Serum Paraoxonase Levels and PON1(192) Polymorphism in Type 2 Diabetes Mellitus Patients

Tip 2 Diyabetli Hastalarda Serum Paraoksonaz Düzeyleri ve PON1(192) Polimorfizmi

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ABSTRACT

Objective: Paraoxonase-1 (PON1) is an HDL-associated enzyme implicated in the pathogenesis of atherosclerosis by protecting lipoproteins against peroxidation. PON1 has two genetic polymorphisms both due to amino acid substitution, one involving glutamine and arginine at position 192 and the other leucineand methionine at position 55. Our study aimed to compare the effect of PON192 polymorphism and PON1 activity in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.
Material and Methods: 50 patients with T2DM and 30 non-diabetic controls were included in this study. The PON192 polymorphism was studied by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). Paraoxonase activity was measured by spectrophotometric method.
Results: The frequencies of the QQ, QR and RR genotypes were found as 36.5 and 14% in type 2 diabetes patients and 26.67, 46.66, 26.67% in control subjects, respectively. The paraoxonase activity was detected at lower levels in diabetics (102.0 ±38.9) than in control subjects (158.1±63.7). PON1192 RR homozygotes had significantly higher PON activity than QR and QQ genotypes among control and type 2 diabetes patients ($p<0.005$).
Conclusion: In comparing the activities of three genotypes of the control and type 2 diabetic groups; all activities were found significantly lower in diabetics. In conclusion, we suggested that paraoxonase activities are affected by PON1 genetic variability in patients with type 2 diabetes mellitus and controls.

Key Words: Paraoxonase, Diabetes Mellitus, polymorphism, HDL

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ÖZET

Amaç: Paraoksonaz-1 (PON1), peroksidasyona karşı lipoproteinleri koruyarak aterosklerozun patogenezinde rol oynayan HDL ile ilişkili bir enzimdir. PON1'in, biri 192. Pozisyonda glutamin ve arginin; diğeri de 55. Pozisyonda lösin ve metyonin olmak üzere her ikisi de iki aminoasit yer değişiminden dolayı olan iki genetik polimorfizmi vardır. Çalışmamız, tip 2 diyabetes mellituslu (T2DM) hastalarda ve diyabetik olmayan kontrollerde PON192 polimorfizmi ve PON1 aktivitesinin etkisini karşılaştırmayı amaçlamıştır.
Gereç ve yöntemler: Bu çalışmaya, 50 T2DM hasta ve 30 diyabetik olmayan kontrol alınmıştır. PON192 polimorfizmi, polimeraz zincir reaksiyonu/restriksiyon parça uzunluk polimorfizmi (PCR/RFLP) ile çalışılmıştır. Paraoksonaz aktivitesi, spektrofotometrik yöntemle ölçülmüştür.
Bulgular: QQ, QR ve RR genotiplerinin sıklıkları tip 2 diabetli hastalarda sırasıyla %36, %50 ve %14, kontrol deneklerde ise %26.67, %46.66 ve %26.67 olarak bulunmuştur. Paraoksonaz aktivitesi, kontrol deneklerine (158.1±63.7) göre diyabetiklerde (102.0 ±38.9) daha düşük saptanmıştır. Kontrol ve tip 2 diabetli hastalar arasında PON1192 RR homozigotlar, QR ve QQ genotiplere göre anlamlı derecede daha yüksek PON aktivitesine sahipti ($p<0.005$).
Sonuç: Kontrol ve tip 2 diyabetik gruplarının üç genotipinin aktiviteleri karşılaştırıldığında tüm aktivitelerin, diyabetiklerde anlamlı ölçüde daha düşük olduğu bulunmuştur. Sonuç olarak, tip 2 diabetes mellituslu hastalar ve kontrollerde paraoksonaz aktivitesinin, PON1 genetik çeşitliliğinden etkilenebileceğini ileri sürmekteyiz.

Anahtar Sözcükler: Paraoksonaz, DiabetesMellitus, polimorfizm, HDL

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INTRODUCTION

Human paraoxonase (PON) is a Ca^{+2}- dependent 45-kDa glycoprotein that binds to high-density liopoprotein (HDL) and has been shown to prevent oxidation of low-density lipoprotein (LDL) in vitro (1). PON1 hydrolyzes organophosphate (OP) insecticides and nerve gases and is responsible for determining the selective toxicity of these compounds in mammals (2,3).

Paraoxonase (PON1) is a member of proteins that also includes PON2 and PON3 genes for which are clustered in tandem on the long arms of human chromosome 7(q21.22) (4,5). PON1 has two common polymorphisms in the coding region: leucine (L) / methionine (M) at position 55 and glutamine (Q) / arginine (R) at position 192 (6). The polymorphisms affect the hydrolytic activity of the PON1 isoenzymes with respect to certain substrates, such as paraoxon and lipid peroxides. The PON1 192 polymorphism has major effect on serum PON1 activity (5-7).

The enzyme has been identified as an independent, genetic risk factor for vascular disease, particularly in T2DM patients (8). Serum PON1 activity is decreased in subjects who have had a myocardial infarction and in subjects with type 1 or type 2 diabetes (9,10). This evidence has given rise to speculation that decreased PON1 activity is associated with the increased lipid peroxidation found in diabetes and could therefore contribute to excess mortality from coronary heart disease (CHD) (11,12,13). Although the pathophysiological mechanism has not been clarified, it is considered to be linked to the anti-oxidant role of PON. This is of particular relevance to diabetic patients where higher risk of oxidative stress is suggested to contribute to greatly increased incidence of vascular disease and other complications (8).

Due to the fact that there are limited data regarding these parameters in the Turkish type 2 diabetics, we studied the relationship between activities of PON1 and PON192 polymorphism with T2DM patients and healthy controls.

MATERIALS AND METHODS

Diabetic patients and controls

Fifty unrelated Turkish patients with T2DM (mean age= 60.8 ,S.D: 9.4 year, sex ratio M/F = 20/30) who fulfilled the World Health Organization criteria for DM (WHO 1999) from Department of International Medicine of Istanbul University Cerrahpasa Medical Faculty were selected for the study.

An additional control group of nondiabetic healthy subjects with a normal glucose tolerance test (n=30, mean age=54.2 (S.D: 8.1) year, sex ratio M/F=16/14) was selected during routine health examination. They were unrelated to the diabetic patients.

Written, informed consent was obtained from the subjects who agreed to participate in the study and the study had the approval of our local ethics committee. The study was conducted in compliance with the Helsinki Declaration.

Blood Sampling and DNA extraction

Venous blood was obtained from the control and diabetic subjects between 9 and 10 AM after a 12-hour fasting. Serum was obtained by low-speed centrifugation. Sera were stored at (-20°C) before further analysis. Genomic DNA was extracted from whole blood using a commercial kit. Plasma concentrations of HDL cholesterol, total cholesterol, triglycerides were measured by Central Laboratory of Istanbul University Cerrahpasa Medical Faculty.

Analysis of paraoxonase activity

The paraoxonase activity was measured by spectrophotometric method (14). Before the analysis of PON1 activity, serum from controls and diabetics was preincubated with 5 mmol/Leserine for 10 min at room temperature to inhibit serum butyrylcholinesterase activity, which is elevated in diabetes and would otherwise interfere with the determination of paraoxonase activity in serum from individuals with diabetes.

PON1 activity was measured by adding serum to 1 ml Tris–HCL buffer (100 mmol/L, pH 8.0) containing 2 mmol/L $CaCl_2$ and 5.5 mmol/Lparaoxon (0,0-diethyl-0-*p*-nitrophenylphosphate; Sigma, Poole, UK). The rate of generation of *p*-nitrophenol was determined at 37°C, with the use of a continuously recording spectrophotometer at 405 nm.

Paraoxonase genotype determination

PON1 genotypes were determined following PCR according to previously published protocols (15). For the 192 polymorphism sense primer 5'-TATTGTTGCTGTGGGACCTGAG-3' and antisense primer 5'-CACGCTAAACCCAAA TACATCTC-3' which encompass the 192 polymorphic region of the human PON1 gene were used. The PCR reaction mixture contained 100 ng DNA template, 0.5 mM of each primer, 1.5 mM $MgCl_2$, 200 mM 4dNTP's and 1 U Taq DNA polymerase. After denaturing the DNA for 5 min at 94°C, the reaction mixture was subject to 46 cycles of denaturing for 1 min at 95°C, 1 min annealing at 60°C and 1 min extension at 72°C for the 192. The 99 bp PCR product was digested with 8 U *Alw*I restriction endonuclease (New England Biolabs, Cambridge) overnight at 37°C and the digested products separated by electrophoresis on 3% agarose gel and visualised using ethidium bromide. The R-genotype contains a unique *Alw*I restriction site which results in 66 and 33 bp products and the Q-genotype will not cut allowing the PON1 192 genotype to be determined.

Statistical analysis

All data are presented as the mean ± SD. A comparison of variables between two groups was performed using the one-way ANOVA. Genotype freqencies were estimated by chi-square test. Variables with a non-gaussian distribution (PON1 activity) were compared using the Mann-Whitney *U* test. $P<0.05$ were considered significant.

RESULTS

Total eighty cases (50 patients and 30 controls) were analysed in terms of some biochemical parameters, paraoxonase activities and PON1 polymorphism.

Some characteristics and biochemical parameters of T2DM patients and control subjects are shown in Table 1. The glucose, total cholesterol, triglyceride levels were higher in T2DM patients compared with the control subjects ($p<0.05$).

PON activities in non-diabetic and T2DM populations are shown in Table 2. It is found that paraoxonase activity was lower in diabetics than in control subjects ($p<0.05$).

Fifty patients and 30 controls were genotyped for PON1 polymorphism. The genotype distribution of the paraoxonase gene Gln-Arg 192 polymorphism in patients and controls are shown in Table 3. The frequencies of the QQ, QR and RR genotypes were found as 36, 50 and 14% in type 2 diabetes patients and 26.67, 46.66, 26.67% in control subjects, respectively. There was no significant difference between genotype frequencies in patients and controls (Table 3) ($p>0.05$).

The relationship between PON1(192) genotypes and PON activity of the T2DM and control group were shown in Table 4. When we compare the PON activities of 3 genotypes for control and T2DM, RR, QQ, QR genotypes have lower enzyme activities at diabetic patients according to controls (102.0±38.9 and 158.1±63.7, respectively). PON1(192) RR homozygotes had significantly higher PON activity than QR and QQ genotypes among control and T2DM patients ($p<0.005$).

Table 1: Characteristics of the T2DM patients and control subjects

	Control mean ± SD	Type 2 DM patients mean ± SD
*Gender,n (male/female)	(16/14)	(20/30)
*Age, (yr)	54.2 ± 8.1	60.8 ± 9.4
**Cholesterol (mg/dL)	186.4± 25.7	234.5 ± 26.23
**Trigliseride (mg/dL)	118.6 ± 31.8	202.6 ± 72.1
**Glucose (mg/dL)	98.2± 8.6	162.1 ± 44.2
**HDL-K (mg/dL)	59.9 ± 9.9	47.8 ± 13.4
**LDL-K (mg/dL)	117.8 ± 25.2	139.2 ± 30.4

* p>0.05
**p<0.05

DISCUSSION

There is a large variation in serum PON activity among individuals and the reason of this variation has been identified as the Q/R polymorphism of the PON gene. PON activity in patients with T2DM has been shown to be low (16). The present study further supports the hypothesis that the paraoxonase 192 Q/R polymorphism is related to low PON1 activity in T2DM. In our results the HDL levels were found to be lower, LDL was found to be higher in patients with T2DM according to control group (Table 1).

Table 3: PON1 genotype distribution in non-diabetic and T2DM populations.

PON1(192)	T2DM patients n (%)	Control n (%)	p
RR	7(14)	8(26.67)	>0.05
QR	25(50)	14(46.66)	

Table 4: PON1 activity in non-diabetic and T2DM populations according to their PON1 (192) genotype.

	Control			T2DM patients			
PON1(192) genotypes	RR	QR	QQ	RR	QR	QQ	P
PON activity (IU/l)	226.0±38.3	164.7± 37.2	78.6±15.1	160.1±29.1	114.6±14.2	61.9±21.5	<0.005

Lipid metabolism is important in the development of atherosclerosis. The oxidative modification of LDL is involved in the initiation of atherosclerosis. Both purified PON and HDL-associated PON inhibit LDL oxidation in vitro. Therefore, HDL-associated PON may protect LDL against oxidation in vivo (16, 17). Low PON1 activity could have significant effect on the ability to metabolize lipid- peroxides in T2DM has been suggested previously (18). This may be a reason for the increased lipid-peroxidation often reported in T2DM (10).

PON has been shown to be associated with the development of atherosclerosis(19). In vitro studies suggest that the paraoxonase 192 Arg/Argalloenzyme is able to less protect LDL against the accumulation of lipid-peroxides than the alloenzymes containing the Gln variant (20). This combination of genetically determined lower paraoxonase-mediated protection from lipid peroxidation, increased nonenzymatic glycation and oxidative damage caused by hyperglycemia, and eventually further impairment of paraoxonase activity by cigarette smoking and from diabetes may lead to an increased entrapment of oxidized LDL particles in the arterial wall, resulting in increased frequency and extent of coronary artery disease (CAD) (1,21) Also, a variety of studies have suggested that low paraoxonase levels are associated with atherosclerosis, hyperlipidemia, and T2DM (21,22). Abbott et al. (14) also revealed that the decreased PON activity was involved in diabetic neuropathy. Some studies suggest that there is an association (23-27), but others show no association between PON gene polymorphisms and coronary hearth disease (28-30). Although some of these controversial results can be explained by factors such as the type of population studied, dietary habits, environmental differences and differences in study design, the answers remain unclear (5).

It has been previously shown that the presence of diabetes severely affects PON1 activity and concentration-independently of a genetic effect on the PON1 phenotypic distribution (31).

Paraoxon hydrolysis activity varies widely among individuals. Part of this variability is due to the polymorphism of PON1 gene. A glutamine (Q) / arginine (R) substitution at position 192 which is determinant for enzyme activity, the R allele coding for a protein displays several-fold higher activity towards paraoxon hydrolysis than the Q allele (32). However, some investigators showed in vitro that low HDL with the RR genotype, which has high serum PON activity, had less ability to protect against LDL oxidation with time compared with that of the QR or QQ genotype (33). This may explain the discrepancy between PON activity and the PON effect on CHD (16). At the same time, Hu et al. (34) showed that the frequency of R allele of PON1 gene in Chinese is significantly higher than that in European and American Caucasians and Indians (15, 35, 36, 37, 38). Furthermore, in a previous study has shown that the low serum paraoxonase activity associated with diabetes was not caused by phenotypic differences in the diabetic population when compared with healthy control subjects, nor it was due to a lower paraoxonase concentration in diabetics (14). But Altuner and Ergun claimed that lower PON activity showed in increased diabetic complication (39, 40).

Table 2: PON activity in non-diabetic and T2DM populations.

	Group	n	mean ± SD	p
PON activity (IU/l)	Control	30	158.1±63.7	<0.05
	T2DM patient	50	102.0±38.9	

In the current study controls and T2DM subjects PON1 activity was significantly highest in the RR genotype and lowest in the QQ with the QR genotype having an intermediate activity. We also found that paraoxonase activity was lower in diabetics than in control subjects (Table 2). These results are in line with the Altuner et al (41). In the present investigation, we have assumed that the polymorphism of PON1 due to amino acid substitution at position 192 may greatly affect the atherosclerosis shown in diabetics together with high LDL and low HDL levels.

CONCLUSION

Our results suggest that paraoxonase activities are affected by PON1 genetic variability in T2DMpatients and controls. Since the individuals with QQ genotype have low serum paraoxonase activities, we believed that the complications of these individuals may observe earlier than the individuals with QR and RR genotype. Further investigations are also required on the role of the PON1 polymorphisms in T2DM and their effects on the anti-atherogenic properties of PON1.

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Conflict of Interest

No conflict of interest was declared by the authors.

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