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Research Article

Genotype Distribution of Angiotensin Converting Enzyme insertion/deletion polymorphism in Sudanese Patients with Ischemic Stroke

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Abstract

There was evidence based on many researches that the D allele in the ACE polymorphism is associated with the risk of ischemic stroke. The aim of this study is to determine the genotypic distribution and the allelic frequency in patients with ischemic stroke. A total of 30 patients with ischemic stroke were enrolled in this study, blood samples were collected from all the patients in ethylene diamine tetra acetic acid (EDTA) containers. DNA was extracted using salting out method, and analyzed for ACE polymorphism using allele specific polymerase chain reaction. ACE genotypes frequencies among patients were DD 21(70%), ID 9(30%) and II (0%). D allele and I allele frequency was found to be (0.85) and (0.15) respectively. In conclusion, DD genotype of the ACE polymorphism was the most frequent among Sudanese patients with ischemic stroke, while II genotype was totally absent. This suggests that Sudanese individuals with II genotype might be at low risk to Ischemic stroke. Furthermore, D allele of the ACE polymorphism might confer increased risk to ischemic stroke.

Keywords: ACE polymorphism, D allele, ischemic stroke, Sudanese patients

INTRODUCTION

Stroke is a cerebrovascular disease which is considered the second commonest overall cause of death and the most important cause of disability among survivors. Cerebrovascular disease is the sixth commonest cause of an ongoing disease burden worldwide, and is expected to progress to the fourth place by 2020[1]. About 80% of all strokes are Ischemic strokes and over 65%of stroke deaths are reported from developing countries[2, 3,4].

The gene encoding angiotensin converting enzyme (ACE), which converts angiotensin I to the vasoconstrictor angiotensin II and inactivates the vasodilator bradykinin, is considered to be an important candidate of cerebral small-vessel disease (SVD) because of its' role in blood pressure regulation, regulation of vascular endothelial function, and smooth muscle proliferation and tone[5].

High ACE concentration was being found in the nigrostriatal pathway and basal gangelia when ACE was mapped within the brain by in vitro autoradiography and immunohistochemical studies[6].

The ACE gene is 21kb long, consisting of 26 exons and 25 introns and located in chromosome 17p23[7]. It is characterized by an insertion/deletion polymorphism based on the presence (insertion I) or absence (Deletion D) of a 287 base pair Alu repeat sequence in intron 16, resulting in three genotypes DD homozygote, II homozygote and ID heterozygote [5].

The DD genotype is associated with a two fold increase in plasma ACE activity over that of II genotype, with intermediate level of heterozygote ID[5].

The D allele of this polymorphism has been investigated as a potential susceptibility factor for ischemic stroke[8, 9].

The aim of this study was to determine the allelic frequency and the genotypic distribution for ACE

gene polymorphism in Sudanese patients diagnosed with ischemic stroke.

MATERIALS AND METHODS

This is a descriptive cross-sectional study conducted in Khartoum state during the period from March to September 2014. 30 patients diagnosed by a neurologist as having ischemic stroke and followed at Alsafa physiotherapy center, Ebrahim Malik hospital and Alribat hospital were recruited for this study.

Blood samples (3ml) were collected from patients- upon their informed consent- in ethylene diamine tetra acetic acid (EDTA) containers and genomic DNA was extracted by salting out method.

The Insertion/Deletion genotyping was performed by using Allele-specific polymerase chain reaction (PCR-TECHNE, TC412, UK) according to Rigat et al[10].

2 µL of the genomic DNA was amplified in a 25 µL reaction mixture containing 5.0 µL master mix (Maxime PCR pre mix kit (i-taq), iNtRON, Korea) and 1 µL of each of the forward (5'CTGGAG ACCACTCCCATCCTTTCT-3'), reverse primer (5'GATGTGGCCATCACATTCGTCAG AT-3') and internalprimer[(5'TGGGATTACAGGCGTGATACAG -3'] , and 15 µL sterile distilled water.

The amplification process consisted of initial denaturation at 94^{o}C/3min; 30 cycles each consist of 94^{o}C for 1 minute, 52^{o}C for 1 minute, and 72^{o}C for 1min; final extension at 72^{o}C for 5minutes.

PCR products were electrophoresed on 2% agarose gel containing ethidium bromide and analyzed under UV light. Three µL of 50 bp DNA ladder was applied with each batch of patients' samples.

A PCR product of 190 bp fragment was consistent with D allele, while a product of 490 bp fragment was consistent with I allele.

Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS). Frequency of the three genotypes was calculated in total patients and in patients with different stroke risk factors. Correlation between ACE I/D genotypes and qualitative variables were tested by chi-square test .The mean age was compared in the patients with the two allelic variants by independent sample T-test.

RESULTS

DNA was analyzed for ACE genotypes from venous blood of 30 ischemic stroke patients; 13(43.3%) were males and 17(56.7%) were females. The patients' ages ranged between 16 to 90 years (Mean ± SD: 56.8 ± 20.4).

According to the results of the molecular analysis two genotypes were determined: DD and ID. The frequency distribution of DD genotype was 70% (21/30) and that of ID was 30 % (9/30). None of the patients was found to have II genotype.

Hypertension has scored the highest frequency between the risk factors followed by smokers, diabetes and family history of thrombosis, patient history of thrombosis and cardiac disease consequently, (Table 1)

There was no statistically significant difference in mean age in patients with DD and those with ID genotypes (Mean±SD: 57.2±20.7 and 55.9±20.8 respectively, *P.value*: 0.878)

DD genotype was more frequent than ID genotype in both males and females. However, the correlation was not statistically significant (Table 2)

The distribution of the ACE genotypes among the different known stroke risk factor showed that, DD genotype was the most frequent. There was no statistically significant correlation between the ACE genotype and the different risk factors (Table 3).

The frequency of D allele in study subjects was 0.85, while the frequency of I allele was 0.15.

DISCUSSION

Recent studies revealed that the D allele of the ACE gene may be a potent risk factor for ischemic stroke and myocardial infarction in human[6, 11- 12].

In this study we determined- for the first time- the frequencies of the ACE DD, ID and II genotypes in Sudanese patients with ischemic stroke.

The present study showed that, the frequencies of DD and ID genotypes were 70% and 30% respectively; the genotype II was totally absent (0%) Our result was consistent with Tascilar *et al*[13] who performed ACE analysis in 97 patients with atherosclerotic stroke and Huriletemuer *et al*[14] who performed ACE analysis in 96 mongolian ischemic stroke patient with hypertension and both of their results reflected that DD>ID>II. These findings might suggest that individuals with II genotype might be at low risk of Ischemic stroke.

Some results of prior studies of ACE polymorphism in stroke patients have been inconsistent with our findings such as Indrajaya *et al*[15] who studied the polymorphism particularly in hypertensive ischemic stroke patients and their results reflected that, ID>II>DD. Miris Dikmen *et al*[16], S al Rajeh *et al*[17] and Prabhakar *et al*[18] results were ID>DD>II. Marshab *et al*[19] reported that II>DI>DD in Zambian patients with stroke.

The inconsistence results may be because of the difference in patients' selection criteria and ethnic variation.

In this study we found that hypertension scored the highest frequency among risk factors followed by smoking, diabetes and family history of thrombosis, patient history of thrombosis, and cardiac diseases consequently. Tuncer *et al* found that hypertension,

hyperlipidemia, smoking, coronary artery disease and diabetes scored the highest frequencies in order[20]. S al Rajeh *et al* reported that hypertension was the most common risk factor among their study population followed by diabetes, cardiopathy, previous transient ischemic stroke attack, smoking and cervical bruit[17.] Our findings agree with both the results that, hypertension was the most frequent risk factor among ischemic stroke patients.

The statistical analysis showed no statistically significant correlation between the ACE genotypes and mean age, gender, hypertension, diabetes, history of thrombosis, family history of thrombosis, cardiac disease and smoking; furthermore, there was insignificant association between the genotypes and overall risk factors of ischemic stroke. Similar results were reported by Hugh *et al* who found no association between genotype and age, sex, smoking history, diabetes or cholesterol level[21].

In the present study, the frequency of D allele in the study subjects was 0.85, while the frequency of I allele was 0.15. In previous researches D allele frequency was determined in different population with ischemic stroke. It was found to be 0.57 in Greek[22], 0.62 in Swedish[11], 0.47 in Japanese[23], 0.64 in Turkish patients with hypertension[20] and 0.352 in Kyrgyzstan with essential hypertension[24].

In comparison the above results, the D allele among Sudanese ischemic stroke patient has the highest frequency; the difference can be due to ethnic variations.

On the other hand, D allele among healthy Sudanese population was found to be 0.64 by Bayoumi *et al*[25]. This means that, the D allele frequency among Sudanese ischemic stroke patients is far higher than healthy populations, and this suggest the D allele as potent risk factor of ischemic stroke among Sudanese. This is more likely to be supported by Catto *et al*, Kostulas *et al*, Margaglione *et al* and Das *et al* [6, 11, 12, 26] who reported same findings.

A limitation of this study was the small sample size; we recommend that further study should be conducted in the future with increased sample size to well establish the correlation between ACE alleles and ischemic stroke.

CONCLUSION

DD genotype of the ACE polymorphism was the most frequent among Sudanese patients with ischemic stroke, while II genotype was totally absent. This suggests that Sudanese individuals with II genotype might be at low risk to Ischemic stroke. Furthermore, D allele of the ACE polymorphism might confer increased risk to ischemic stroke.

Table 1
Frequencies of stroke risk factors among study subjects

Risk factor		Frequency	Percent (%)
Hypertension	Yes	11	36.7
	No	19	63.3
Diabetic	Yes	3	10
	No	27	90
History of thrombosis	Yes	2	6.6
	No	28	93.3
Family history of thrombosis	Yes	3	10
	No	27	90
Cardiac disease	Yes	1	3.3
	No	29	96.7
Smokers	Yes	5	16.7
	No	25	83.3
Known risk factor	Yes	15	50
	No	15	50

Table 2
Correlation of ACE genotypes with gender

Gender	total	DD N (%)	ID N (%)	P. value
Male	13	11 (84.6)	2 (15.4)	
Female	17	10 (58.8)	7 (41.2)	0.229

Table3
The distribution of DD and ID genotype among the different risk factors.

Risk factor		N	DD N (%)	ID N (%)	*P.value*
Hypertension	Yes	11	6 (54.5)	5 (45)	0.160
	No	19	15 (78)	4 (21.1)	
Diabetic	Yes	3	2 (66.7)	1(33.3)	0.894
	No	27	19 (70.4)	8 (29.6)	
History of thrombosis	Yes	2	2 (100)	0 (0%)	0.338
	No	28	19 (67.9)	9 (32.1)	
Family history of thrombosis	Yes	3	2 (66.7)	1 (33.3)	0.894
	No	27	19 (70.4)	8 (29.6)	
Cardiac disease	Yes	1	1 (100)	0 (0%)	0.506
	No	29	20 (69)	9 (31)	
Smokers	Yes	5	5 (100)	0 (0%)	0.109
	No	25	16 (64)	9 (36)	
Known risk factor	Yes	15	10 (66.7)	5 (33.3)	0.690
	No	15	11(73.3)	4 (26.7)	

REFERENCES

1. Menken M, Munsat TL, Toole JF. The global burden of study: implications for neurology. J. Arch Neurol, 2000;57(3):418-20.
2. John Marx, Robert Hockberger, Ron Walls. Rosen's Medicine: concept and clinical practice, Stroke Vol.1 (7th ed) Mosby Elsevier, Philadelphia 2010. pp1333.
3. Bonita R, Mendis S, Truelsen T, Bogousslavsky J, TooleJ, Yatsu F. The global stroke initiative. J. Lancet Neurol, 2004; 3(7):391-3.
4. Feigin VL. Stroke epidemiology in the developing world. J. Lancet, 2005; 1;365(9478):2160-1.
5. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin Invest, 1990; 86(4):1343-6.
6. Catto A, Carter AM, Barrett JH, Stickland M, Bamford J, Davies JA, Grant PJ. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke, 1996; 27(3):435-40.
7. Hubert C, Houot AM, Corvol P, Soubrier F. Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. J. Biol Chem, 1991; 15;266(23):15377-83.
8. Sharma P, Carter ND, Barley J, Brown MM. Molecular approach to assessing the genetic risk of cerebral infarction: deletion polymorphism in the gene encoding angiotensin 1-converting enzyme. J. Hum Hypertens, 1994; 8(8):645-8.
9. Kalita J, Somarajan BI, Kumar B, Mittal B, Misra. A study of ACE and ADD1 polymorphism in ischemic and hemorrhagic

stroke. J. Clin Chim Acta, 2011; 18;412(7-8):642-6.
10. Rigat B et al. PCR detection of the polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). J. Nucl Ac Res, 1992; 20(6):1.433.
11. Kostulas K, Huang WX, Crisby M, et al. An angiotensin-converting enzyme gene polymorphism suggests a genetic distinction between ischemic stroke and carotid stenosis. Eur J Clin Invest 1999 ;29(6):478-83.
12. Margaglione M, Celentano E, Grandone E, et al. Deletion polymorphism in the angiotensin-converting enzyme gene in patients with a history of ischemic stroke. J. Arterioscler Thromb Vasc Biol, 1996; 16(2):304-9.
13. Tascilar N, Dursun A, Ankarali H, et al. Angiotensin-converting enzyme insertion/deletion polymorphism has no effect on the risk of atherosclerotic stroke or hypertension. J. Neurol. Sci, 2009; 285(1-2):137-141.
14. Huriletemuer H, Zhang C, Niu G, Zhao S, Hurile H. Gene polymorphisms and related risk factors in Mongolian hypertensive stroke patients. J. Neurosciences (Riyadh), 2010;15(3):184-189
15. Taufik Indrajaya. The role of ACE gene polymorphism on pathogenesis of ischemic stroke. J. Acta Med Indones, 2011; 43(3):152-7.
16. Miris Dikmen, Hasan Veysi Günes, Irfan Degirmenci, Gazi Özdemir, Ayse Basaran. Are the angiotensin-converting enzyme gene and activity risk factors for stroke?. J. Arq Neuropsiquiatr, 2006; 64(2-A):211-216.
17. Al Rajeh S, Awada A, Niazi G, Larbi E. Stroke in a Saudi Arabian National Guard community. Analysis of 500 consecutive cases from a population-based hospital. J. Stroke 1993; 24(11):1635-9.
18. Prabhakar P, De T, Nagaraja D, Christopher R. Angiotensin-converting enzyme gene insertion/deletion polymorphism and small vessel cerebral stroke in Indian population. Int J Vasc Med, 2014; 2014:305309.
19. Masharip Atadzhanov, Mwila HM et al. Association of the APOE, MTHFR and ACE Genes Polymorphisms and Stroke in Zambian Patients. Neurol Int, 2013; 5(4): e20.
20. Nese Tuncer, Serhan Tuglular, Gamze Kılıç, Ali Sazcı, Önder Us, İhsan Kara. Evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischaemic stroke. J. Clin Neurosci, 2006;13(2):224-7
21. Hugh S. Markus, Jackie Barley, Ros Lunt. Angiotensin-Converting Enzyme Gene Deletion Polymorphis A New Risk Factor for Lacunar Stroke but not Carotid Atheroma. J. Stroke, 1995; 26(8):1329-33.
22. Karagiannis A, Balaska K, Tziomalos K, Tokalaki-Nikolaidou L, Papayeoryiou A, Zamboulis C. Lack of an association between angiotensinconverting enzyme gene insertion/deletion polymorphism and ischaemic stroke. J. Eur Neurol, 2004;51(3):148-152.
23. Kario K, Kanai N, Saito K, Nago N, Matsuo T, Shimada K. Ischemic stroke and the gene for angiotensin-converting enzyme in Japanese hypertensives. J. Circulation 1996;93(9):1630-33.
24. Andrey Polupanov, Abdimutalip Halmatov, Oleg Pak, Tatyana Romanova. The I/D polymorphism of the angiotensin converting enzyme gene as a risk factor for ischemic stroke in patients with essential hypertension in Kyrgyz population. J. Türk Kardiyol Dern Arfl - Arch Turk Soc Cardiol, 2007;35(6):347-353.
25. Bayoumi RA, Simsek M, Yahya TM, Bendict S, Al-Hinai A, Al-Barwani H, Hassan MO. Insertion-deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among Sudanese, Somalis, Emiratis, and Omanis. J. Hum Biol. 2006;78 (1):103-8.
26. Das S, Roy S, Sharma V, Kaul S, Jyothy A, Munshi A. Association of ACE gene I/D polymorphism and ACE levels with hemorrhagic stroke: comparison with ischemic stroke. J. Neurol Sci, 2015;36(1):137-142.