Angiotensin Converting Enzyme I/D Polymorphism in Egyptian Patients with Type 2 Diabetes Mellitus

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Abstract

Objective: The deletion (D) allele of the angiotensin-I converting enzyme (ACE) is associated with higher ACE activity, it has been studied in various populations in relation to hypertension and type 2 diabetes mellitus (DM) with contradictory results. The objective of this study was to determine the ACE insertion/deletion polymorphism, genotype distribution in Egyptian patients with type 2 DM and to evaluate the possible association of ACE insertion/deletion polymorphism with hypertension in diabetic patients.

Subjects and Methods: A total of 48 patients with type 2 DM, 23 of them had hypertension and 21 healthy subjects age and sex matched with the patients, as control group were included in this study. Genotyping was performed by polymerase chain reaction (PCR).

Results: The frequency of DD genotype was significantly higher in diabetic patients compared to controls (\(p=0.008\)). The DD genotype (Vs DI and II genotypes) was associated with increased risk of diabetes (OR: 3.647, 95% CI: 1.235-10.773, \(p=0.016\)) and the D allele was more frequent in diabetic patients and was associated with increased risk of diabetes (OR: 3.939, 95% CI: 1.782-8.709, \(p<0.001\)). No significant difference in genotype distribution or allele frequency was detected between diabetic patients with and without hypertension.

Conclusion: We can conclude that a significant association between ACE gene I/D polymorphism and type 2 DM is present in Egyptian patients and the D allele is associated with increased risk for type 2 DM.

Key Words: ACE polymorphism – Diabetes mellitus – Hypertension.

Introduction

THE angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin system (RAS), modulating the synthesis of angiotensin II and inactivation of bradykinin [1]. Interindividual differences in serum ACE levels are due in part to the presence of an insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene, with the D allele being associated with higher ACE levels. Individuals homozygous for the deletion allele (DD) have serum ACE levels almost twice that of individuals homozygous for the insertion allele (II) [2]. Stephens and colleagues showed that the DD genotype leads to a higher ACE expression and activity and therefore might predispose individuals to type 2 DM and its complications [3].

Studies regarding the association between ACE gene I/D polymorphism and manifestations of metabolic dysregulation, including DM revealed contradictory results [4,5,6]. Some studies revealed an association between D/D polymorphism and type 2 DM [5,6]. However, it should be noted that other studies have reported the I allele of the ACE gene is associated with insulin resistance [7,8]. In addition, Jeng and colleagues [9] reported no difference in insulin sensitivity between those individuals with the D or I allele of the ACE gene.

The association of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism with hypertension has not been confirmed, with some studies have described a linkage between angiotensin converting enzyme (ACE) gene polymorphism and hypertension [10] and the DD genotype of the ACE gene has been reported to be a genetically predisposing factor for hypertension in a large general population [11] and other reports concluded that ACE polymorphism does not appear to have any significant association with blood pressure [12,13]. Inconsistencies may be due to the differences of background population characteristics.
**Aim:** To determine the ACE insertion/deletion polymorphism in Egyptian patients with type 2 diabetes mellitus and to compare the genotype distribution between subjects with type 2 diabetes and non-diabetic healthy control. We aimed also to evaluate the possible association of ACE insertion/deletion polymorphism with hypertension in diabetic patients.

**Patients and Methods**

The studied population consisted of 48 patients with type 2 diabetes mellitus, 23 of them had hypertension and 21 healthy subjects age and sex matched with the patients, as control group. The patients were recruited from Kasr El Aini hospital and outpatient clinic of Medical Services Unit at National Research Centre.

Each patient was subjected to detailed history taking, thorough clinical examination and laboratory investigations in the form of: Fasting blood glucose, total cholesterol, serum triglycerides, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) using Hitachi 912 and angiotensin converting enzyme gene I/D polymorphism by polymerase chain reaction method.

Blood pressure was determined after five minutes rest and systolic and diastolic blood pressures were calculated from mean value after two readings. Hypertension was defined as a mean systolic blood pressure (SBP) $\geq 140$ mmHg, mean diastolic blood pressure (DBP) $\geq 90$ mmHg or the patient is taking antihypertensive medication.

We excluded any patients with history or symptoms suggestive of secondary diabetes or secondary hypertension.

**Determination of ACE genotypes:**

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method (14 Marre et al., 1977). The purity of DNA was checked.

To determine the ACE-ID genotype; polymerase chain reaction (PCR) technique was performed with 20pm of each primer (sense primer: 5'-CTGGAGACCCTCCTTCT-3' and antisense primer: 5'-GATGGCCCATCTCTGG-3') in a final volume of 25 µl containing 100ng genomic DNA, 2mM MgCl$_2$, 10mM Tris-HCl (pH=8.3), 0.4mM of each dNTP and 1 unit of Taq polymerase. PCR was done with an initial denaturation at 94°C for 30s, annealing at 58°C for 1min and extension at 72°C for 2min followed by final extension at 72°C for 8min before the storage of the sample at 4°C. After electrophoresis in a 2% ethidium bromide-stained agarose gel, the PCR products were visualized under UV light. In the case of the deletion (D allele) and insertion (I allele), a 190bp fragment and a 490bp fragment were obtained, respectively. Therefore, there will be three genotypes after electrophoresis: A 490bp band (genotype II), a 190bp band (genotype DD) and both 490bp and 190bp band (ID genotype) (Figs. 1, 2).

![Fig. (1): Detection of I/D polymorphism of ACE gene in 2% agarose gel electrophoresis. Lanes 1 and 6 show homozygous DD genotype; lanes 2, 3, 4, 5 and 7 show heterozygous ID genotype of I/D polymorphism. M represents a Φ174bp DNA ladder.](image1)

![Fig. (2): Detection of I/D polymorphism of ACE gene in 2% agarose gel electrophoresis. Lanes 1, 2, 3, 7, 8 and 9 show homozygous DD genotype; lane 4 show homozygous II genotype; lanes 5 and 6 show heterozygous ID genotype of I/D polymorphism. M represents a Φ174bp DNA ladder.](image2)

**Statistical method:** The SPSS 10.0 for windows was used for data management and analysis. Quantitative data were presented as mean $\pm$ SD & qualitative data as frequency and percentage. Association of ACE genotypes and alleles with DM & different clinical and laboratory parameters were calculated using the $\chi^2$ test. Risk estimate was done by odds ratio. $p$ value was considered significant at $<0.05$.

**Results and Analysis of the Results**

Among 48 patients with type 2 diabetes, 25 were men and 23 were women, their mean age was $52.62\pm11.27$ years. The control group consisted of
21 subjects, 12 men and nine women their mean age was 46.4±14.3 years. Demographic, clinical and laboratory data of the patients are represented in Table (1).

Table (1): Demographic, clinical and laboratory data of the patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male, female)</td>
<td>25, 23</td>
</tr>
<tr>
<td>Age, years (mean±SD)</td>
<td>52.62±11.27</td>
</tr>
<tr>
<td>Family history of DM n (%)</td>
<td>42 (78.5)</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>23 (47.9)</td>
</tr>
<tr>
<td>SBP mmHg (mean±SD)</td>
<td>131.56±12.08</td>
</tr>
<tr>
<td>DBP mmHg (mean±SD)</td>
<td>82.6±7.36</td>
</tr>
<tr>
<td>Total Cholesterol, mg/dl (mean±SD)</td>
<td>198.78±53.14</td>
</tr>
<tr>
<td>Triglycerides, mg/dl (mean±SD)</td>
<td>167.8±97.27</td>
</tr>
<tr>
<td>LDL-c mg/dl (mean±SD)</td>
<td>127.25±42.07</td>
</tr>
<tr>
<td>HDL-c mg/dl (mean±SD)</td>
<td>40.94±10.18</td>
</tr>
</tbody>
</table>

SBP: Systolic blood pressure.  
DBP: Diastolic blood pressure.  
LDL-c: Low density lipoprotein cholesterol.  
HDL-c: High density lipoprotein cholesterol.

The frequency of DD genotype was significantly higher in diabetic patients compared to controls (p=0.008) (Table 2). The DD genotype (Vs DI and II genotypes) is associated with increased risk of diabetes (OR: 3.647, 95% CI: 1.235-10.773, p=0.016).

Table (2): Genotype distribution in diabetic patients and control group.

<table>
<thead>
<tr>
<th>ACE genotype</th>
<th>Diabetic patients (n=48)</th>
<th>Controls (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD n (%)</td>
<td>31 (64.6)</td>
<td>7 (33.3)</td>
<td>0.008*</td>
</tr>
<tr>
<td>DI n (%)</td>
<td>16 (33.3)</td>
<td>8 (38.1)</td>
<td>0.351</td>
</tr>
<tr>
<td>II n (%)</td>
<td>1 (2.1)</td>
<td>6 (28.6)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p significant.

As regard allele frequency, the D allele was more frequent in diabetic patients (Table 3). The D allele (Vs the I allele) is associated with increased risk of diabetes (OR: 3.939, 95% CI: 1.782-8.709, p<0.001).

Table (3): Allele frequency in diabetic patients and controls.

<table>
<thead>
<tr>
<th>ACE gene allele</th>
<th>Diabetic patients (n=48)</th>
<th>Controls (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D n (%)</td>
<td>78 (81.3)</td>
<td>22 (52.4)</td>
<td></td>
</tr>
<tr>
<td>I n (%)</td>
<td>18 (18.8)</td>
<td>20 (47.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

We divided the diabetic patients into two groups according to the presence or absence of hypertension: 25 patients without hypertension and 23 patients with hypertension. Comparing the frequency of DD genotype and DI genotype in both groups no significant difference was found (Table 4).

Table (4): Genotype distribution in diabetic patients with and without hypertension.

<table>
<thead>
<tr>
<th>ACE genotype</th>
<th>Diabetics without hypertension (n=25)</th>
<th>Diabetics with hypertension (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD n (%)</td>
<td>17 (68)</td>
<td>14 (60.9)</td>
</tr>
<tr>
<td>DI n (%)</td>
<td>7 (28)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>II n (%)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

p=0.48

By comparing allele frequency, there was no significant difference between both groups (Table 5).

Table (5): Allele frequency in diabetic patients with and without hypertension.

<table>
<thead>
<tr>
<th>ACE gene allele</th>
<th>Diabetics without hypertension (n=25)</th>
<th>Diabetics with hypertension (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D n (%)</td>
<td>41 (82)</td>
<td>37 (80.44)</td>
</tr>
<tr>
<td>I n (%)</td>
<td>9 (18)</td>
<td>9 (19.56)</td>
</tr>
</tbody>
</table>

p=0.84

Discussion

Type 2 diabetes is a complex disorder with strong substantial genetic contribution to the susceptibility to this disease. One possible genetic factor that has attracted much attention is the ACE gene, as previous studies have shown that ACE inhibitors may improve glucose utilization and suppress hepatic glucose production in subjects with type 2 DM [15]. The deletion (D) allele of (ACE) gene has been studied in various populations in relation to type 2 DM with contradictory results, Several studies have reported the association of the D allele of ACE gene with type 2 DM in various populations [5,6,16,17]. However, some studies have failed to show the association between the D allele and type 2 diabetes mellitus [18,19]. The discrepancies may be due to the racial differences or heterogeneity of the population sampling or possibly environmental factors may contribute to the negative associations.

Therefore, this study was initiated to determine the relationship of I/D polymorphism of the ACE gene to type 2 DM in Egyptian diabetic patients, we found that both the DD genotype & the D allele were strongly associated with type 2 diabetes and the presence of the D allele confers a significant increased risk for the type 2 diabetes. A recent
study conducted on Malaysian population concluded that the D allele of the ACE gene is associated with essential HTN and type 2 DM in Malaysian subjects [17] and a study of Stephens and colleagues demonstrated clearly an association between the ACE I/D common gene variant and type 2 diabetes in Caucasian population [3].

However, similar studies failed to establish such an association in other populations like the recent study on Tunisian type 2 diabetic patients whose results showed that there was no significant statistical difference between the genotype distribution and allele frequencies of the (I/D) polymorphism in all type 2 diabetic subjects compared to non-diabetic controls [18] and the study carried out by Grammer and his associates [19] on large number of Caucasian population, showed that: The genotypes ACE II, ID, DD occurred at similar frequencies in patients with type 2 diabetes mellitus compared to non-diabetic individuals also, There was no association of the ACE D allele with all type 2 diabetes mellitus patients, nor with newly diagnosed cases with diabetes [19].

Regarding the association of angiotensin converting enzyme (ACE) I/D polymorphism with hypertension, it has not been confirmed, with several studies have shown positive association [20, 21, 22] and other studies have shown negative association [12, 13]. Moreover, the relationship between I/D polymorphism and hypertension in the diabetic population has not been sufficiently studied, so, we aimed in this study to evaluate for the possible association between I/D polymorphism and hypertension in Egyptian diabetic patients. In the present study We could not find a significant association between DD genotype and hypertension in diabetic patients. By comparing allele frequency, the D allele was not found to be associated with increased risk of hypertension in diabetic patients, this is in agreement with the previous study in Turkey where there was no significant association between ACE gene polymorphism and hypertension in diabetics [23].

However, several studies support the hypothesis that the DD genotype & D allele have a strong association with hypertension in type 2 diabetes, including a study performed in Sweden and suggested that the DD genotype increases the risk of hypertension in diabetic patients [24] and a study conducted on Iranian diabetic population which concluded that the DD polymorphism in the ACE gene is independently associated with hypertension and diabetic patients with the DD genotype seem to be more prone to hypertension [25]. Also, the results of a recent study conducted on Malaysian population provided a strong evidence for the association of I/D polymorphism of ACE gene in Malaysian hypertensive and type 2 diabetic subjects and the D allele of the I/D polymorphism of ACE gene is proved to be an important genetic marker for essential hypertension and type 2 DM in Malaysian subjects [17].

In summary, this study showed a significant association between ACE gene I/D polymorphism and type 2 DM in Egyptian patients with type 2 diabetes mellitus and the D allele is associated with increased risk for type 2 DM. These results suggest that subjects carrying the D allele may benefit from earlier therapy with ACE inhibitors or angiotensin-II receptor blocker to reduce ACE activity and subsequently the future risk of type 2 diabetes and its associated complications.

Taking our results and the results of similar studies in other populations together we can observe that ethnic background appears to influence ACE gene I/D polymorphism globally, so, the analysis of the ACE gene polymorphism and activity within and across the major human groups with larger sample size appears to be useful in identifying the mechanisms contributing to the emergence of common chronic diseases such as hypertension and type 2 DM.

References


