



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

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**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 0.22 for 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

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## **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).

3  $\mu$ l (~150ng) of prepared DNA template was added to 7  $\mu$ l master mix (Bioline, UK), and 0.5  $\mu$ l 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  $\mu$ l 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  $55.4 \pm 11.1$  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             |         |

**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).

### 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.

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.

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