International Journal of Biomedical Materials Research
2016; 4(2): 6-10
http://www.sciencepublishinggroup.com/j/ijbmr
doi: 10.11648/j.ijbmr.20160402.11
ISSN: 2330-7560 (Print); ISSN: 2330-7579 (Online)

ACE Insertion/Deletion (I/D) Polymorphism in Hypertensive Patients of Palestinian Population

Lamia'a Sobhi Saqer

Medical Sciences Department, University College of Science and Technology, Gaza Strip, Palestine

Email address:
lamiaa1912@yahoo.com

To cite this article:
Lamia'a Sobhi Saqer. *ACE Insertion/Deletion (I/D)* Polymorphism in Hypertensive Patients of Palestinian Population. *International Journal of Biomedical Materials Research.* Vol. 4, No. 2, 2016, pp. 6-10. doi: 10.11648/j.ijbmr.20160402.11

Received: September 5, 2016; **Accepted**: September 21, 2016; **Published**: October 10, 2016

Abstract: Hypertension is a risk factor for coronary heart disease, stroke, and renal failure; resulting from interaction of several genes with each other and with environmental factors. The renin-angiotensin system(RAS) plays an important role in the regulation of blood pressure. Angiotensin-converting enzyme (ACE) is an enzyme of the RAS. *ACE ID* gene polymorphism has been associated in the pathogenesis of cardiovascular diseases. The objective of this work was to determine the frequencies of the *ACE* gene alleles *D* and *I* and any associations to hypertension risk factors in Palestinian population. Genomic DNA was isolated from 293 subjects who have participated in a case–control study. *ACE* gene *ID* polymorphism was analyzed by polymerase chain reaction in 193 hypertension cases and 100 healthy controls. The frequency of ACE genotype were: *DD* 62.2%, *II* 6.7% and *ID* 31.1%,while as in control group the *DD* frequency is 54.0%, *II* 4. 0% and *ID* 42.0%. The frequencies of the *ACE D* and *I* alleles of the study population were 0.78 and 0.22for case group, 0.75 and 0.25 for control group respectively. There was no statistically significant difference between the groups with respect to genotype distribution. Furthermore, we did not find any significant difference in the frequency of *ACE ID* polymorphism in hypertension subjects when stratified by gender ($p = 0.61$).The results showed that there was no significant association between the *ACE ID* gene polymorphism and hypertension in Gaza strip.

Keywords: Angiotensin Converting Enzyme Gene, Hypertension, Polymerase Chain Reaction, Polymorphism

1. Introduction

Hypertension is a common disorder resulting from interaction of several genes with each other and with environmental factors such as obesity, dietary salt intake, and alcohol consumption [1]. Genetic factors like polymorphism of genes encoding various proteins [2]. As one of the genetic factor, polymorphisms of the angiotensin I-converting enzyme (*ACE*) gene [3]. ACE is an enzyme of the renin-angiotensin system (RAS) catalyzing the conversion of angiotensin I to angiotensin II which is involved in fluid and electrolytes balance. [4]. Angiotensin II is a potent vasoconstrictor. It also acts on the adrenal cortex, causing the release of aldosterone, which stimulates tubules in the kidneys, allowing them to reabsorb more sodium and water from the urine [5]. These effects act to increase the amount of fluid in the blood and to increase blood pressure [6], it also inhibits the release of acetylcholine and has pro-inflammatory effect [7].Angiotensin II mediates cell growth and proliferation by stimulating various cytokines and growth factors [6]. An *insertion/deletion* (*I/D*) polymorphism of the *ACE* gene has been identified in humans [7, 8].The *ACE* gene consists of 26 exons and spans 21 Kb on chromosome 17 [9], and the *I/D* polymorphism is characterized by the presence or absence of a 287 base pair *(Alu* repeat) fragment in intron 16 giving three possible genotypes (*DD*, *DI* and *II*) [8].

The physiological importance of the I/D polymorphism relies on the fact that the *DD* genotype is associated with increased circulating [7] and tissue ACE levels [10].

The highest serum ACE activity was in the *DD* genotype as opposed to *II* genotype in which the lowest activity was found [8]. The *ACE DD* genotype increases the plasma ACE concentration and the risk for numerous cardiovascular-renal diseased states, such as myocardial infarction, cardiomyopathy and diabetic nephropathy [11]. In addition,

characterization of the *ACE ID* gene polymorphism has also been suggested for decision making regarding antihypertensive treatment regimens [12]. To the best of our knowledge this is the first study to investigate the association of *ACE* gene *I/D* polymorphism and hypertension in Palestinians. So that, a case-control study carried out in Palestinian population to determine if this *ACE I/D* polymorphism is associated with an improved risk of hypertension in our population.

2. Materials and Methods

2.1. Study Population

This study included 193 hypertensive patients. All patients were recruited from Nasser Medical Complex and Ashifa hospital. Hypertension was defined by the use of one or more antihypertensive medications and/or a blood pressure not less than 140 mm Hg systolic or 90 mm Hg diastolic. Blood samples of 100 age and sex matched cases were collected to serve as external controls.

Data on all patients and controls were obtained from personal interviews with patients and/or guardians and medical records. All participants were informed about the study and their will to participate in this study was taken on predesigned questionnaire.

2.2. DNA Extraction and Polymerase Chain Reaction

DNA extraction was performed using Wizard DNA extraction kit (Promega, USA) following the manufacturer's instructions from fresh EDTA whole blood cells. Polymorphism in intron 16 of the *ACE* gene was assessed by polymerase chain reaction (PCR) under conditions that have been previously described by Salem and Batzer [13]. The specific segment of *ACE* gene was amplified by using the following oligonucleotide primers:

Forward: 5'CTGGAGACCACTCCCATCCTTTCT'3'

Reverse: 5'GATGTGGCCATCACATTCGTCAGATTT'3

which amplified 490 bp in case of homozygous *II* genotype,190 bp amplicon in case of homozygous *DD* genotype and both in case of heterozygous *ID* genotype (Fig. 1).

3µl (~150ng) of prepared DNA template was added to 7 µl master mix (Bioline, UK), and 0.5 µl of each primer (5 pmol) in 0.2 ml thin walled microfuge tube. PCR was performed in a thermal cycler (Biometra, Germany). The cycling conditions were: an initial denaturation for 1 min at 95°C, followed by 35 cycles of 15s at 95°C, 15s at 59°C, 10s at 72°C and an additional 10 min at 72°C for final extension. The quality of the isolated DNA was determined by running 5 µl of each sample on ethidium bromide stained 2.0% agarose gels and the DNA was visualized on a short wave U. V. transilluminator.

Figure 1. *Representative gel picture of ACE gene polymorphism by differential amplification of intron 16 of the ACE gene. Lane M :50bp ladder amplification product,490 bp for II,490 bp and 190 bp for ID, 190 bp for DD.*

2.3. Statistical Analysis

Observed frequencies of genotypes in hypertensive patients were compared to controls using chi-square. The chi-square test was used to verify whether genotype distributions were in Hardy-Weinberg equilibrium. Independent t test, ANOVA and Odd's ratio were also used. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with SPSS version 20.

3. Results

3.1. Study Population

A total of 193 hypertensive patients and 100 control subjects were included in this study. The patients comprised 91 (91/193; 47.15%) males and 102(102/193; 52.85%) females and the control subjects consisted of 59 (59/100; 59%) males and 41(41/100; 41%) females. The mean age of subjects was 55.4 ± 11.1 years. Furthermore, among

hypertensive patients 19.2 % were smokers and 80.8% nonsmokers. 78.5% of the participants were non-smokers (Table 1).

Table 1. *Frequency Distribution of Selected Demographic in Hypertensive Cases and Controls.*

Variable	Cases (n=193)	Control (n=100)	P-value
Age group			
> 50	148	39	0.000
≤ 50	45	61	
Gender			
Male	91	59	0.054
Female	102	41	
Smoking status			
Never	26	74	0.177
Ever	37	156	

3.2. Frequencies of Alleles and Genotypes

In this study, among 193 hypertensive cases we found the frequency of ACE *DD* genotype to be 62.2% (120/193), *II* 6.7% (13/193) and *ID* 31.1% (60/193), while as in general control (100) population the *DD* frequency is 54.0% (54/100), *II* 4.0% (4/100) and *ID* 42.0% (42/100). The association of *ACE I/D* polymorphism with the hypertensive cases was not found to be significant (p=0.15) (Table 2). The frequencies of the *ACE D* and *I* alleles of the study population were 0.768 and 0.232 respectively. The observed frequencies of 0.594 (n=174), 0.348 (n=102) and 0.580 (n=17) for the *DD*, *ID* and *II* genotypes were in Hardy–Weinberg's equilibrium (p=0.68). No significant differences were found between men and women (p=0.51) (Table 2). The present study showed similar *D* allele frequency in patients and healthy controls (78% and 75%, respectively) (Table 2).

However, there was an observed difference in the distribution of *ACE DD* genotype, being higher among patients compared to healthy volunteers.

Table 2. *Distribution of ACE genotypes and allelic frequencies of the study population.*

Study group	*ACE* genotypes			Total	Allelic frequencies		Total
	DD	*ID*	*II*		*D*	*I*	
Control n(%)	54 (54.0%)	42 (42.0%)	4 (4.0%)	100	150 (0.75)	50 (0.25)	200
Patients n(%)	120 (62.2%)	60 (31.1%)	13 (6.7%)	193	300 (0.78)	86 (0.22)	386
P-value		0.15			0.46		
Male	83 (55.3%)	61 (40.3%)	6 (4.0%)	150	227 (0.757)	73 (0.243)	300
Female	91 (63.6%)	41 (28.7%)	11 (7.7%)	143	223 (0.780)	63 (0.220)	286
P-value		0.61			0.51		

The distribution of genotype frequencies associations of *ACE* gene polymorphisms between male and female among control and hypertensive subjects are given in table 3. The results showed that among three genotypes within control group, *DD* genotype was more prevalent in male and female among study groups as compared to other two genotypes However, these prevalent are not statistically significant.

Table 3. *Distributions of ACE genotypes between male and female.*

ACE genotype	Control		Case	
	Male (n=59)	Female (n=41)	Male (n=91)	Female (n=102)
DD	30	24	53	67
ID	28	14	33	27
II	1	3	5	8
ID+II	29	17	38	35
p-value	0.202		0.316	

3.3. Genotype Frequencies of ACE Polymorphism in Cases and Controls

To check the association of *DD* genotype with hypertension, a comparison was made between the wild type (*DD*) and the variant types (*ID + II*) as shown in Table 4.

Moreover, the Odd's ratio of *ACE DD* genotype in hypertensive cases was found to be 1.4 times of control population when compared to other genotypes (Table 4).

70.5% of hypertensive cases diagnosed as Coronary Heart Disease (CHD) patients. The most frequent genotype among CHD patient was *DD* (62.5%) followed by *ID* (30.1%).

Table 4. *Comparison between various genotypes (D/D, I/D, I/I).*

ACE genotypes	Odds Ratio(CI 95%)	P-value
DD vs *II*	0.86 (0.21-2.2)	0.522
DD vs *ID*	1.6 (0.93-2.6)	0.088
ID vs II	0.44 (0.13-1.4)	0.175
DD vs ID+II	1.4 (0.85-2.2)	0.177
DD + ID vs II	1.7 (0.55-5.5)	0.346

4. Discussion

Systemic hypertension is a risk factor for various cardiovascular disease, mainly myocardial infarction, and stroke. Many studies showed that the hypertension is a polygenic disease, the genetic and environmental factors were also involved in the pathogenesis of hypertension. Genetic cause is one of the main predisposing factors, but the exact mechanism is unclear [13]. The RAS has been identified to be the most important of the endocrine systems that affect the control of blood pressure by many studies [14-16]. In the present study, a case – control study used to analyze the relationship between the *ACE* gene polymorphism and hypertension. The role of *ACE* gene in

essential hypertension remained unclear.

The frequency of *DD*, *ID* and *II* polymorphism among controls was: 54, 42 and 4%. The frequency among hypertensive patient was: 62.2, 31.1 and 6.7 % respectively (Table 2). On statistical comparison of *DD* genotypes between the controls and cases, the cases showed an increase of frequency. But no statistical significance was observed between the *ACE* gene *I/D* polymorphism and hypertension. This was in tune with other studies [16-18].Woo et al. [19] reported *ACE* gene was not observed to be associated with hypertension. On the other hand, in another study, the *D* allele was found to be associated with hypertension [20]. Moreover, Kenric et al. also found that the *D* allele was associated with hypertension in a group of African Americans [20].A significant association of the *ACE D* allele with hypertension in Egyptian, Indian, Chinese and Japanese population has been also reported [22-25]. Alternatively, many studies failed to expose such correlation as the study of Bhavani et al. revealed, they did not found a positive correlation between the *DD* genotype of *ACE* gene polymorphism and hypertension [26]. Another study on Turkish population also found no significant association between *ACE* gene polymorphism and hypertension [27]. This variation may be due to ethnic factors, since the distribution of the *ACE I/D* polymorphism is known to be different between various ethnic populations [28] and environmental backgrounds across the numerous populations [29]. No association reported with the *ACE I/D* polymorphism with hypertension in Lebanon, while the *D* allele frequency was high (77%) in Lebanese hypertensive patients [30].

The present study showed that the Odd's ratio of *ACE DD* genotype in hypertensive cases was found to be 1.4 times of control population this can be explained by the fact that *DD* genotype is associated with high ACE levels. ACE is responsible for the conversion of Angiotensin I to Angiotensin II; which is a potent vasoconstrictor, and a stimulator of aldosterone synthesis which causes increased blood pressure.

5. Conclusion

In conclusion, the present study did not show an association between *ID* polymorphism of *ACE* gene and hypertension in the Palestinian populations, with high frequency in *D* allele among study groups. However, more knowledge about the genetics of hypertension can be obtained by performing a study to detect the ACE levels and analyzed along with *ACE* polymorphism so the relationship can be investigated.

Acknowledgement

The author is grateful to all patients for their cooperation and gratefully acknowledge to the University Collage of Science and Technology-Medical Department for its facilitations to achieve this research.

References

[1] Pereira AC, Mota GF, Cunha RS, Herbenhoff FL, Mill JG, Krieger JE: Angiotensinogen 235T allele "dosage" is associated with blood pressure phenotypes. Hypertension 2003, 41: 25-30.

[2] Mourad JJ, Ducailar G, Rudnicki A, Lajemi M, Mimran A, Safar ME. Age-related increase of pulse pressure and gene polymorphisms in essential hypertension: a preliminary study. Journal Renin Angiotensin Aldosterone Syststem 2002; 3: 109-115.

[3] Higaki, J., Baba, S., Katsuya, T., Sato, N., Ishikawa, K., Mannami, T., Ogata, J. and Ogihara T. (2000) Deletion allele of Angiotensin-Converting Enzyme Gene increases risk of essential hypertension in Japanese men. Circulation 101, 2060-2065.

[4] Wang JG, Staessen JA. Genetic polymorphisms in the renin-angiotensin system: relevance for susceptibility to cardiovascular disease. European Journal of Pharmacology 2000; 410 (2-3): 289-302.

[5] Brewster UC, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. American Journal of Medicine. 2004; 116: 263–272.

[6] Carluccio M, Soccio M, De Caterina R. Aspects of gene polymorphisms in cardiovascular disease: the renin-angiotensin system. European Journal of Clinical Investigation. 2001; 31: 476–488.

[7] 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. Journal of Clinical Investigation 1990, 86: 1343–1346.

[8] Rigat B, Hubert C, Corvol P, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxipeptidase 1). Nucleic Acids Research 20: 1433, 1992.

[9] Hubert H, Houot Am, Corvol P, Soubrier F: Structure of the angiotensin I converting enzyme gene. Journal of Biological Chemistry 266: 15377–15383, 1991.

[10] Danser Ahj, Schalekamp Madh, Bax Wa, Van Den Brink Am, Saxena Pr, Riegger Gaj, Schunkert H: Angiotensin converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 92: 1387–1388, 1995

[11] So WY, Ma RC, Ozaki R, Tong PC, Ng MC, Ho CS,Lam CW, Chow CC, Chan WB, Kong AP. Angiotensin-converting enzyme (ACE) inhibition in type 2 diabetic patients: Interaction with ACE insertion/deletion polymorphism. Kidney International 2006; 69 (8): 1438-1443.

[12] Salem AH, Batzer MA. High frequency of the D allele of the angiotensin-converting enzyme gene in Arabic populations. BMC Res Notes 2009; 2 (99).

[13] Luft FC. Molecular genetics of human hypertension. Journal Hypertension 1998; 16: 1871-1878.

[14] Sipahi T, Budak M, Şen S, Ay A, Şener S. Association between ACE gene Insertion I/deletion D polymorphism and primary hypertension in Turkish patients of Trakya region. Biotechnology & Biotechnology Equepment. 2006; 20: 104-108.

[15] Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, Tsai JH. Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan. Nephrology Dialysis Transplantation 2000; 15(7): 1008-1013.

[16] Mondry A, Loh M, Liu P, Zhu AL, Nagel M. Polymorphisms of the insertion/deletion ACE and M235T AGT genes and hypertension: surprising new findings and meta-analysis of data. BMC Nephrology 2005; 6 (1): 1.

[17] Pamies Andreu E, Palmero Palmero C, Garcia Lozano R, Stiefel Garcia-Junco P, Miranda Guisado ML, Martin Sanz V, Villar Ortiz J, et al. The effect of the angiotensinogen M235T and the angiotensin-converting enzyme I/D polymorphisms on arterial hypertension and other cardiovascular risk factors. Medicina Clínica 1999; 113(5): 164-168.

[18] Dzida G, Sobstyl J, Puzniak A, Golon P, Mosiewicz J, Hanzlik J. Polymorphisms of angiotensin-converting enzyme and angiotensin II receptor type 1 genes in essential hypertension in a Polish population. Medical Science Monitor 2001; 7 (6): 1236-1241.

[19] Woo SW, Bang S, Chung MW, Jin SK, Kim YS, Lee SH: Lack of association between ACE and bradykinin B2 receptor gene polymorphisms and ACE inhibitor-induced coughing in hypertensive Koreans. Journal of Clinical Pharmacy and Therapeutics 2009, 34 (5): 561–7.

[20] He Q, Fan C, Yu M, Wallar G, Zhang ZF, Wang L, Zhang X, Hu R: Associations of ACE Gene Insertion/Deletion Polymorphism, ACE Activity, and ACE mRNA Expression with Hypertension in a Chinese Population. PLoS One 2013, 8 (10): e75870.

[21] Zivko M, Kusec R, Galesić K: Impact of angiotensin-converting enzyme gene polymorphism on proteinuria and arterial hypertension. Collegium Antropologicum 2013, 37: 765–770.

[22] Zarouk W, Hussein I, Esmaeil N, Raslan H, Reheim H, Moguib O, Emara N, Aly A, Hamed M. Association of angiotensin converting enzyme gene (I/D) polymorphism with hypertension and type 2 diabetes. Bratisl Lek Listy 2012; 113 (1): 14-18.

[23] Choudhury I, Jothimalar R, Patra A K. Angiotensin Converting Enzyme Gene Polymorphism and its Association with Hypertension in South Indian Population. Indian Journal Clinical Biochemistry 2012; 27(3): 265–269.

[24] ZhouY, Yan H, Hou XP, MiaoJL, Zhang J, Yin QX, Li JJ, Zhang XY, Li YY, Luo HL. Association study of angiotensin converting enzyme gene polymorphism with elderly diabetic hypertension and lipids levels. Lipids in Health and Disease 2013; (12): 187-190.

[25] Higaki J., Baba S., Katsuya T., Sato N, Ishikawa K, Mannami T, Ogata J, Ogihara T "Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita study," Circulation 2000; 101 (17): 2060–2065.

[26] Bhavani BA, Padma T, Sastry BKS, Krishna-Reddy N, Nausheen K. The insertion/deletion polymorphism of ACE gene increase the susceptibility to hypertension and/or diabetes. International Journal of Human Genetics 2005; 5: 247 – 252.

[27] Gunes HV, Ata N, Degirmenci I, Basaran A, Timuralp B, Dikmen M, Ustuner C, Kudaiberdieva G. Frequency of angiotensin-converting enzyme gene polymorphism in Turkish hypertensive patients. International Journal of Clinical Practice 2004; 58 (9): 838–843.

[28] Saab YB, Gard PR, Overall AD. The geographic distribution of the *ACE II* genotype: a novel finding. Genetics Research Journal 2007; 89: 259-267.

[29] Ono K, Mannami T, Baba S, Yasui N, Ogihara T and Iwai N. Lack of association between angiotensin II type 1 receptor gene polymorphisms and hypertension in Japanese. Hypertension Research 2003; 26: 131–134.

[30] Saab YB, Gard PR and Overall ADJ. The association of hypertension with renin–angiotensin system gene polymorphisms in the Lebanese population. Journal of the Renin-Angiotensin- Aldosterone System 2011; 12 (4): 588-94.