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Investigation of the glutathione S-transferase gene M1/T1 and angiotensin converting enzyme gene I/D polymorphism in type 1 diabetic patients and possible association with diabetic microvascular complications

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ABSTRACT

Objectives: Glutathione S-transferase (GST) polymorphism may play a role in the etiology of type 1 diabetes, as GST is involved to detoxification of reactive oxygen radicals and synthesis of proinflammatory mediators. Genetic polymorphisms in the renin-angiotensin aldosterone system, including angiotensin converting enzyme (ACE) gene insertion-deletion (I/D) polymorphism, can affect the progression of diabetes and diabetic complications. In our study we aimed to investigate the GST and ACE gene I/D polymorphism in type 1 diabetic patients for comparison with population and relationships with diabetic complications.

Methods: A total of 116 type 1 diabetic patients were included to study. ACE polymorphism analyzed in the 71 subjects and GST polymorphism analyzed in the 62 subjects as control groups. Polymorphism of DNA samples was studied by PCR technique. Results compared with control groups and studied according the diabetic complications.

Results: ACE gene DD genotype and D allele ratio in the patient group were significantly higher than control group. GST T1 and GST M1 ratios were similar between patient and control groups. ACE genotype group distributions and GST M1/T1 genotype ratios were not different in terms of obesity, glycemic control, duration of diabetes and hypoglycemia frequency and not changed according to diabetic complications.

Conclusions: DD genotype and D allele ratio in diabetic patient group were found to be significantly higher and so a significant relationship was observed between and ACE I/D gene polymorphism and type 1 diabetes. On the other hand, it was observed that ACE I/D and GST gene polymorphism did not have any significant effect on diabetic microvascular complications.

Keywords: Type 1 diabetes, ACE gene polymorphism, GST gene polymorphism, diabetic complications

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Diabetes mellitus (DM), is a major public health problem that genetic and environmental factors play a role in the pathogenesis, is increasingly prevalent throughout the world [1]. According to IDF 2017 data, there are 451 million diabetic patients worldwide and it is estimated that the number of diabetic patients will reach 693 million by 2045 [2]. Type 1 diabetes accounts for 5-10% of all diabetes cases. The rate of β-cell damage leading to the disease may vary slightly between individuals, with being faster in infants and children and slower in adults [3]. Auto-reactive T cells and autoimmunity that caused by proinflammatory cytokines and reactive oxygen radicals, are responsible for pancreatic β-cell damage. Studies in monozygotic twins have reported that environmental components may play a role in 20-60% of pathogenesis [4].

Oxidative stress resulting from impaired balance between free oxygen radicals and antioxidants plays an important role in the etiopathogenesis of type 1 diabetes and in the development of diabetic complications [5]. Glutathione, which is a major antioxidant synthesized in many cells, plays a role in the neutralization of free radicals and in the immune response. Glutathione-s-transferase (GST) represents the enzyme family that catalyzes conjugation and elimination of the substrates such as free radicals or xenobiotics. Polymorphisms in the glutathione-s-transferase mu 1 (GSTM1) and glutathione-s-transferases theta 1 (GSTT1) genes lead to decreased enzymatic activation and homozygous deletion (null) in both genes leads to a complete loss of enzyme activity. GSTM1 and T1 polymorphism are thought to be associated with many diseases such as allergy, bronchial asthma, coronary artery disease and hypertension [4, 6]. Considering the role of reactive oxygen radicals and proinflammatory mediators in the pathogenesis of pancreatic beta cell damage, GST polymorphism may play a role in the etiology of type 1 diabetes, as GST is involved in processes such as detoxification of reactive oxygen radicals and synthesis of proinflammatory mediators.

Angiotensin converting enzyme (ACE) insertion-deletion (I/D) polymorphism occurs at the intron 16 of the ACE gene, localized on chromosome 17. While, ACE activity was highest in the presence of D allele, it has lowest activity with I allele. Circulating ACE levels in plasma are 30% higher in ID heterozygotes and 60% higher in DD homozygotes when compared to II homozygotes [7, 8]. Genetic polymorphisms in the renin-angiotensin aldosterone system (RAAS), including ACE I/D polymorphism, can affect the progression of diabetes and diabetic complications [9].

In our study, it was planned to investigate the GST and ACE gene I/D polymorphism in type 1 diabetic patients and to investigate possible association with diabetic microvascular complications.

METHODS

Patients and Protocol

A total of 116 patients between 18 and 65 years of age who were followed up in the endocrinology outpatient clinic with the diagnosis of type 1 diabetes for at least one year were included in this study. Patients with malignancy or chronic disease which is not associated with diabetes and pregnant patients were not included in the study. The control group was selected for age and gender matched patients without diabetes. ACE polymorphism analyzed 71 subjects and GST polymorphism analyzed 62 subjects were included in the study as separate control groups. Patients' files, including height, weight, HbA1c, daily insulin doses, hypoglycemic episodes, presence of diabetic microvascular complications, findings at the last visits were recorded and examined in detail. Hypoglycemic events are classified as minor hypoglycemia (often: more than once per week, rare: one per week or less) and major hypoglycemia (often: more than once a year, rare: one per year or less). Patients, those with urinary microalbuminuria/creatinine levels between 30 and 100 mg were classified as microalbuminuria, those over 100 mg as macroalbuminuria, those with a glomerular filtration rate of less than 50 ml/min were classified as chronic renal insufficiency. Presence of retinopathy was assessed according to the final retinal examination. The presence of neuropathy was evaluated with complaints of the patient and physical examination findings in detail. Clinical and laboratory parameters recorded patients' blood samples was taken for genetic analysis and stored at -20 °C in EDTA.

Genetic Analysis

Genomic DNA isolation was performed using the DNA isolation kit (Dr. Zeydanli Life Sciences, Turkey) procedure from blood samples. The ACE

gene I/D polymorphism of DNA samples was detected by PCR technique. F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT 3 ', R: 5' GAT GTG GCC ATC ACA TTC GTC AGA T-3' primary was used for the ACE I/D polymorphism, F: 5 '-TGG GAC CAC AGC GCC CGC CCG CCA CTA C-3', R: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3 ' was used as insertion region specific primer to confirm DD genotype. A 30 µL volume PCR mix was prepared for amplification of the ACE gene region from DNA samples. For each sample, the mixture contained 2.5 µl of 10X Taq polymerase buffer, 0.5 µl of dNTP mixture of 10 mM, 2 µl of MgCl2 of 25 mM, 1 µl of 10 pmol of primary pair, 0.2 µl of Taq polymerase enzyme (Bioron) and 20 µL of ddH2O. Approximately 3 µl (100 ng) DNA sample was added to the mix. The samples in the ACE DD genotype were confirmed by PCR for the second time to avoid false DD genotyping. PCR conditions consisted of denaturation at 94 °C for 1 minute followed by denaturation at 94°C for 5 minutes, 1 minute at 57 ° C for connection (63 °C to confirm the DD genotype), extension for 1 minute at 72 °C for 35 cycles and the final elongation was performed at 72 °C for 10 minutes. For PCR amplification, samples were run on 2% agarose gel electrophoresis, stained with ethidium bromide and then photographed. In the agarose gel, 190 base pairs (bp) amplification bands were observed in the samples with DD genotype, 490 and 190 bp with ID genotype and 490 bp in samples with genotype II (Fig. 1). In the second PCR analysis for DD confirmation, amplification band of 335 bp was observed in the samples with the insertion band.

In isolated DNA, the multiplex PCR method was used to determine the polymorphism of the GST M1 and GST T1 genes. Primers for forward 5'-TTCCT-TACTGGTCCTCACATCTC-3 'and reverse 5'-TCACCGGATCATGGCCAGCA-3' for GST-T1 polymorphism, forward 5'-GAACTCCCT-GAAAAGCTAAAGC-3 'and reverse 5'-GTTGGGCTCAAATATACGGTGG-3' primers for GST-M1 polymorphism and for the purpose internal controls, the primers albumin forward 5'-GCCCTCT-GCTAACAAGTCCTAC-3 'and reverse 5'-GCCC-TAAAAAGAAAATCCCCAATC-3' were used. 15, 16th PCR conditions were performed as 35 cycles of 1 min at 94 ºC (denaturation), 1 min at 58 ºC (annealing), 1 min at 72 ºC (elongation) and finally 10 min at 72 ºC (last extension) after first denaturation at 94 ºC for 5 minutes. 350 bp for albumin, 219 bp for GSTM1 and 459 bp for GSTT1 were expected to occur. The resulting products were run on ethidium bromide containing 2% agarose gel for evaluation. First, a control 350-bp product line of the albumin gene, which indicates whether the reaction has taken place, was checked. If there is no control product, PCR is repeated. The genotype determination was made as a

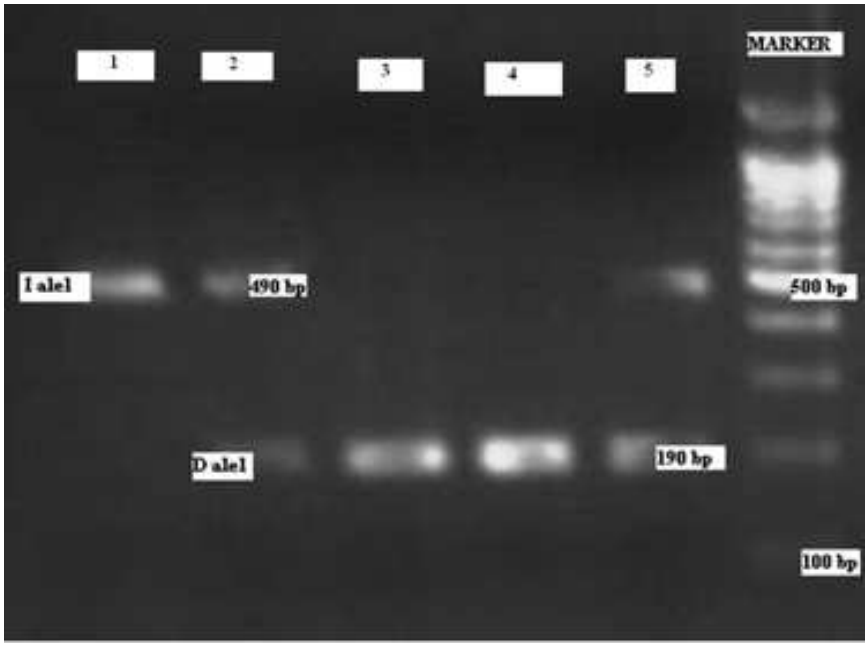

Fig. 1. Agarose gel image of PCR products made with ACE primers. The final well is a 100 bp DNA ladder (Marker), first well is case with genotype II, 2nd and 5th wells with DD genotype, 3rd and 4th wells with ID genotype.

'positive genotype' if the GSTT1 gene (459 bp) and the GSTM1 gene (219 bp) were present, or as a 'negative (null) genotype' indicating the presence of homozygote deletion.

Statistical Analysis

Demographic characteristics, HbA1c, age (years), gender, diabetes duration (years), BMI (Kg/m2) are summarized with descriptive statistics, including mean and standard deviation (values are given in parentheses) for continuous variables and frequency and percentages for categorical variables. Chi-square, Kruskal-Wallis and Mann-Whitney U variance analysis tests were used where they are convenient in the rates of D allele and I alleles in DD, ID, and II genotype groups in ACE gene polymorphism, in evaluation of GST T1 and M1 polymorphism-positive patients' rates in patients' classified according to patient / control, glycemic control and microvascular complications. Pearson and Spearman's correlation coefficients were calculated to assess the associations between variables. All analyses were performed using IBM SPSS 20™ (SPSS Inc., Chicago, IL, USA) and *p* values < 0.05 were considered statistically significant.

RESULTS

The mean age of the 116 patients was 31 ± 9.4 years and the female / male ratio was 72/44. For ACE gene polymorphism 74 control subjects and for GST polymorphism 62 control subjects were included in the study. The age and sex distribution of the patients and control groups are given in table 1. The mean duration of diabetes was 12.5 ± 8.1 years and the HbA1c level was 75 mmol/mol (9.0 ± 1.9%) in the patients. In the control group, ACE gene D/I genotype distributions were 35.1% DD, 41.9% ID and 23% II. In type 1 diabetes patients, the DD genotype was 44%, the ID genotype 45.7% and the II genotype 10.3%. The D allele rate was 66.8% in the patient group and 56.1% in the control group. The weighted genotypes and alleles were the same in both groups (ID genotype and D allele). On the other hand, DD genotype and D allele

Table 1. Clinical characteristics and genotypes of diabetic and healthy control group

		Patients n = 116	Control I n = 74	Control II n = 62	*p* value	Odds Ratio	% 95 CI
Age (years)		31.0 ± 9.4	33.0 ± 5.1	31.0 ± 5.6	0.10[a] 0.93[b]		
Gender (F/M)		72/44	37/37	38/24	0.10[a]/0.92[b]		
DD (years)		12.5 ± 8.1					
Weight (kg)		61.5 ± 9.5					
BMI (kg/m^2)		22.3 ± 3.3					
HbA1c %		9.0 ± 1.9					
mmol/mol		75					
ACE gene D/I genotype	DD %	44.0	35.1		**0.04**	1.45	0.79-2.64
	ID %	45.7	41.9			1.17	0.65-2.10
	II %	10.3	23.0			0.35	0.15-0.80
ACE gene D/I allel rates	D all %	66.8	56.1		**0.04**	1.49	0.97-2.27
	I all %	33.2	43.9		**0.04**	0.63	0.41-0.97
GST genotype	T1 %	78.4		75.8	0.73		
	M1 %	37.9		45.2	0.35		

DD = Diabetes duration, BMI = Body Mass Index, ACE = Angiotensin converting enzyme, D/I = deletion/insertion, GST = Glutathione S-transferase

Table 2. Relationships between I/D polymorphism allelic frequencies and disease releated characters in diabetic patients

		n	DD %	ID %	II %	*p* value	D allel %	I allel %	*p* value
BMI (Kg/m^2)	Normal	21	44.2	45.3	10.5	0.98	69.7	33.3	0.96
	High	95	42.9	47.6	9.5		69.8	33.2	
HbA1c *	Low	14	35.7	57.1	7.1	0.65	64.3	35.7	0.67
	High	102	45.1	44.1	10.8		67.2	32.8	
DD (years)	Short (<10)	56	44.6	39.3	16.1	0.11	64.3	35.7	0.61
	Long (>10)	60	43.3	51.7	5.0		69.2	30.8	
Minor hypoglycemia	Rare	55	40.0	50.9	9.1	0.56	65.5	34.5	0.58
	Often	61	47.5	41.0	11.5		68.0	32.0	
Major hypoglycemia	Rare	101	44.6	45.5	9.9	0.90	67.3	32.7	0.68
	Often	15	40.0	46.7	13.3		63.3	36.7	
Nephropathy	No	73	43.8	43.8	12.3	0.64	65.8	34.2	0.74
	Yes	43	44.2	48.8	7.0		68.6	31.4	
Neuropathy	No	91	47.3	42.9	9.9	0.39	68.7	31.3	0.21
	Yes	25	32.0	56.0	12.0		60.0	40.0	
Retinopathy	No	78	44.9	44.9	10.3	0.96	67.3	32.7	0.80
	Yes	38	42.1	47.4	10.5		65.8	34.2	

DD = Diabetes duration, *Low = < 7% or 53 mmol/mol, High = >7% or 53 mmol/mol

ratio in the patient group were significantly higher and II genotype and I allele ratio were significantly lower than control group (p = 0.04) (Table 1). When GST gene polymorphism was examined, T1 positivity was 78.4% and 75.8% and M1 positivity was 37.9% and 45.2% in the patient and control group respectively. There was no difference between GST T1 and GST M1 ratios between patient and control groups.

ACE gene polymorphism genotype group distributions were not different in obese and non-obese patients, in patients with and without good glycemic control, in patients with diabetes duration of less ten years or more than 10 years, and in those who had frequent hypoglycemic episodes. Similarly, when assessed for microvascular complications, in terms of genotype distribution and allele ratios, no significant difference was found according to patients with or without nephropathy, retinopathy and neuropathy (Table 2). GST gene M1/T1 genotype ratios were not different in terms of obesity, glycemic control, duration of diabetes and hypoglycemia frequency. T1 and M1 ratios were similar to those in patients with or without nephropathy, neuropathy and retinopathy (Table 3). There was no statistically significant difference between ACE genotype distribution and allele ratios and nephropathy levels. In patients with microalbuminuria, macroalbuminuria and chronic renal insufficiency, ACE genotype distributions and D/I alleles ratios were not different. Similarly, the GST T1 and GST M1 genotype ratios were similar in the nephropathy subgroups (Table 4).

DISCUSSION

Oxidative stress is defined as oxidative damage re-

Table 3. Relationships between glutathione-S-transferase genotype and disease releated characters in diabetic patients

		n	T1 %	*p* value	M1 %	*p* value
BMI (kg/m^2)	Normal	21	85.7	0.37	38.1	0.97
	High	95	76.8		37.9	
HbA1c*	Low	14	78.6	0.99	28.6	0.44
	High	102	78.4		39.2	
DD (years)	Short (< 10)	56	80.4	0.63	39.3	0.77
	Long (> 10)	60	76.7		36.7	
Minor hypoglycemia	Rare	55	76.4	0.60	36.4	0.74
	Often	61	80.3		39.3	
Major hypoglycemia	Rare	101	80.2	0.23	34.8	0.059
	Often	15	66.7		60.0	
Nephropathy	No	73	79.5	0.73	38.4	0.90
	Yes	43	76.7		37.2	
Neuropathy	No	91	80.2	0.38	38.5	0.82
	Yes	25	72.0		36.0	
Retinopathy	No	78	79.5	0.70	35.9	0.52
	Yes	38	76.3		42.1	

DD = Diabetes duration, *Low = < 7% or 53 mmol/mol, High = >7% or 53 mmol/mol

Table 4. ACE I/D polymorphism allelic frequencies and glutathione-S-transferase genotype according to the diabetic nephropathy risk

		No Nephropathy n = 73	Micro-albuminuria n = 19	Macro-albuminuria n = 11	Chronic Renal Failure n = 13	*p* value
ACE	DD %	43.8	47.4	45.5	38.5	0.97
	ID %	43.8	47.4	45.5	53.8	
	II %	12.3	5.3	9.1	7.7	
	D allel %	65.8	71.1	68.2	65.4	0.95
	I allel %	34.2	28.9	31.8	34.6	
GST	T1 poz %	79.5	68.4	90.9	76.9	0.53
	M1 poz %	38.4	31.6	45.5	38.5	0.90

ACE = Angiotensin converting enzyme, D/I = deletion/insertion, GST = Glutathione S-transferase

sulting from the disruption of the balance between reactive oxygen radicals and antioxidants. Oxidative stress plays an important role in the pathogenesis of diabetes and in the development of diabetic complications as in malignancies, cardiovascular diseases, kidney diseases and neurodegenerative diseases [1]. Glutathione is one of the most effective antioxidants to prevent cell damage caused by environmental toxins and reactive oxygen radical accumulation. Free oxygen radicals and xenobiotics are neutralized by glutathione through the glutathione-s-transferase enzyme. Glutathione-s-transferase enzyme polymor-

phisms are known to increase or decrease susceptibility to many diseases [10].

In a study comparing type 1 diabetic patients with healthy controls, the GSTT1 null genotype was found to be twice as frequent in the diabetic group, but there was no significant difference in the incidence of GSTM1 between the two groups [11]. In another study, GST T1 null genotype was found to be statistically more frequent in type 1 diabetic group compared to healthy volunteers, and the result was concluded that GST T1 null genotype increases the frequency of type 1 diabetes by 4.2-fold [10]. In the study conducted by Hori *et al*., the relationship between GST and type 2 diabetes was investigated, it is concluded that GST T1 and GST M1 null genotypes was an independent risk factor for the development of type 2 diabetes [12]. The current results were described as GST null genotypes may be associated with low antioxidant enzymatic activity [13]. On the other hand, in a study conducted in type 1 diabetic patients between 0-35 years of age and healthy volunteers in the Caucasian community, the GST M1 null genotype was associated with protection from type 1 diabetes [4]. In our study, at the aspect of GST gene polymorphism, in patient and control groups, T1 positivity were 78.4% and 75.8%, and M1 positivity was 37.9% and 45.2% respectively. GST T1 and GST M1 rates were not different between the groups. The prevalence of GST M1 null genotype frequency (45.2%) in the healthy control group was similar to that in European countries (38-62%) [14].

DD genotype was 44.0%, ID genotype was 45.7% and II genotype was 10.3% in type 1 diabetes patients included in our study. The D allele ratio was 66.8% in the patient group and 56.1% in the control group. The weighted genotypes and alleles were observed to be identical in both groups (ID genotype and D allele). On the other hand, the DD genotype and D allele ratio in the patient group were significantly higher than the control group, and the II genotype and I allele ratio were significantly lower than the control group. In the study of Hibbert *et al*., the homozygous genotype II was found to be significantly lower in long-term type 1 diabetic patients [15].

There was no significant difference between ACE gene polymorphism and glutathione-s-transferase polymorphism, obesity, duration of diabetes, glycemic control and hypoglycemia rates in type 1 diabetic patients included in our study.

Diabetes mellitus is the most common cause of chronic kidney disease. It occurs in 20-40% of diabetic patients and may progress to end-stage renal failure. Many environmental, genetic and epigenetic factors are responsible for the pathogenesis of diabetic nephropathy [3, 7]. Studies show that renal tubulointerstitial injury plays an important role in the pathogenesis and progression of diabetic nephropathy. Glutathione-s-transferases are thought to play a role in the pathogenesis of diabetic nephropathy as they are present in high concentrations in renal tubules [16]. In our study, T1 positivity was 76.7% in the diabetic nephropathy patient group while 79.5% in the non-nephropathy group and M1 positivity was 37.2% in the nephropathy patient group while 38.4% in the non-nephropathy group. The results were not statistically significant.

In the literature, the results of studies investigating the role of ACE I/D gene polymorphism in the pathogenesis of diabetic nephropathy are controversial [7]. In a meta-analysis evaluating the results of twelve studies, ACE I/D gene polymorphism was associated with end-stage renal failure in type 2 diabetic nephropathy patients, the same relationship was not observed in type 1 diabetic patients [17]. In another meta-analysis, data from 17 case-control studies in the literature were evaluated and ACE I/D polymorphism was associated with nephropathy in type 1 diabetes, particularly in Asian populations [18]. In our study, the distributions of genotype groups in diabetic nephropathy group was examined, the percentages of DD, ID and II were 44.2%, 48.8% and 7.0% respectively, and in the non-nephropathy group the percentages were 43.8%, 43.8% and 12.3% respectively. There was no statistically significant difference between the two groups in terms of ACE gene polymorphism genotype distributions.

In the study by Yang *et al*. [19], they found that GST T1 homozygote deletion is create a risk for end-stage renal failure in diabetic patients, but the same risk was not observed in GST M1 homozygote deletion. When the patients included in the study were grouped according to the presence of nephropathy, microalbuminuria, macroalbuminuria and chronic renal failure, no statistically significant differences were observed between the groups in terms of ACE gene polymorphism and glutathione-s-transferase

polymorphism.

Diabetic retinopathy is the most common cause of new blindness in the 20-74 age group in developed countries. In addition to the duration of diabetes, poor glycemic control, concomitant nephropathy, hypertension, and dyslipidemia increase the risk of developing retinopathy [3]. In the study conducted by Hovnik *et al*., the GSTM1 gene was found to be associated with diabetic retinopathy. As a result of this study, the authors suggested that GSTM1 gene deletion is protective for diabetic retinopathy [20]. Bekris *et al*. [4], found that GSTM1 gene deletion is protective against the development of type 1 diabetes in children aged 14-20 years. It is thought that the protective effect of GSTM1 null genotype on diabetic retinopathy in type 1 diabetic patients could be explained by the upregulation of the other antioxidant enzymes such as manganese superoxide dismutase with GSTM1 gene deletion [21]. In our study, while T1 positivity was 76.3% in the diabetic retinopathy group, it was 79.5% in the non-retinopathy group and, while M1 positivity was 42.1% in the retinopathy group, it was 35.9% in the non-retinopathy group. There was no statistically significant difference between the groups.

No significant association was found between ACE I/D polymorphism and the presence of diabetic retinopathy in patients with type 1 diabetes in the Caucasian race [22]. In our study, DD, ID and II genotype groups' percentages of ACE gene polymorphism were 42.1%, 47.4% and 10.5% in diabetic retinopathy group, respectively, whereas it was 44.9%, 44.9% and 10.3% in non-retinopathy group respectively. No statistically significant difference between the two groups in terms of ACE gene polymorphism genotype distributions was observed.

Diabetic peripheral neuropathy is asymptomatic up to approximately 50% in diabetic patients [3]. In type 1 diabetic patients, good glycemic control can effectively prevent diabetic peripheral neuropathy and cardiac autonomic neuropathy [23]. Babizhayev *et al*. [24] suggested that polymorphism in genes encoding antioxidant enzymes might cause genetic susceptibility in type 1 diabetic patients. In our study, while T1 positivity was 72.0% in the diabetic neuropathy patient group, it was 80.2% in the non-neuropathy group and while M1 positivity was 36.0% in the neuropathic patient group and 38.5% in the neuropathy group, respectively. There was no significant difference between the groups. Peripheral nerve damage in diabetic patients has been associated with polyol accumulation, AGE products and oxidative stress. One of the mechanisms involved in pathogenesis is that hyperglycemia stimulates NADP oxidase by raising tissue angiotensin levels by RAAS activation, thereby increasing oxidative stress and vascular injury [25]. In our study, the percentages of DD, ID and II genotype groups of ACE gene polymorphism were 32.0%, 56.0% and 12.0% in the diabetic neuropathy group, respectively, and 47.3%, 42.9% and 9.9% in the non-neuropathy group, respectively. No statistically significant difference in terms of polymorphism genotype distributions was observed.

In diabetic patients with DD genotype, serum ACE levels are higher and increased RAS activity in diabetic patients is associated with increased ACE levels. The high rate of DD genotype in the patient group in our study supports this. It is possible that increased RAS activity may increase the progression of nephropathy, and some of the cited studies have suggested a relationship between the occurrence of complications, particularly nephropathy, and the DD genotype. In our study, however, DD genotype was not found to be associated with complications. This situation shows that other factors besides the increased RAS activity also affect the complications.

CONCLUSION

In this study, the relationship between GST genotype and ACE I/D gene mutation and age, gender, duration of diabetes, presence of hypoglycemia and diabetic microvascular complications such as nephropathy, retinopathy and neuropathy in type 1 diabetic patients was investigated. As a result of our study, DD genotype and D allele ratio in diabetic patient group were found to be significantly higher than that of healthy control group, and II genotype and I allele ratio were found to be significantly lower in diabetic patients, and a significant relationship was observed between and ACE I/D gene polymorphism and type 1 diabetes. On the other hand, the same relationship was not detected in the GST gene polymorphism. However, as a result of our study, it was observed that ACE I/D and GST gene polymorphism did not have any significant effect on diabetic mi-

crovascular complications. Considering the different results on the subject, studies in the literature suggest that studies involving a higher number of patient groups will be important for the detection of susceptibility genes and for assessing the risk of complications in type 1 diabetic patients in which complex factors play a role in etiopathogenesis.

Ethics Approval and Consent to Participate

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Authors' Contribution

Study Conception: SC,; Study Design: SC,; Supervision: CE,; Funding: SC; Materials: SC; Data Collection and/or Processing: SC, ÖÖG, PŞ, AD; Statistical Analysis and/or Data Interpretation: SC, MK; Literature Review: SC, PŞ; Manuscript Preparation: SC, NK, PŞ and Critical Review: NK, CE.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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