people

over 50 years old were separated into hypertension and normotension groups parallel with JNC VIII. The number of samples has been calculated and adjusted according to the inclusion and exclusion criteria. The inclusion criteria for participants were those aged over 50 years and committed to participating in the research, while the exclusion criteria were participants who had liver disease, cancer, and autoimmune diseases.

RNA isolation

Vein blood was collected about 2 ml. It is isolated into RNA with the Quick RNA Miniprep kit. The entire extraction process follows the kit's procedures. Initially, the blood is added with cell lysis buffer and Proteinase K. After incubation, the sample is added with isopropanol and centrifuged. Finally, the sample is washed with an RNA wash buffer. The extraction results are added with RNA-Free Water and stored at -80 degrees Celsius. Before being used for cDNA production, the concentration is measured at a wavelength of 260/280 nm using a nanophotometer.¹¹

cDNA synthesis and quantitative Real Time Polymerase Chain Reaction

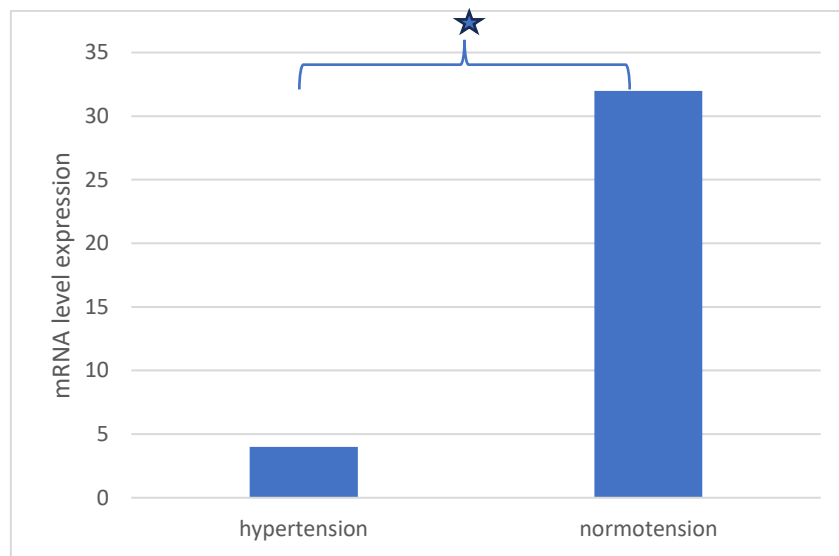
cDNA was developed by SENSOQUEST PCR machine. 200ng total RNA was added into SensiFAST cDNA Synthesis kit along with reverse transcriptase enzyme and nucleus free water (NFW). cDNA construction began with a denaturation process at 95 degrees Celsius and annealing at 60 degrees Celsius. The total reaction was 40 cycles. Product was kept in -20 degrees Celsius. FOXO3A mRNA expression was investigated with 2 step qRT PCR (QiAquant 96 5 plex). cDNA for qRT PCR was made by adding 9 ul NFW and 1 ul cDNA. SensiFAST SYBR Green NO-ROX was added into cDNA with forward and reverse FOXO3A primer including NFW up to 20 ul. Reaction was made duplo and the PCR cycle was up to 50. FOXO3A primer sequences were TTGGAAGAACTCCATCCGACA (forward) and CCACGGCTCTTGGTGTACTT (reverse).¹² House keeping gene was GAPDH and primer sequences were GTC TCC TCT GAC TTC AAC AGC G (forward) and ACC ACC CTG TTG CTG TAG CCA A (reverse). Result was displayed in Ct (cycle threshold) value. It was used to appraise FOXO3A expression at both groups. Livak method was ruled out to count level expressions.⁸

Statistical analysis

Data analysis was performed with SPSS Ver 29. Normality test using Shapiro Wilk. Comparison Data was analyzed with Mann-Whitney Test.¹³

RESULTS

A total of 60 participants were included in the final analysis, comprising 30 subjects in the hypertension group and 30 subjects in the normotension.



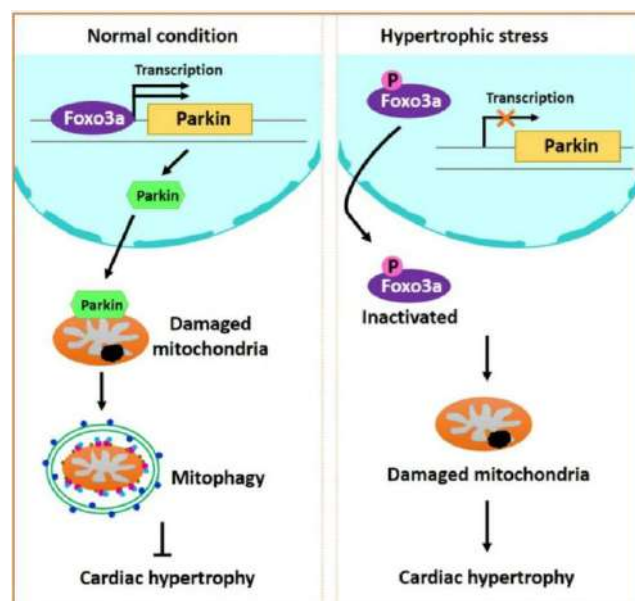
Graphic 1. mRNA FOXO3A level expression through GADPH (($p < 0.001$).

Using the Mann–Whitney U test, the distribution of expression values differed significantly between the hypertension and normotensive groups. As depicted in the graph 1, mRNA FOXO3A expression was 7.98 folds lower in hypertension than normotension. It significantly decreased in hypertension people over 50 years old.

DISCUSSION

This study found that mRNA expression FOXO3A was lower in hypertension people compared to normotension people. This finding is in line with previous studies where FOXO3A expression was found to decrease significantly in the elderly group over 70 years compared to the 50-60 years age group. Increasing age decreases the expression of the FOXO3A gene, which plays a role in cell survival and defence against ROS through regulating the expression of antioxidants such as catalase and MnSOD as well as redox balance in mitochondria.¹³ Our previous study also showed a decrease in catalase expression in the hypertensive group compared to normotensive. Although not significantly decreased, catalase as an antioxidant enzyme has the ability to convert ROS compounds into neutral.¹¹ Different results were found in patients with acute myeloid lymphocytic cancer, where FOXO3A expression was found to increase compared to controls. Other genes whose expression also increased in acute myeloid lymphocytic were Sirt-1, MnSOD and Catalase. There is evidence of a strong positive correlation between Sirtuin-1 and FOXO3A. It stated that overexpression in cancer cells will consume higher energy than normal cells, thus having high resistance to oxidative stress, cell survival, and inhibiting cell apoptosis.¹⁴ Another study on FOXO3A expression in radiation enteritis showed the ability of apoptosis and the antioxidant activity of SOD2 and Catalase. This study used resveratrol, which is hypothesized to be able to strengthen the work of the FOXO3A transcription factor through the PI3K/AKT pathway and deacetylate FOXO3A through Sirt1 activation.¹⁵

Recent research has shown FOXO3A as a transcription factor for mitophagy. Mitophagy is a form of homeostasis of autophagy that balances free radical scavenging, thus preventing mitochondrial damage. Increased FOXO3A expression was found in cardiac fibroblast cells treated with Angiotensin II, but decreased phosphorylated FOXO3A expression. Knocked-down FOXO3A and treated with Angiotensin II significantly inhibited proliferation and migration. Increased expression of PINK1 and Parkin factors, but decreased expression of p62 in cardiac fibroblast cells.¹⁶ Another pathway that demonstrates the link between mitophagy and FOXO3A is the condition of cardiac dysfunction in patients with diabetes mellitus, where decreased mitophagy activity was found. Decreased Sirt3-FOXO3A-Parkin pathway reduces the effect of mitophagy, which plays a role in cardiomyopathy in diabetes mellitus.¹⁷



Graphic 2. Mitophagy signalling in cardiac hypertrophy

Another study found that FOXO3A activation under normal conditions causes Parkin to be expressed, allowing mitophagy to occur in dysfunctional

mitochondria, preventing cardiac muscle hypertrophy. Graphic 2 shows that cardiac muscle hypertrophy is caused by FOXO3A being phosphorylated, preventing Parkin from being expressed, preventing the elimination of damaged mitochondria. Mitophagy plays a crucial role in cardiac muscle aging and the development of heart failure, including changes in cardiac muscle cells, development, and remodelling. FOXO3A could inhibit the development of cardiomyopathy in cardiac muscle cells treated with Angiotensin II. Sarcomere formation, increased cell surface area, and increased cardiac hormone markers are indicate the absence of Angiotensin II.¹⁸

The forkhead box O type 3 family plays a role in longevity. This has been demonstrated through various studies of variations associated with longevity. One study conducted on Okinawans found the rs2802292 variant to be significantly associated with longevity exceeding 95 years. The variant is associated with the G allele compared to the T allele, where the variation is associated with telomere effects. Various chronic diseases are also associated with pro-inflammatory and anti-inflammatory cytokine activity that can lead to senescence. FOXO3A variations are said to protect the risk of coronary heart disease, especially those with the G allele. In women over 55 years old who have the G allele have lower IL-6 levels than those with the T allele. Results by gender that women and men have similar telomere length trends in the G allele and the T allele. For ages 19-54 years and those over 55 years old, variations in the G allele were also found to be significantly associated with FOXO3 expression.¹⁹ On the other hand, FOXO3A expression was found to be significantly lower in those over 70 years of age compared to those aged 60-70 years. Different age groups, races, and genders may have different outcomes through different mechanisms.¹³

CONCLUSION

This study found FOXO3A downregulate in people with hypertension over 50 years old. As a transcription factor, FOXO3A plays a role in various processes that contribute to heart disease, including mitophagy, a specific autophagy pathway for damaged mitochondria. Proposing FOXO3A as an aging dan vascular disease marker could open up promising diagnostic opportunities and the development of therapeutic targets. Further protein level examination is needed to support the diagnosis.

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