Vol 8 No 2 June 2023

E-ISSN: 2528-410X

RESEARCH ARTICLES

Cholesterol Levels in Angiotensin Converting Enzyme Gene Polymorphisms in Schizophrenia Patients

Isra Thristy[1*], Nanda Sari Nuralita[2], Debby Mirani Lubis[3]

[1]Department of Biochemistry, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, jalan Gedung Arca No. 53 Medan 20217 Sumatera Utara
[2]Department of Psychiatry, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, , jalan Gedung Arca No. 53 Medan 20217 Sumatera Utara
[3]Departement of Physiology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, , jalan Gedung Arca No. 53 Medan 20217 Sumatera Utara

Corresponding email: israthristy@umsu.ac.id

Abstract: Angiotensin-converting enzyme (ACE) is widely distributed on the surface of endothelial and epithelial cells. The renin-angiotensin system in the brain has implications for various functions, including cerebral blood circulation and brain protection, stress, depression, and the aetiology of schizophrenia. ACEs and angiotensin receptors can be found on dopaminergic neurons in the basal ganglia. Elevated levels of triglycerides and cholesterol can also be found in schizophrenic patients related to treatment and duration of the course of the disease. The purpose of this study was to determine the relationship between ACE gene polymorphism and cholesterol levels in schizophrenia patients. The design of this study was observational analytic with a cross-sectional approach. The sample in this study was schizophrenia patients who sought treatment at Madani Medan Hospital with a total of 42 people. The data obtained is then processed with the ANOVA one-way test. The results of the study were obtained from 42 samples, the most types of ACE gene polymorphisms were type II (76.2%), followed by the type of DD polymorphism (19%) and the least was the type of ID polymorphism (4.8%). The average value of cholesterol levels of the sample was 235.40 mg/dl with a standard deviation value of 47.618. Statistical test results of ACE gene polymorphism with cholesterol levels with $p = 0.770$ ($p > 0.05$). It can be concluded that there is no relationship between the polymorphism of the ACE gene and the cholesterol levels of schizophrenic patients.

Keywords: Angiotensin-converting enzyme (ACE) gene, cholesterol, schizophrenia

INTRODUCTION

Angiotensin-converting enzyme (ACE) has an important role in the conversion of angiotensin I to angiotensin II and the degradation of bradykinin, a powerful vasodilator, which mediates various cellular functions in different tissues. ACEs are widely distributed on the surface of endothelial and epithelial cells.[1]

The renin-angiotensin system in the brain has implications for a variety of functions, including cerebral blood circulation and brain protection, stress, depression, seizures, alcohol consumption, memory, and the aetiology of alzheimer's disease and schizophrenia.[2] ACEs and

E-ISSN: 2528-410X

angiotensin receptors can be found on dopaminergic neurons in the basal ganglia.[2,3]

Schizophrenia is a mental disorder that can occur at a young age, symptoms of which vary including impaired cognitive function, mood disorders, and psychosis.[4]

The genetic aetiology of schizophrenia has been widely studied, including the ACE gene which is thought to play a role in changes in brain ACE levels in schizophrenia patients.[4] ACEs that have a role in the release and interaction of dopamine.[4] Dopamine is a neurotransmitter that is closely related to the pathophysiology of schizophrenia.[5] In previous studies found increased levels of brain fluid ACE in schizophrenia patients.[4,5]

The ACE gene is located on chromosome 17q23. Insertion/deletion (I/D) of the ACE gene polymorphism in either the presence (insertion, I) or absence (deletion, D) of the 287 pair entering the gene base at intron 16. The D allele is associated with higher ACE activity in serum and tissues than the I allele. The D allele of ACE I/D polymorphism causes higher expression of ACE mRNA. ACE I/D polymorphism is associated with the development of multifactorial diseases such as diabetes mellitus, hypertension, coronary artery disease, diabetic nephropathies, pre-eclampsia, and cerebral infarction.[6] Previous research suggested that ACE gene polymorphisms had no association with improvements in left ventricular ejection fraction in chronic heart failure patients. [7]

Elevated levels of triglycerides and cholesterol can be found in schizophrenic patients related to treatment and duration of the course of the disease. In addition, in schizophrenia patients, many metabolic syndrome abnormalities are found associated with increased cholesterol levels which can cause an increased risk of cardiovascular disease which is closely related to the ACE gene.[8] In addition, risk factors for ACE gene polymorphism were also found for the appearance of depressive symptoms in coronary heart disease patients.[9]

There have been no previous studies in Indonesia looking for a link between ACE gene polymorphisms and blood cholesterol levels of schizophrenic patients in Indonesia. So, this study aims to find out whether angiotensin gene polymorphism in schizophrenia patients is one of the risk factors for increased blood cholesterol levels of patients by knowing the relationship between the two variables.

METHODS

The design of this study was observational analytic with a cross-sectional approach. The population in this study was schizophrenia patients who sought treatment at Madani Medan Hospital. The study sample was subjects taken from a population that met the inclusion criteria in the form of outpatients with a diagnosis of schizophrenia based on the diagnosis criteria of the Guidelines for Classification and Diagnosis of Mental Disorder / *Pedoman Penggolongan dan Diagnosa Gangguan Jiwa* (PPDGJ) III or DSM 5 2, patients aged 20 to 60 years, and willing to participate in the study by signing informed consent. While the exclusion criteria in this study were patients taking anti-cholesterol drugs.

BULETIN FARMATERA
E-ISSN: 2528-410X

Sampling was carried out at Madani Hospital Medan with the total sample in this study being 42 people who then took the patient's blood and divided into two for examination of ACE gene polymorphism and blood cholesterol levels. Blood tests for ACE gene polymorphism and blood cholesterol levels were carried out at the Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara.

Examination of ACE gene polymorphism levels is carried out by first isolating DNA using procedures and materials from the DNA purification kit genomic wizard. [10] After the DNA sample was stored at a temperature of 2-8ºC, it was continued with the PCR (Polymerase Chain Reaction) method which used primers 3 F 5' – GAT, GTG, GCC, ATC, ACA, TTCGTC, AGAT -3' and Primers 3, R 5' – CTG, GAC, ACC, ACT, CCC, ATC, CCT, TCT -3' by producing products in the form of DNA bands at 490 bp and 190 bp. The PCR method used is with a thermal cycler tool with the first order of 2 minutes at a temperature of 98 º C then carried out 31 cycles at a temperature of 98 º C for 15 seconds, 58 º C for 1 minute and 75 º C for 30 seconds. After 31 cycles entered a temperature of 75ºC for 5 minutes and the results were stored at 4ºC. PCR results were read using 2% agarose gel electrophoresis stained with gel red. The interpretation of the results on this PCR result is polymorphism II when only one DNA band is found at 490 bp, DD polymorphism when there is only one DNA band at 190 bp and ID polymorphism when 2 DNA bands are found, namely at 490 bp and 190 bp. [7]

Examination of blood cholesterol levels is carried out using spectrophotometric tools that use materials and procedures from the Dyasis factory. [11]

Univariate analysis is seen to describe each variable studied, both dependent and independent variables. This analysis is used to look at the frequency distribution table to determine the number and percentage of each variable. Data analysis includes descriptive analysis and hypothesis testing.

To determine the magnitude of the correlation between the numerical scale and the categorical scale, a correlative hypothesis test is used, namely the Annova one-way test.[12]

RESULTS

This research has received approval from the ethics commission of Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara for the implementation of research activities with letter number 576/KEPK/FK UMSU/2021. This sampling was carried out from July to August 2021.

Table 1. Frequency distribution of ACE gene polymorphisms in schizophrenic patients

Polymorphism Gen ACE	Subject (person)	Percentage (%)
II	32	76.2
ID	2	4.8
DD	8	19
Total	**42**	**100**

Based on Table 1. It can be seen that of the 42 samples collected, the most types of ACE gene polymorphisms are type II with 32 samples (76.2%), followed by the DD polymorphism type of 8 samples (19%)

E-ISSN: 2528-410X

and the least is the ID polymorphism type of 2 samples (4.8%).

Table 2. Description of cholesterol levels of schizophrenic patients

	N	Minimum	Maximum	Mean	Std. Deviation
Cholesterol	42	145	341	235.40	47.618
Valid N	42				

Based on Table 2, it was obtained that the lowest cholesterol level in the sample was 145 mg/dl while the highest cholesterol level in the sample was 341 mg/dl. The average value of cholesterol levels of the sample was 235.40 mg/dl with a standard deviation value of 47.618.

Based on the statistical results of the homogeneity test, a value of $p = 0.694$ ($p > 0.05$) was obtained so that it met the criteria to be continued with a hypothesis test between the ACE gene polymorphism variable and total cholesterol value using the Annova test so that results were obtained with a value of $p = 0.770$ ($p > 0.05$).

DISCUSSION

The results of this study are related to the frequency distribution of genotypes found in most schizophrenia patients is the insertion type (II) ACE gene polymorphism, which is 76.2% of samples.

This is not in line with the results of a study conducted in Persia which showed that of all samples of schizophrenia patients, the most common type of polymorphism genotype was ID (±75%), followed by II and DD.[13] In research conducted in Brazil also showed results, the type of genotype polymorphism most commonly found in patients with psychotic symptoms, namely ID (53%), followed by II (25%), and DD (22%).[14] Meta-analysis research states that the DD homozygous genotype is associated with an increased risk of depressive symptoms in Caucasian and Asian populations. ID polymorphism also has an association with schizophrenia risk, although this is debatable.[15] Still a contradiction between allele I may lower the risk of schizophrenia in Turkish women, but allele D in the Spanish population.[16.17]

Several things can be taken into consideration in differences in the results of this study, including heterogeneity factors, confounding factors and biases, ACE gene expression in other locations and ethnic factors from the study population when compared to other studies. [18]

The results of this study showed high average values of total cholesterol levels in schizophrenia patients. This is following the results of previous studies, with high average total cholesterol levels in schizophrenia patients.[19.20]

In this study, there was no significant relationship between ACE gene polymorphism and total cholesterol levels in schizophrenia patients. This is following the results of a previous study conducted on schizophrenia patients in Croatia. This study showed that I/D polymorphism was significantly unrelated to several metabolic syndrome parameters such as cholesterol, triglycerides, HDL, LDL, glucose and BMI levels in schizophrenic patients receiving antipsychotic therapy. [4,21]

ACE enzyme can be found in several tissues in the body such as the kidneys, heart, lungs, endothelium of blood vessels,

E-ISSN: 2528-410X

skin, joints, and testicular tissue.[4] The ACE enzyme has an important role in the regulation of RAS and angiotensin II conversion, as well as directly increasing vascular contraction of smooth muscle cells.[21] This enzyme also affects smooth muscle proliferation, monocyte adhesion, platelet adhesion and its aggregation, and as a modulator of pro-inflammatory and neurotransmitters.[22] The I/D polymorphism of ACE is located within the introns of the ACE gene and is closely related to genetic factors that affect ACE levels in serum.[6] Schizophrenia and blood cholesterol levels are complex conditions that can be influenced by multiple gene factors, inherited genetic factors, and environmental factors. Based on the study, genetic heterogeneity to genotype ACE gene polymorphisms in schizophrenia patients varies greatly in different populations.[23] In addition, differences in the patient's clinical condition are also considered to influence. [9,22,23]

CONCLUSION

There was no significant association between ACE gene polymorphism and total cholesterol levels in schizophrenia patients. The most common type of ACE gene polymorphism found in this study was the insertion genotype (II) followed by the deletion genotype (DD) and the least was the mixed genotype (ID). In addition, high cholesterol levels were also found in the average schizophrenic patient.

REFERENCES

1. Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. Journal of medical virology. 2020 Jul; 92 (7):726-30.
2. Nuralita NS, Thristy I, Lubis DM. Relationship between polymorphism of the angiotensin-converting enzyme gene with symptoms of depression in schizophrenic patients. Buletin Farmatera. 2022 Feb 27;7(1):13-8.
3. Santiago TC, Parra L, Nani JV, Fidalgo TM, Bradshaw NJ, Hayashi MA. Angiotensin converting enzymes as druggable features of psychiatric and neurodegenerative disorders. Journal of Neurochemistry. 2023 Mar 12.
4. Nadalin S, Dević Pavlić S, Peitl V, Karlović D, Zatković L, Ristić S, Buretić-Tomljanović A, Jakovac H. Association between insertion-deletion polymorphism of the angiotensin-converting enzyme gene and treatment response to antipsychotic medications: a study of antipsychotic-naïve first-episode psychosis patients and nonadherent chronic psychosis patients. International Journal of Molecular Sciences. 2022 Oct 12; 23(20):12180.
5. McCutcheon, R. A., Marques, T. R., & Howes, O. D. 2020. Schizophrenia—an

E-ISSN: 2528-410X

overview. JAMA psychiatry, 77(2), 201-210.
6. Montes-de-Oca-García A, Perez-Bey A, Velázquez-Díaz D, Corral-Pérez J, Opazo-Díaz E, Rebollo-Ramos M, Gómez-Gallego F, Cuenca-García M, Casals C, Ponce-González JG. Influence of ace gene I/D polymorphism on cardiometabolic risk, maximal fat oxidation, cardiorespiratory fitness, diet and physical activity in young adults. International Journal of Environmental Research and Public Health. 2021 Mar 26;18(7):3443.
7. Handayani A; Thristy I; Andina M. Association of angiotensin-converting enzyme polymorphism with improved left ventricular ejection fraction in patients with chronic heart failure. Buletin Farmatera, 2021, 6.2: 108-115.
8. Wójciak P, Domowicz K, Rybakowski JK. Metabolic indices in schizophrenia: association of negative symptoms with higher HDL cholesterol in female patients. The World Journal of Biological Psychiatry. 2021 Aug 9; 22 (7):552-6.
9. Meyer T, Rothe I, Staab J, Deter HC, Fangauf SV, Hamacher S, Hellmich M, Jünger J, Ladwig KH, Michal M, Petrowski K. Length polymorphisms in the angiotensin I-converting enzyme gene and the serotonin-transporter-linked polymorphic region constitute a risk haplotype for depression in patients with coronary artery disease. Biochemical genetics. 2020 Aug;58:631-48.
10. Cholesterol Fs, DiaSys Diagnostic Systems GmbH Alte Strasse 9 65558 Holzheim Germany, 2021.
11. Technical Manual; Isolating genomic DNA; Isolating genomic DNA. Wizard® Genomic DNA Purification Kit. 2019.
12. Ichsan B; Pengantar metodologi penelitian kedokteran dan kesehatan masyarakat; Muhammadiyah University Press; 2022.
13. Akbari M, Eghtedarian R, Hussen BM, Eslami S, Taheri M, Ghafouri-Fard S. Angiotensin I converting enzyme gene polymorphisms and risk of psychiatric disorders. BMC psychiatry. 2022 May 23;22(1):351.
14. Nani JV, Dal Mas C, Yonamine CM, Ota VK, Noto C, Belangero SI, Mari JJ, Bressan R, Cordeiro Q, Gadelha A, Hayashi MA. A Study in First-Episode Psychosis Patients: Does angiotensin I converting enzyme activity associated with genotype predict symptom severity reductions after treatment with atypical antipsychotic risperidone? international journal of neuropsychopharmacology. 2020 Nov;23(11):721-30.
15. Nadalin S, Jakovac H, Peitl V, Karlović D, Buretić-Tomljanović A. Dysregulated inflammation may predispose patients with serious mental illnesses to severe COVID-19. Molecular Medicine Reports. 2021 Aug 1;24(2):1-9.

E-ISSN: 2528-410X

16. Boulenouar H, Benhatchi H, Guermoudi F, Oumiloud AH, Rahoui A. An actualized screening of schizophrenia-associated genes. Egyptian Journal of Medical Human Genetics. 2022 Apr 16;23(1):81.
17. Kong LN, Shen YL, Chen YL, Chen X, Su GM, Wang JH, Xiao GB, Guo QW, Zhang JC, Fang DZ, Lin J. Insertion/deletion polymorphism at angiotensin-converting enzyme gene in PTSD individuals and their reciprocal effects on blood pressure. Clinical and Experimental Hypertension. 2022 Apr 3;44(3):208-14.
18. Pillinger, Toby, et al. "Cholesterol and triglyceride levels in first-episode psychosis: systematic review and meta-analysis." The British Journal of Psychiatry 211.6., 2017, 339-349.
19. Kanagasundaram P, Lee J, Prasad F, Costa-Dookhan KA, Hamel L, Gordon M, Remington G, Hahn MK, Agarwal SM. Pharmacological interventions to treat antipsychotic-induced dyslipidemia in schizophrenia patients: a systematic review and meta-analysis. Frontiers in psychiatry. 2021 Mar 17;12:642403.
20. Wójciak P, Domowicz K, Rybakowski JK. Metabolic indices in schizophrenia: Association of negative symptoms with higher HDL cholesterol in female patients. The World Journal of Biological Psychiatry. 2021 Aug 9;22(7):552-6.
21. João V. Nani, Camila M. Yonamine, Diego Castro Musial, Caroline Dal Mas, Jair J. Mari & Mirian A. F. Hayashi. ACE activity in blood and brain axis in an animal model for schizophrenia: Effects of dopaminergic manipulation with antipsychotics and psychostimulants, The World Journal of Biological Psychiatry, 2020, 21:1, 53-63.
22. Fjukstad KK, Athanasiu L, Bahrami S, O'Connell KS, van der Meer D, Bettella F, Dieset I, Steen NE, Djurovic S, Spigset O, Andreassen OA. Genetic variants associated with cardiometabolic abnormalities during treatment with selective serotonin reuptake inhibitors: a genome-wide association study. The Pharmacogenomics Journal. 2021 Oct;21(5):574-85.
23. Mio M, Goldstein BI. Bipolar disorder and cardiovascular dysfunction: Mechanisms and implications. In Neurobiology of Bipolar Disorder 2021 Jan 1 (pp. 223-233). Academic Press.