ACE I/D Polymorphisms in patient with Diabetic Nephropathy and Non-Diabetic Nephropathy

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Abstract: Diabetic nephropathy (DN) is a complication that develops due to progressive diabetes, and it is an important cause of morbidity and mortality in both type I and type II diabetes. Many genes are known to contribute to the development of DN. Angiotensin converting enzyme (ACE) is the most common single nucleotide polymorphism and the ACE gene is thought to be crucial in the DN' pathogenesis. In this work, we aimed to evaluate the ACE gene I/D polymorphisms, which we think that it is effective in diabetic nephropathy. Ninety DN patients (30 Male/60 Female), and 45 Non-Diabetic Nephropathy patients (15 Male/30 Female) and 30 healthy individuals (control groups) were included in the work. DNA genotyping was performed by PCR amplification using primers specific for ACE I/D. I/D genotypes and allele distributions were significantly different in DN compared to non-DN patient (x^2=21.174, P=0.0000142). DN patients had a significantly higher prevalence than patients with non-DN patients according to D allele and DD genotype. In our studies, a significant relationship is observed between ACE I/D polymorphism and DN.

Keywords: Diabetic nephropathy, diabetes mellitus, angiotensin converting enzyme (ACE), polymorphism

1. Introduction

Diabetic nephropathy (DN) is one of the main microvascular complications in diabetes and is a crucial cause of end-stage renal disease (Rahimi et al, 2012; Aldukhayel, 2017). DN is a critical cause of morbidity and mortality for type I and type II diabetes mellitus (DM) patients (Li et al, 2019). In addition, environmental and genetic factors are probably effective in the development of DN (Rahimi, 2012). Some genes involved in the pathogenesis of DN have attracted the attention of researchers and various studies have been conducted (Khan, 2011; Rahimi, 2012; Li et al, 2019; Samsu, 2021). An important issue that draws attention in the studies is that the genes that may be involved in the pathogenesis of DN have not been studied together. However, many genes are thought to contribute to the development of DN. These genes also play a role in the regulation of familial hyperlipidemia, familial hypertension, blood pressure and other cardiovascular system diseases (Samsu, 2021). Almost one-third of patients with type 2 DM develop DN. Therefore, it is suggested that there may be a genetic predisposition in diabetic nephropathy.

Angiotensin II is stimulated by variables such as hyperglycemia, increased glomerular filtration, some inflammations and oxidative stress in diabetic patients (Ruggenenti et al, 2008; Vallon and Komers, 2011). DN begins to develop with various renal functional changes and increased glomerular filtration rates. After this development, it mostly manifests itself with microalbuminuria, which can progress to macroalbuminuria (Rahimi, 2012). Meanwhile, angiotensin converting enzyme (ACE) emerges as an important component of the renin-angiotensin system (RAS), which plays crucial role in blood pressure homeostasis through producing the vasoconstrictor peptide angiotensin II and inactivating the vasodilator peptides bradykinin and angiotensin (Movva, 2007). The ACE gene, which is one of the most frequently studied genes in the pathogenesis of DN, is in the long arm of the 17th chromosome and carries 26 exons and 25 introns. Many polymorphisms are known in the ACE gene, the most common of which is single nucleotide polymorphism (SNP). ACE polymorphism is one of topics investigated in DN. There are differences in the frequency of ACE alleles, and there are highly variable results in the literature regarding the role of the ACE (I/D) polymorphism in DN (Felehgari, 2011). For the first time, Rigat et al. (1990) reported the ACE I/D polymorphism in the 287 bp DNA sequence of the gene intron 16. In this study, we aimed to evaluate ACE gene I / D polymorphisms which are effective in Diabetic Nephropathy patients and non-Diabetic nephropathy patients.

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Material and Methods

Subjects

Ninety Diabetic Nephropathy patients (30 Male/ 60 Female), and 45 Non- Diabetic Nephropathy patients (15 Male/ 30 Female) and 30 healty invidiuals (15 Male/ 15 Female) for control group were included in the study.

Methods

DNA extracts from blood samples taken from EDTA-coated tubes were performed by commercial DNA extraction kit (Biorad, United Kingdom). After extraction, purity and quantification of DNAs were determined spectrophotometrically. PCR Master Mix (Biorad, United Kingdom) was used for amplification and reverse (GGATGGCTCTCCCCGCCTTCTCTC-R) and forward (GCCCTGCAGGTGTCTGCAGCATGT-F) primers were used to determine target gene expressions. The primers contain 20-80% guanine (G) - cytosine (C), especially from nucleotides that work like G and do not have G base at the 5 'end of the probe, C base is more than G in the selected sequence, temperature of melting temperature (Tm) is 65 between 85 ^{o}C. In our study, BioRad Real-Time (Biorad, United Kingdom) device and SYBR Green method were used. Real-Time PCR protocol; 95 °C 5 min., 95 °C 20 sec. (45 cycles), 60 °C 45 sec.

Statistical Analysis

The data obtained in result of the studies were analyzed using the Statistical Package (SPSS) program (version 20.0, SPSS Inc., Chicago, IL, USA). Results were reported as mean values ± standard deviation and standard error of mean (SEM). Non-parametric tests were used to calculate the Chi-square values of the data. Statistically significant value was accepted as $p<0.05$. 95% confidence interval was used in the studies.

Results

A total of 165 individuals were included in our work. Table 1 includes some characteristic and clinic findings data of DN patients, non-DN patients and control groups. When the age data were examined, a statistical difference was observed between the control group and the diabetic nephropathy patient group, and between the control group and the non-diabetic patient group ($p<0.05$). Moreover, there were a significant differences in fasting blood sugar, bun, creatinine and HbA1c between control groups both DN patients and non-DN pattients ($p<0.05$). However, there were no significant differences in gender and body mass index (BMI) between the three groups ($p>0.05$).

Table 1. The characteristic and clinic findings data of the study groups

Parameters	Control (n=30)	Hemodialysis	
		Diabetic Nephropathy (n=90)	Non-Diabetic Nephropathy (n=45)
Age (years)	54.1±6.4[a]	63.9±10.4[b]	58.1±11.6[c]
Gender (Male/ Female)	15/15	30/60	15/30
Body mass index (kg/m^2)	24.3±3.4	28.7±4.7	25.2±3.5
Fasting Blood Sugar (mg/dl)	89.12±9.74[d]	194.8 ±51.7[e]	90.2 ±16.7[f]
Bun (mg/dl)	8.7± 2.6[g]	59.1 ±19.3[h]	74.6 ±11.1[ı]
Creatinine (mg/dl)	0.6±0.3*	4.8 ±1.8*	11.3 ±1.9
Potassium (mmol/L)	3.8±0.4	4.6±0.8	5.4±0.6
Sodium (mmol/L)	138±19.3	138.4±5.5	139.8±3.4
HbA1c (mg/dl)	4.2± 1.1	7.81±1.13	5.17±1.12

a-b; a-c; d-e, d-f; g-h; g-ı, $p<0.05$

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In this work, ACE I/D gene polymorphisms were evaluated in 90 DN patients and 45 non-DN patients. The genotypes distribution and allele frequencies of DN patients and non-DN shown in Table 2 and Table 3. Between genotype distribution and some characteristic/clinic findings data were not significantly different between DN and non-DN patients ($p>0.05$). However, both the genotype and allele distributions of the ACE I/D polymorphism of DN patients were significantly different as compared with non-DN patients ($x^2=21.174$, $P=0.0000142$).

Table 2. Genotype distribution

Genotype	Non-Diabetic Nephropathy	Diabetic Nephropathy
II	4 (8.9%)	8 (9.0%)
ID	18 (40.0%)	35 (38.8%)
DD	23 (51.1%)	47 (52.2%)
Total Genotypes	45	90

Table 3. Allele frequencies

Allele	Non-Diabetic Nephropathy	Diabetic Nephropathy with type 2 DM
I	21 (23.3%)	47 (26.1%)
D	69 (76.7%)	133 (73.9%)
Total alleles	90	180

Discussion

Diabetic nephropathy (DN) is one of the critical adversity of diabetes and emerges as a significant cause of end-stage renal disease (ESRD) (Rahimi, 2012). It is thought that genetic factors may play a role in DN. There are studies reporting that the genotype DD or D allele seen in the ACE gene polymorphism is related with increased DN stimulation in type 2 DM patients. DM patients with nephropathy have higher ACE activity than diabetic patients without nephropathy (Rahimi, 2011; Li et al, 2019). In our study, ACE gene polymorphism which is thought to be effective in DN patients and non-DN patients was investigated. As result, both the genotype and allele distributions of the ACE I/D polymorphism of DN patients were significantly different as compared with non-DN patients. A similar study investigated the effect of ACE I/D genotypes on the progression of DN in 239 patients. In this study, patients were divided into two groups. Group1 ($n=99$, stable renal function) and Group2 ($n=140$, reduced renal function). The frequency of DD in group 2 was found to be significantly higher than group 1 (30.7%, 9.1%, respectively; $p<0.05$). No significant differences were observed in age, blood pressure, hemoglobin A1c levels or lipid profiles between ACE genotype groups, and it was suggested in this study that the DD genotype in the ACE gene may be an important risk factor for the progression of DN (Ha et al., 2003). In another study investigating the ACE polymorphism of diabetic nephropathy patients, 54 diabetic nephropathy patients and 74 healthy individuals were included. ACE polymorphism, genotype distribution and allele frequency were analyzed. As a result, the frequencies of the DD and ID genotypes, the frequency of the D allele were higher in those diagnosed with diabetic nephropathy, but the allele I was lower ($p<0.05$). In this study, ACE I/D polymorphism was associated with the onset of DN in T2DM patients (Yuying et al., 2016).

The ACE I/D polymorphism influences susceptibility to renal vascular modulation and DN with a direct effect on cellular hypertrophy. Literatures on the relationship between this polymorphism and DN have conflicting data. When the relationship of ACE I/D polymorphism with a possible risk for the development of DN is evaluated in studies published until 2018, a significant relationship is observed for ACE I/D polymorphism. The D allele appears to be a predisposing factor for DN in diabetic patients (Silveira, 2018).

In conclusion, diabetic nephropathy patients had a significantly higher prevalence than patients with Non - Diabetic Nephropathy according to D allele and DD genotype. In our studies, a significant relationship is observed between ACE I/D polymorphism and diabetic nephropathy.

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