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Overexpression of Apolipoprotein-E and Angiotensin Converting Enzyme Gene mRNA in First Descendant of Coronary Heart Disease Patients

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Abstract

Coronary Heart Disease (CHD) has several risk factors including dyslipoproteinemia, obesity, smoking, hypertension and genetic disorders. Recent research on gene expression shows that the Apolipoprotein-E (Apo-E) and the Angiotensin Converting Enzyme (ACE) gene are proven to have a role in the process of CHD disease. The aim of this study to find out the level of Apo-E and ACE gene expression in the first descendant of CHD and non-CHD patients in Bengkulu City. The method used is analytical observational with a cross sectional research design using techniques purposive sampling. The results of this study show that the expression of the Apo-E and ACE genes in the first descendant of CHD is significantly higher than non-CHD with a significance value of P=0.039; P<0.05 for the Apo=E gene and P=0.018; P<0, 05 in the ACE gene. This shows that comparing the expression of the Apo-E gene, it was found that the CHD first descendant group had a higher expression of around 1.88 times compared to non-CHD first descendant. Meanwhile, when comparing the ACE gene, it was found that the expression of the CHD first descendant group was 1.02 higher than the non-CHD first descendant group. In conclusion, the first descendant of CHD sufferers have a higher vulnerability to CHD events due to over expression of Apo-E and ACE genes. This highlights the need for prompt implementation of preventive interventions.

Keywords: CHD, Apo-E, ACE, gene expression, first descendant

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1. Introduction

Coronary Heart Disease (CHD) is a cardiovascular disease caused by impaired function of the heart and blood vessels, where in this condition there is narrowing or blockage in the form of plaque in the coronary blood vessels, known as coronary artery atherosclerosis [1]. Atherosclerosis is an endothelial dysfunction that causes progressive changes in the arterial walls and characterized by fat deposits, recruitment and accumulation of leukocytes, formation of foam cells, migration and proliferation of myocytes and extracellular matrix deposits that cause thickening and stiffness of the arteries [2]. This chronic condition where the arteries harden through plaque accumulation can be caused by several risk factors including dyslipoproteinemia, obesity, smoking, hypertension and genetic disorders [30]. In previous studies, it was discovered that one factor causing CHD that cannot be controlled was genetic factor. The discoveries state that all diseases involve changes in genetic structure which can have an impact on changing the susceptibility of a population to disease [4-5] Previous research regarding the expression of the Apo-E gene and the ACE gene has been shown to increase in CHD patients [6-7]. The expression analysis that has been carried out can determine the quantity

of the Apo-E gene and ACE gene in CHD patients, so that it can identify patients who are at risk of CHD and preventive measures can be taken. However, research regarding the expression of the Apo-E gene and the ACE gene in the first descendant of CHD patients has never been studied, so this research was conducted to analyze the relationship between the expression of the Apo-E gene and the ACE gene in the first descendent of CHD patients .

2. Materials and Methods

The research was conducted from February – July 2023 with a cross-sectional research design and using purposive sampling techniques. The sample used was the first descendant of CHD and non-CHD sufferers in Bengkulu City. The number of samples used in this study was 54 subjects, 27 CHD first derivatives obtained from 10 families with a maximum of 4 children and 27 Non-CHD first derivatives obtained from 13 families with a maximum of 5 children. The research sample size was determined using the categorical descriptive formula [8]. The inclusion criteria for this study were the first child of patients with CHD in Bengkulu City (from one or both parents who had CHD), the first child of patients without CHD in Bengkulu City and expressing willingness to participate in the research and providing informed consent. The study's exclusion criteria encompassed those with a documented history of psychiatric illnesses, those currently receiving ACE inhibitor therapy, and those who declined participation as research subjects.
The research samples were obtained from catheterization laboratory at M. Yunus Hospital, Bengkulu City. This data was taken from the results of a coronary artery catheterization examination, which is the gold standard for CHD examination. Completing the WHO Rose Angina questionnaire is given to partners of non-CHD patients. Blood sampling in this study was carried out in the city of Bengkulu.

2.1 RNA isolation

Blood samples were isolated using the buffer contained in the Geneaid Total RNA Mini Kit (Blood) QAIC/TW/50077 isolation kit, to obtain total RNA using the guidelines from the original protocol. RNA isolation was carried out at the Research Laboratory of the Faculty of Medicine and Health Sciences, Bengkulu University. The isolation results obtained a volume of 30 µL of pure RNA. After RNA isolation is complete, the sample continues with the process of measuring its concentration and purity levels.

2.2 Sample Concentration and Purity

Measurement of RNA isolation results using Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer. Nanodrop spectrophotometry has a working principle by calculating the difference in UV light absorption with the wavelength used. The wavelength to determine RNA content on a spectrophotometer is 260nm, while contaminants such as protein use a wavelength of 280nm. A purity value of 1.8-2.0 and a concentration above 100 ng/µL indicate good sample quality [9].

2.3 RT-PCR

The process of measuring the expression of ApoE , ACE and GAPDH mRNA as housekeeping genes with specific primer sequences (Table 1) was measured using Applied Biosystems 7500 Fast Real-Time PCR Thermo Scientific™. The amplification process was carried out at the PCR Laboratory at M. Yunus Hospital, Bengkulu City. The process of measuring mRNA expression levels was adapted to the original SensiFAST™ SYBR ® Lo-ROX One-Step Kit protocol. mRNA is reverse transcribed into cDNA according to the protocol: Temperature holding stage 1 reverse transcription cycle for 10 minutes at 45 °C, polymerase activation cycle for 2 minutes at 95 °C; 40 cycling stages in the denaturation cycle 5 seconds at 95 °C, annealing and extension 20 seconds at 63 °C, melt curve stage at step and hold settings at 95 °C for 1 second, 60 °C for 20 seconds, and 95 °C for 1 second.

2.4 Data analysis

The amplification results were analyzed using the Livak method by comparing the target Ct with the calculation of ΔCt = Ct target gene – Ct housekeeping gene. Then a comparison of expression levels was obtained using 2-ΔΔCt. The statistical test for amplification results uses the Mann-Withney test using the Statistical Program for Social Science (SPSS) for Windows version 25 statistics.

3. Results and Discussion

3.1 Apo-E and ACE gene mRNA expression

The mRNA expression of the Apo-E gene were analyzed using Livak method for CHD first descendant group was around 3.81 ± 0.78 and for non-CHD first descendant 1.93 ± 0.71. Results of analysis using Mann-Whitney test, expression of Apo-E mRNA gene obtained P=0.039 or P<0.05 which shows there were significant difference between groups. From these results, the expression of the ApoE gene in the CHD first descendant group had a higher expression of around 1.88 times compared to the non-CHD first descendant group. The mRNA expression of the ACE gene using Livak method for CHD first descendant group was around 2.45 ± 0.46 and for non-CHD first descendant 1.43 ± 0.39. Results of analysis using Mann-Whitney test, expression of ACE mRNA gene obtained P=0.018 or P<0.05 which shows there were significant difference between groups. From these results, the expression of the ACE gene in the CHD first descendant group had a higher expression of around 1.02 times compared to the non-CHD first descendant group. The median value of Apo-E gene expression obtained for CHD first descendant samples was 2.736 and non-CHD first descendant was 0.469. The median value of ACE gene expression obtained for CHD and non-CHD first descendant samples were 2.172 and 0.340. The results of calculations of Apo-E and ACE gene expression showed that 14 samples or 52% of CHD first descendant samples experienced increased gene expression .

Table 1. Nucleotide Sequences in Forward and Reverse Primers

Gen	Primer	Sequences
APOE	Forward	5'-TGGACAAGTCTGGGATCCTT-3′
	Reverse	5'-CATCTTCCTGCCTGTGATTG-3′
ACE	Forward	5′ -AAGCAGGACGGCTTCACAGA -3′
	Reserve	5′-GGGTCCCCTGAGGTTGATGTAT-3′
GAPDH	Forward	5'-TGCACCACCAACTGCTTAGC-3'
	Reserve	5'-GGCATGGACTGTGGTCATGAG-3'

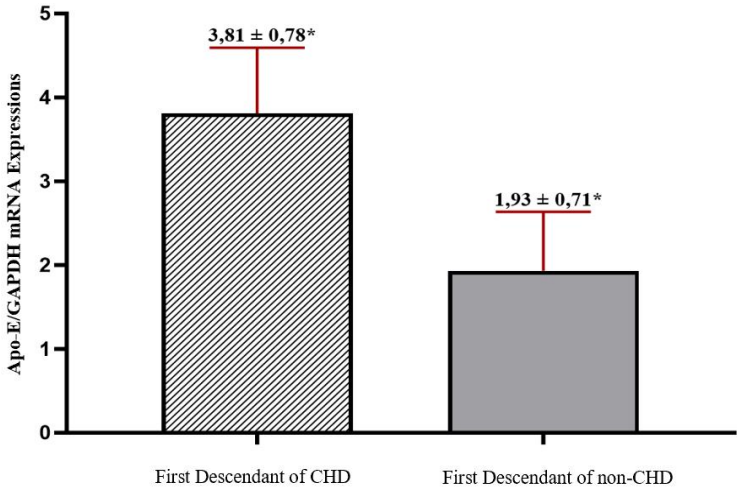

Figure 1. Results of ApoE Gene mRNA Expression *;* Description: *Mann-Whitney test* for measuring ApoE/GAPDH mRNA expression $p = 0.039$ or $p < 0.05$; *Mean* ± S.E

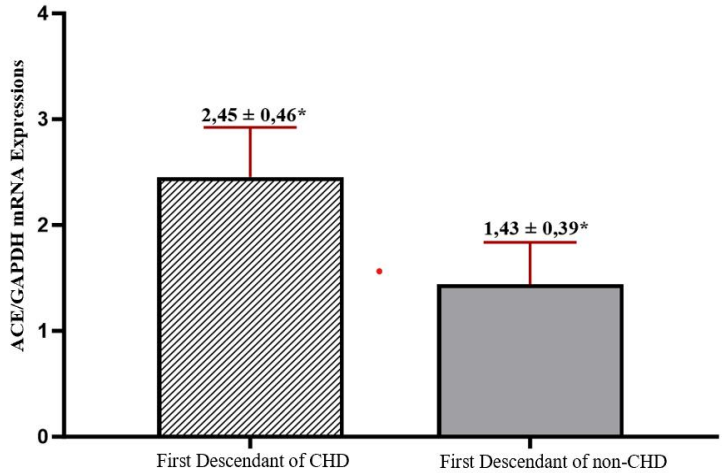

Figure 2. Results of ACE Gene mRNA Expression *;* Description: *Mann-Whitney test* for measuring ACE/GAPDH mRNA expression $p = 0.018$ or $p < 0.05$; *Mean* ± S.E

3.2 APO-E Gene mRNA Expression in CHD and Non-CHD First Descendant

The results in this study show that there were significance difference in the average level of mRNA expression of the ApoE gene in the first CHD and non-CHD groups, where there was an increase in the mRNA expression level of the ApoE gene in the first CHD group. This indicates that the relative expression of ApoE gene mRNA in the CHD first descendant group tends to be higher than in the non-CHD group. High and low levels of ApoE mRNA expression are associated with the incidence of neurogenerative diseases. In previous research, ApoE had significantly higher mRNA expression than the control group [10]. The high level expression in the first descendant CHD group, making it possible for ApoE to be a risk factor for coronary heart disease. One study regarding ApoE expression revealed the relative ApoE mRNA levels in the Alzheimer Disease group were significantly higher than those in the control group [11]. This is in line with this study that the mRNA expression in the diseased first-generation group was higher than in the non-sick first-generation group. The high level of mRNA expression of the ApoE gene in the first generation group can be concluded to be due to genetic influence originating from parents in the first generation of CHD. ApoE is a protein that has three alleles consisting of the E2, E3, and E4 alleles. One of these alleles will be inherited from parents to their children [12].

Prior research has validated the mRNA expression of the Apo-E gene in patients with coronary heart disease (CHD), suggesting that Apo-E4 may serve as a promising candidate gene for investigating the risk of cardiovascular development.6 The observed outcomes can be attributed to the potential interaction between Apo-E and additional genetic or environmental risk factors in determining the risk of coronary heart disease (CHD). These findings indicate that E4 can be regarded as a beneficial component in the progression of atherosclerosis [13]. Apo-E genotype E4 in groups with CHD and non-CHD parents proves the existence of dominant genetics that are passed from parents to children according to research results [14]. Additionally, a separate study found notable disparities in the Apo-E gene and genotype frequencies between patients and controls. This suggests that the E2 and E3 alleles do not pose a substantial risk for coronary heart disease in Bengkulu. A different case was shown in the E4 genotype analysis. This study demonstrates that children in Bengkulu Province who possess the Apo-E gene polymorphism type are at a higher susceptibility to developing coronary heart disease. The genotype of the first descendent of CHD and non-CHD patients exhibits dissimilarities. The prevalence of the E4 genotype was higher in the first generation of individuals with coronary heart disease compared to the first generation of those without coronary heart disease [15]. The mRNA expression of the Apolipoprotein E gene can serve as an alternate method for identifying people who are at risk of coronary heart disease (CHD), allowing for the implementation of preventive interventions.

3.3 ACE mRNA Expression in CHD and Non-CHD First Descendant

The expression of the ACE gene in CHD first descendant in this study also higher compared to the expression results in the non-CHD first descendant group. The results of ACE gene amplification in this study by calculating 2-ΔΔCt (fold change) for the first descendant of CHD and non-CHD were respectively 2.45 ± 0.46 and 1.43 ± 0.39 (mean ± SE). These results are similar to the results of an ACE gene expression study conducted in India in 2014 in hypertensive patients.16 Based on the Ct value obtained quantitatively, ACE gene expression is higher in the first descendant of CHD. This is in line with the theory that genetic variations are passed from parent to child in the DNA of egg and sperm cells. The parents' genetic code is then copied into every cell of the child's body during development [17]. The excessive ACE gene in the first generation may appear due to excessive ACE genetic variations inherited from parents suffering from CHD. This is supported by studies which say that first degree relatives of individuals with type 2 Diabetes Mellitus (DM) are about 3 times more. likely to develop the disease than individuals without a family history [18-19]. In this study, genetic variations are thought to play a major role in increasing ACE activity, especially in CHD disease. This is in line with a study conducted by Sahin et al in Turkey, which stated that ACE levels in CHD sufferers were higher than the control group [20]. The results of one meta-analysis study show that higher circulating ACE levels are also known to be associated with the ACE I/D gene polymorphism which has been identified as a risk factor for Behcet's disease (BD) or Behcet's Syndrome, which has signs and symptoms in the heart as CHD [21-22]. Studies conducted in South India also show that ACE gene expression increases in hypertension sufferers, where hypertension is a risk factor for CHD.3,23 So in this research, ACE gene expression can be an alternative for identifying the risk of CHD so that preventive action can be taken.

4 Conclusions

The genetic variations of the Apo-E and ACE genes in the CHD first descendant group were higher or overexpression compared to the expression results of non-CHD first descendant. This proves that the Apo-E and ACE genes as genetic factors can be inherited. This gene can also be a factor that can cause the first group of CHD to have a greater risk of CHD disease. So that the overexpression of the Apo-E and ACE genes can be a step in early detection of risk factors for CHD events, so that preventive measures can be implemented immediately .

Acknowledgements

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Ethical Approvals

The study obtained ethical clearance from the Bengkulu University Health Research Ethics Committee with the reference number 51/UN30.14.9/LT/2023.

Declaration of Interest Statement

The writers affirm that they do not have any conflicts of interest.

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