Sumatera Medical Journal (SUMEJ)Vol. 3, *No.* 1, 2020 | 41 - 47

SUMEJ
Sumatera Medical Journal

Correlation of ACE Gene Polymorphism and Hypertension in Stroke Ischemic Patients

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Abstract. Stroke is the third highest cerebrovascular disease in the world with high mortality and disability rate that is mostly dominated by ischemic stroke. Genetic factor that had been reported to have an indirect effect in increasing the incidence of ischemic stroke is ACE gene polymorphism. ACE gene polymorphism is characterized by the insertion marked by letter (I) or deletion marked by letter (D) on intron 16, chromosome 17. ACE gene polymorphism has drawn a lot of attention from scientists and had been reported to have an indirect effect in increasing the ischemic stroke incidence through pathogenesis of hypertension and atherosclerosis. In this study, 78 subjects of ischemic stroke consist of 43 subjects with hypertension and 35 subjects with normotension. I allele of ACE gene polymorphism was more dominant than D allele in hypertensive ischemic stroke patients (72.1% > 27,9%), and this dominance was also seen in the incidence of hypertension vs normotension (55.4% > 44.6%). However, the correlation of ACE gene polymorphism with the incidence of hypertension was not statistically significant when compared based on its genotype (p=0.280) and allele (p=0.948).

Keyword: ACE; gene; hypertension; ischemic stroke; polymorphism

Received 22 December 2019 | Revised 4 January 2020 | Accepted 14 January 2020

1. Introduction

Stroke is the second most killer in the world and causes more than five million deaths per year, and more than three and a half is dominated by ischemic stroke [1][2]. Based on the International Classification of Disease (ICD)-11 in WHO, ischemic stroke is defined as an acute partial neurological dysfunction in one or several parts of the brain. Ischemic stroke can be diagnosed if the duration of symptoms lasts more than 24 hours or by neuroimaging of the brain [3]. The main causes of ischemic stroke are hypertension, and the presence of plaque in the arteries called atherosclerosis [4].

In previous studies, genetic factors have been known to be involved in the occurrence of ischemic stroke. Genetic factors such as ACE gene polymorphism may influence by modulating other

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ischemic stroke risk factors, such as hypertension [5][6]. The ACE gene polymorphism was first discovered by Rigat et al. in 1990 and this gene located on the long arm of chromosome 17 at position 23.3 that consists of 26 exons and 25 introns [7][8]. The ACE gene polymorphism was marked by insertion (I allele) or deletion (D allele) at 287 base pairs (bp) on intron 16, which results in three different types of genotypes: II, ID and DD. Identification of intron 16 from the ACE gene resulted in a PCR product of 490 bp for I allele or 190 bp for D allele [8][9].

In the renin-angiotensin-aldosterone system, ACE has an important role in vascular remodelling, the formation of atherosclerosis and hypertension, which results in stroke. D allele can directly affect the risk of stroke by influencing other factors, such as vascular endothelium modification [10]. DD genotypes have an increased risk of ischemic stroke. However, many genetic and environmental risk factors may contribute to this complex condition [11].

In 2011, a research conducted in Yogyakarta found that the frequencies of DD genotype and D allele of the ACE gene polymorphism were higher in the hypertension group compared to normotension. Therefore, there was a correlation between ACE gene polymorphism and hypertension [12][13]. On the contrary, in 2017, there was no significant correlation between ACE gene polymorphism and hypertension in ischemic stroke patients [14].

In this study, it is proposed to determine the correlation between ACE gene polymorphism and hypertension in ischemic stroke patients in North Sumatra, that has never been done before. The results of this study are expected to provide education, the information in therapy and prognosis to patients.

2. Materials and Methods

2.1. Place and Year Work

The present study was carried out from July – September 2019. The ACE genes were obtained and approved by previous researcher Arina et al., [15]. The determination of PCR products from ACE gene polymorphism was carried out in Integrated Laboratory from the Department of Biomedical Science, Faculty of Medicine, Universitas Sumatera Utara.

2.2. Ethics Statement

The research was approved by The Health Research Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara. Written informed consent was obtained prior to the investigation.

2.3. Subjects

The study included a total of 78 stroke ischemic patients of both sexes, consisting of 43 hypertensive patients and 35 normotensive patients. Each individual was recruited form the General Hospital H. Adam Malik, North Sumatra, Indonesia. Inclusion criteria include the admission of stroke ischemic patient at age > 18 years old; the neurological deficits were confirmed in all cases by computerized tomography (CT) scan [15].

2.4. DNA Studies

The genotype of the ACE gene was determined by the polymerase chain reaction (PCR) using Thermal Cycler. The total volume of 25 μL PCR tube was used, consisting of 2 μL isolated DNA, 12.5 μL GoTaq® Green Master Mix, 1 μL forward primer, 1 μL reverse primer and 8.5 μL nuclease-free water. The ACE gene in DNA samples was amplified with an initial denaturation temperature of 95ºC for 1 minute, and continued with 32 cycles consisting of denaturation at 95ºC for 30 seconds, annealing at 59ºC for 60 seconds and extension at extension 72ºC for 90 seconds with final extension for 5 minutes. The PCR products were analyzed on a 2% agarose gels, stained with ethidium bromide and 50 bp DNA Ladder (Promega), showed one band of 490 bp corresponding to the homozygous (II), two bands of 190, and 490 bp for the heterozygous (ID), one band of 190 bp for the homozygous (DD). All DD genotypes were reamplified in order to avoid the probability of mistyping ID heterozygotes as DD homozygotes by using new primer sense specific for the insertion, and the result was negative.

2.5. Statistical Analysis

The values of the data on clinical characteristics of the subject groups were expressed in percentages. Allele and genotype frequencies in ischemic stroke subjects with history of hypertension and normotension were analysed using chi-square and Fisher exact test. All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25.

3. Results

The characteristics of the 43 hypertensive and 35 normotensive of stroke ischemic patients who met the criteria are summarized in **Table 1**. In this study, both of 39 men and women had an ischemic stroke, 33 patients at the age below 55 y.o, while 45 patients at the age above 55 y.o.

Table 1 Distribution of characteristic frequency ischemic stroke patients with hypertension and normotension

Variable	Category	Ischemic Stroke					
		Hypertension		Normotension		Total	
		N	%	N	%	N	%
Gender	Male	21	26.9	18	23.1	39	50
	Female	22	28.2	17	21.8	39	50
Age	≤ 55 y.o	16	20.5	17	21.8	33	42.3
	> 55 y.o	27	34.6	18	23.1	45	57.7

The distribution of stroke ischemic subjects based on genotype and allele are presented in **Table 2.** In this study, the majority of the subjects are distributed by the presence of I allele.

Table 2 Distribution of ACE gene polymorphism in ischemic stroke patients based on genotype and allele

ACE gene polymorphism	Category	N=78	(%)
Genotype	II	43	55.1
	ID	27	34.6
	DD	8	10.3
Allele	I (Insertion)	56	71.8
	D (Deletion)	22	28.2

Polymorphism was detected as a 490 bp PCR product corresponding to the insertion allele (I) and 190 bp corresponding to the deletion allele (D). Genotype and allele frequencies are shown in **Table 3**. Among 78 ischemic stroke patients with hypertension and normotension, the II genotype was found in 43 patients; 55.1% (25 patients with hypertension, 18 patients with normotension). The ID genotype was found in 27 patients; 34.6% (12 patients with hypertension, 15 patients with normotension). The DD genotype was found in 8 patients; 10.3% (6 patients with hypertension and 2 patients with normotension). Based on the allele, I allele was found in 56 patients; 71.8% (31 patients with hypertension, 25 patients with normotension). The D allele was found in 22 patients; 28.2% (12 patients with hypertension, 10 patients with normotension). Fisher exact test was done for genotype (p = 0.280), and chi-square was done for allele (p = 0.948), the results showed that the correlation was not significant respectively.

Table 3 Distribution of ACE gene polymorphism in ischemic stroke patients with hypertension and normotension based on genotype and allele

ACE gene polymorphism	Category	Ischemic Stroke						P valaue
		Hypertension		Normotension		Total		
		F	*%*	*F*	*%*	*F*	*%*	
Genotype	II	25	32	18	23.1	43	55.1	0.280*
	ID	12	15.4	15	19.2	27	34.6	
	DD	6	7.7	2	2.6	8	10.3	
Allele	I	31	39.7	25	32.1	56	71.8	0.948**
	D	12	15.4	10	12.8	22	28.2	

**Fisher Exact Test; **Chi-square Test*

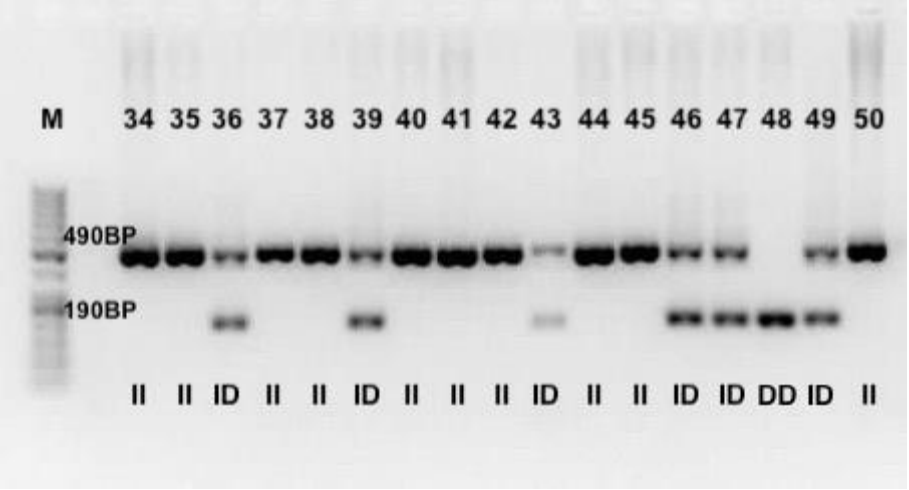

Figure 1 Electrophoresis results of ACE gene polymorphism on 2% agarose gel (samples number 34-50)

4. Discussion

The distribution of ACE gene polymorphism is still varied among several ethnicities in the world. In the study of Heidari et al., the distributions of ACE I/D polymorphism in Asians and other populations were differences between each other [16]. Studies of ACE gene polymorphism on homogeneous populations are expected in future studies.

In He et al. analysis, the II genotypes seen to have the highest ACE activities, but not in agreement with majority of the researchers [17]. Many studies have shown that D allele carrier has high plasma ACE level that lead to hypertension [6][8][18]. The study of Ljungberg et al. did not support that the ACE level associated with hypertension both in men and women. There was no correlation between ACE level and systolic, diastolic and pulse pressure as well [19]. However, Rasyid et al. found a significant correlation of ACE I/D polymorphism with pulse pressure [9].

Our analysis showed that both hypertension and normotension subjects, II carriers were highest frequencies followed by ID and lowest were DD. Based on DD genotype, the ratio between both subjects was 3:1 for hypertension. The distribution of the alleles in subjects was showed not much difference between the two groups. Hence, there was no difference between ACE gene polymorphism and hypertension in ischemic stroke population, and this result was consistent with Indrajaya's and Shaleh's [5][14]. There were more negative than positive studies from the effect of ACE gene in relation to ischemic stroke [7]. According to Indrajaya, ACE gene plays a role of around 5% in the development of ischemic stroke [5]. In 2017, study of Malueka et al. showed significant correlation between ACE gene polymorphism and ischemic stroke by measuring the functional outcome [20].

Inconsistencies were still visible between researchers about the ACE gene polymorphism in ischemic stroke patients. Therefore, further studies are still needed, for example, the relationship to other factors such as gene or protein expression that plays role in the process of atherosclerosis that lead to ischemic stroke.

5. Conclusion

Based on the allele and its genotype, there is no significant correlation between ACE gene polymorphism and hypertension in ischemic stroke patients. In this study, majority of the subjects are dominated by I allele.

6. Acknowledgement

The authors would like to express their gratitude to Department of Biomedical Science, Universitas Sumatera Utara and TALENTA Universitas Sumatera Utara for providing the opportunity for us to conduct this experimental study.

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