The relationship between the insertion/deletion polymorphism of the ACE gene and hypertension in Iranian patients with type 2 diabetes

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Abstract

Background. Observations on the association between the ACE gene polymorphism and hypertension have been inconsistent, which might be due to ethnic and geographical variations. Moreover, the relationship between insertion/deletion (I/D) polymorphism and hypertension in the diabetic population has not been sufficiently studied. The aim of this study was to evaluate for the first time the possible association between I/D polymorphism and hypertension in an Iranian diabetic adult population.

Methods. A total of 82 consecutive patients with type 2 diabetes and hypertension (Group A) and 87 patients with type 2 diabetes but without hypertension (Group B) were included. Patients who had a history of hypertension before the onset of diabetes and those with findings suggesting secondary hypertension were excluded. The following variables were determined for each patient: age, sex, body mass index (BMI), diabetes duration and the drugs used, history of coronary artery disease and its complications, blood pressure (systolic and diastolic), fasting blood sugar (FBS), haemoglobin A1c (HbA1c), total cholesterol (Chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), plasma creatinine (Crt) and 24 h urine albumin excretion. Polymerase chain reaction (PCR) was used to detect the I/D alleles. Univariate (chi-squared and t-test) and multivariate (multivariate binary logistic regression with adjusted odds ratios) analyses were applied to determine the association between I/D polymorphism (with genotype II as reference) and hypertension. P < 0.05 was considered statistically significant.

Results. In univariate analysis, the groups were statistically similar in all variables except for diabetes duration (156.05 ± 73.54 months in Group A vs 121.74 ± 65.53 months in Group B; P = 0.002), Crt (1.04 ± 0.25 mg/dl in Group A vs 0.93 ± 0.23 mg/dl in Group B; P = 0.003), albuminuria (486.25 ± 484.60 mg/d in Group A vs 316.50 ± 459.56 mg/d in Group B; P = 0.021) and the frequency of DD genotype (27 cases in Group A vs 11 cases in Group B; P = 0.026). Multivariate logistic regression (using age, sex and BMI as clinically significant variables and diabetes duration, Crt, albuminuria and genotype as statistically significant variables) was then used to determine independent associations and adjusted odds ratios (OR). The DD genotype was the strongest independent predictor of hypertension [P = 0.029, OR = 3.122, 95% confidence interval (CI) = 1.127–8.647], followed by log (albuminuria) (P = 0.042, OR = 1.183, 95% CI = 1.006–1.391). Considering albuminuria as a categorical variable did not change the results significantly.

Conclusion. The DD polymorphism in the ACE gene is independently associated with hypertension in the diabetic population.

Keywords: albuminuria; ACE; diabetes; hypertension; polymorphism

Introduction

The pathophysiology of the association between essential hypertension and type 2 diabetes is poorly understood. Both conditions are thought to result from an interplay between multiple genetic and environmental factors [1,2]. One possible genetic determinant is the angiotensin-converting enzyme gene polymorphism, which gives three different genotypes II, ID and DD, where I and D stand for insertion and deletion, respectively [3].

Most (but not all) studies on the association of ACE gene polymorphism with type 2 diabetes have shown that the DD genotype is associated with increased risk of developing type 2 diabetes [4,5]. On the other hand, the association between I/D polymorphism and
hypertension is still controversial. Some studies have proposed that the DD genotype increases the incidence of essential hypertension [6–8], while others have not found a significant association [9–12]. The authors are aware of only two case-control studies, in Swedish [13] and Turkish populations [14], that have evaluated whether I/D polymorphism alters the risk of developing hypertension in diabetic patients. However, caution should be exercised in extrapolating an association found in one population to others. The presence or absence of an observed association in any ethnic, racial or geographic population may be related to a number of other factors including gene-gene and gene-environmental interactions.

We therefore opted for the first time to elucidate the possible association between the I/D polymorphism and hypertension in an Iranian diabetic adult population. Studies of this type can confirm whether the observed associations are consistent over different ethnicities and populations.

Subjects and methods

Study population

A total of 82 consecutive patients with type 2 diabetes and hypertension (Group A) and 87 patients with type 2 diabetes but without hypertension (Group B) who referred to our diabetes clinic between March 2005 and March 2006 were enrolled into the study. Patients who had a history of hypertension before the onset of diabetes and those with findings suggesting secondary hypertension such as renovascular, renal parenchymal, thyroid or adrenal diseases were excluded. All patients gave their written informed consent and the local ethics committee at Tehran University of Medical Sciences approved the study protocol.

The following variables were determined for each patient: age, sex, body mass index (BMI) according to Quetelet equation by using BMI = weight in kilograms/height in metres squared, diabetes duration and the drugs used (oral agents, insulin or both), history of coronary artery disease and its complications, blood pressure (systolic and diastolic), fasting blood sugar (FBS), haemoglobin A1c (HbA1c), total cholesterol (Chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), plasma creatinine (Crt) and 24h urine albumin excretion with albuminuria defined as an albumin excretion of ≥30 mg per day. The diagnosis of diabetes was based upon the WHO criteria [15]. Blood pressure was recorded by an electronic device on the right arm in the sitting position after 5 min rest. Systolic and diastolic blood pressures were calculated from two recordings with a minimal interval of 10 min. Hypertension was defined as a mean systolic blood pressure of ≥140 mmHg, mean diastolic blood pressure of ≥90 mmHg or taking antihypertensive medications. Patients with a plasma creatinine ≥1.5 mg/dl were excluded.

ACE gene I/D polymorphism

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method [16]. For amplification, a flanking primer pair was used and when it was necessary, a primer pair that recognizes the insertion specific sequence was also employed for confirmation of the specificity of the amplification reactions [17–19]. PCR was performed with 20 pm of each primer (sense primer: 5’-CTG GAG ACC ACT CCC ATC CCT TCT-3’ and antisense primer: 5’-GAT GTG GCC ATC ACA TTC AGT AGAT-3’) in a final volume of 25 μl, containing 0.5 μg genomic DNA, 2 mM MgCl₂, 10 mM Tris-HCl (pH = 8.3), 0.2 mM of each dNTP and 0.5 unit of Taq polymerase. PCR was done with an initial denaturation at 94 °C for 1 min. Then the DNA was amplified for 30 cycles with denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min followed by final extension at 72 °C for 8 min. 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 190 bp fragment and a 490 bp fragment were obtained, respectively. Therefore, there will be three genotypes after electrophoresis: A 490 bp band (genotype II), a 190 bp band (genotype DD) and both 490 bp and 190 bp band (ID genotype). Mistyping of ID heterozygote as D homozygotes may occur. Thus, each sample which had the DD genotype was submitted to PCR amplification using the forward 5’-TG CAC AGC GCC CCC TAC-3’ and the reverse 5’-TCC CCA GCC CTC CCA TGCA TAA-3’ primers with identical PCR conditions except for an annealing temperature of 67 °C. The reaction yielded a 335 bp amplicon only in the presence of an I allele and no product when the samples were homozygous for DD.

Statistical analysis

Data were analysed with SPSS statistical program (SPSS Inc., SPSS/PC+, Chicago: Illinois, USA). Results are presented as mean ± SD. All tests were two-sided and P < 0.05 was considered statistically significant. Significance of differences between group means was tested by the Student’s t-test and differences in proportions were assessed by the chi-square test. Multivariate binary logistic regression was used to determine the independent association between genotype (with the II genotype considered as reference) and hypertension. Age, sex and BMI as clinically significant variables as well as the statistically significant variables extracted from the univariate analysis were included in the multivariate analysis. Associations were expressed as adjusted odds ratio (OR) with 95% confidence interval (95% CI).

Results

Among 169 individuals included in the study, there were 86 (50.9%) men and 83 (49.1%) women. The mean age of patients was 56.90 ± 6.50 years (range: 43–76 years) and the mean BMI was 26.50 ± 4.93 (range: 16.05–43.70). Systolic/Diastolic blood pressures were 150.19 ± 12.81/90.02 ± 9.08 and 121.55 ± 9.66/77.49 ± 5.09 mmHg in groups A and B respectively (P < 0.001). There was no significant
The results of our study showed that the DD genotype is strongly associated with increased risk of hypertension in the diabetic population. The DD genotype (vs the II genotype) independently increased the risk of hypertension in diabetes 3.1-fold, while the ID genotype did not alter the risk significantly. This pattern suggests a recessive mode of inheritance in allele D of the ACE gene polymorphism, as also suggested in other studies [8].

By excluding patients with features suggesting secondary hypertension and those who had hypertension before their diabetes was diagnosed, we confined our study to diabetes-related (and possibly essential) hypertension. The pathogenesis of hypertension in diabetes is complex and involves several interrelated factors that collectively increase the propensity to develop hypertension. Multiple maladaptive pathways that involve renal sodium retention, increased sympathetic nervous system activity, vascular dysfunction and increased rennin–angiotensin–aldosterone system activity play roles in this process [20]. In our series, the diabetes-related component of hypertension was partly reflected in the independent association

### Table 1. Characteristics of the two groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 82)</th>
<th>Group B (n = 87)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>57.32 ± 6.11</td>
<td>56.51 ± 6.86</td>
<td>0.419</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>40:42</td>
<td>46:41</td>
<td>0.646</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>26.82 ± 4.96</td>
<td>26.21 ± 4.91</td>
<td>0.421</td>
</tr>
<tr>
<td>Systolic BP (mean ± SD)</td>
<td>150.19 ± 12.81</td>
<td>121.55 ± 9.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diastolic BP (mean ± SD)</td>
<td>90.02 ± 9.08</td>
<td>77.49 ± 5.09</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>History of CAD (%)</td>
<td>12 (14.63)</td>
<td>7 (8.05)</td>
<td>0.233</td>
</tr>
<tr>
<td>History of CCU admission (%)</td>
<td>11 (13.41)</td>
<td>7 (8.05)</td>
<td>0.330</td>
</tr>
<tr>
<td>Diabetes duration (month; mean ± SD)</td>
<td>156.05 ± 73.54</td>
<td>121.74 ± 65.53</td>
<td>0.002*</td>
</tr>
<tr>
<td>Oral agent(s); Insulin: Both (%)</td>
<td>62.16:25.68:12.16</td>
<td>79.27:15.85:4.88</td>
<td>0.052</td>
</tr>
<tr>
<td>FBS (mean ± SD)</td>
<td>192.45 ± 56.42</td>
<td>186.20 ± 60.20</td>
<td>0.493</td>
</tr>
<tr>
<td>HbA1c (mean ± SD)</td>
<td>8.64 ± 1.84</td>
<td>8.42 ± 1.93</td>
<td>0.445</td>
</tr>
<tr>
<td>TG (mean ± SD)</td>
<td>196.92 ± 86.32</td>
<td>188.31 ± 85.60</td>
<td>0.519</td>
</tr>
<tr>
<td>Chol (mean ± SD)</td>
<td>211.76 ± 39.25</td>
<td>208.57 ± 42.03</td>
<td>0.614</td>
</tr>
<tr>
<td>LDL (mean ± SD)</td>
<td>116.80 ± 31.00</td>
<td>114.25 ± 31.02</td>
<td>0.599</td>
</tr>
<tr>
<td>HDL (mean ± SD)</td>
<td>42.31 ± 9.47</td>
<td>43.87 ± 10.13</td>
<td>0.308</td>
</tr>
<tr>
<td>Crt (mean ± SD)</td>
<td>1.04 ± 0.25</td>
<td>0.93 ± 0.23</td>
<td>0.003*</td>
</tr>
<tr>
<td>24 h urine albumin (mean ± SD)</td>
<td>484.25 ± 484.60</td>
<td>316.50 ± 459.56</td>
<td>0.021*</td>
</tr>
<tr>
<td>Albuminuria (%)</td>
<td>51 (62.20)</td>
<td>32 (36.78)</td>
<td>0.003*</td>
</tr>
<tr>
<td>DD:ID:II ratio</td>
<td>27:33:22</td>
<td>11:51:25</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

*P < 0.05.
OR, odds ratio; CI, confidence interval.

The DD genotype increases the risk of hypertension in diabetic patients.

Both diabetes and hypertension are multifactorial diseases, with many different interacting parameters existing in their pathophysiology. The only way to assess the independent role of any potential determinant in these conditions is by eliminating the potentially confounding effects of other involved parameters. One appropriate analytical method is multivariate regression. Of the two studies mentioned above, regression analysis was used only in the latter. We repeated that study with the same method in an Iranian population, inspired by the fact that an association found in one population might not be true in others. In contrast to the mentioned study, which analysed obese and non-obese patients separately, we included BMI (along with age and sex as clinically significant variables) in the covariate list of regression analysis. Although the two groups in our study were similar in sex, age and BMI, we considered these variables as potential confounders in multivariate analysis. We also included in the regression model the variables with significant associations in univariate analysis (Crt, diabetes duration, albuminuria). Our results support those obtained by Bengtsson et al. [13].

In conclusion, we showed for the first time in an Iranian diabetic population that the DD polymorphism in the ACE gene is independently associated with hypertension. Clustering of risk factors associated with insulin resistance in hypertensive diabetic patients (compared to patients with diabetes alone) has been previously suggested [23]. The results of the present study along with those obtained by Bengtsson et al. [13] support the hypothesis that patients with both hypertension and type 2 diabetes are different from those with type 2 diabetes alone. Diabetic patients with the DD genotype seem to be more prone to hypertension based on these results.

**Conflict of interest statement.** None declared.

### References


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