Original Article

Association of Paraoxonase 55 and 192 Gene Polymorphisms on Serum Homocysteine Concentrations in Preeclampsia

(paraoxonase / preeclampsia / genetic polymorphism / homocysteine)

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Abstract. Paraoxonase 1 (PON1) is thought to influence serum homocysteine concentrations, at least in part, due to its homocysteine thiolactonase activity and to play a role in preeclampsia and atherosclerosis. We investigated the effects of *PON* 55 and *PON* 192 polymorphisms on plasma total homocysteine (tHcy) concentrations in preeclamptic and healthy pregnants among Turkish population (N = 106). *PON* 55 and 192 genotypes were determined by PCR RFLP techniques. Plasma tHcy concentrations were measured by high-performance liquid chromatography. No differences were observed in the distribution of *PON1* 55/192 genotypes and allele frequencies between the preeclamptic and healthy pregnants. tHcy level in the plasma of preeclamptic women was found to be increased in comparison with healthy pregnants ($P < 0.01$). Preeclamptic women bearing the mutated *PON* 192RR and wild-type *PON1* 55LL genotypes had higher tHcy levels than those of the healthy pregnants with the corresponding genotypes, supporting the possibility that the hyperhomocysteinaemia seen in preeclamptic women is associated with the *PON* genotypes. However, no influence of the allelic distribution on plasma tHcy concentrations was detected in either group. Our results suggest that *PON1* 55 and 192 genotypes might have an important role in developing hyperhomocysteinaemia and may also have a role in the pathogenesis of preeclampsia in a Turkish population.

Introduction

Preeclampsia is a syndrome that is usually defined as the onset of hypertension and proteinuria after 20 weeks of gestation in pregnant women (Noris et al., 2005). Preeclampsia is thought to involve several different susceptibility genes and environmental interactions with endothelial cell dysfunction as a common endpoint (Gratacos, 2000). Since it has been shown that homocysteine (Hcy) or its metabolites are associated with endothelial dysfunction and cardiovascular disease, hyperhomocysteinaemia was suggested to have a role in promoting endothelial dysfunction in preeclampsia (Dekker and van Geijn, 1996). At high concentrations, Hcy is first converted by methionyl-tRNA synthetase to homocysteine thiolactone (HTL), which then reacts with lysine residues in proteins, thereby causing damage in their structure and impairment in their physiological activities (Jakubowski, 2002). HTL appears to be more toxic to human cells than Hcy itself. The extent of HTL synthesis and protein homocysteinylation, which have detrimental effects on human vascular endothelial cells, mainly depends on the levels of Hcy (Dekker and van Geijn, 1996; Jakubowski et al., 2001).

Human serum paraoxonase 1 (PON1, aryl-dialkylphosphatase, EC 3.1.8.1) is a high-density lipoprotein (HDL)-bound enzyme that is considered as the major determinant of the antioxidant action of HDL (Mackness et al., 1998). PON1 is a multifunctional antioxidant enzyme that not only can destroy oxidized low-density lipoprotein (ox-LDL), but can also detoxify the homocysteine metabolite, homocysteine thiolactone (Mackness et al., 1998; Jakubowski et al., 2001). Two major genetic polymorphisms of human PON have been described, due to glutamine (Q) or arginine (R) at position 192 and methionine (M) or leucine (L) at position 55. The paraoxonase activity of the Gln192 (Q allele) and Met55 (M allele) isoforms has been reported to be lower

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Abbreviations: EDTA – ethylene diamine tetra-acetic acid, Hcy – homocysteine, HTase – homocysteine thiolactone hydrolase, HTL – homocysteine thiolactone, PON1 – paraoxonase 1, tHcy – total homocysteine.

than that of the Arg192 (R allele) and Leu55 (L allele) isoforms. An ML substitution at position 55 has a lesser effect on activity, while a strong linkage disequilibrium exists between L55 and Q192 (Eckerson et al., 1983; Adkins et al., 1993). Furthermore, Jakubowski (Jakubowski et al., 2001) and Lacinski (Lacinski et al., 2004) reported that high HTase activity was associated with *PON1* 192R and *PON1* 55L alleles, whereas low HTase activity was associated with *PON1* 192Q and *PON1* 55M alleles. Although the association of *PON1* gene polymorphisms and cardiovascular disease has been implicated in some studies (Serrato and Marian, 1995; Schmidt et al., 1998), others failed to show such a relation (Antikainen et al., 1996; Herrmann et al., 1996; Gardemann et al., 2000).

The aim of this study was to assess the association of the *PON1* Q192R and L55M polymorphisms with serum homocysteine concentrations and to investigate the relationship between hyperhomocysteinaemia and paraoxonase gene polymorphisms in patients with preeclampsia in a Turkish population.

Material and Methods

Patients. One hundred and six pregnant women were included in this study. This included 64 preeclamptic patients (mean age, 28.2 ± 6.31) and 42 healthy pregnant controls (mean age, 25.7 ± 5.27). The patients and the controls had similar distribution of age, 42 healthy subjects were complication-free pregnants in their third trimester. Preeclampsia was defined as a blood pressure of ≥ 140/90 mmHg on two different occasions more than 4 h apart in a previously normotensive woman, associated with proteinuria in excess of 300 mg/l in a 24-h collection. The healthy pregnants were followed up until delivery to assess that they did not develop preeclampsia. Since pregnant women may be prone to alterations in some biochemical parameters, a group of healthy pregnants was also included in the study in order to evaluate serum homocysteine concentrations during the third trimester.

All the subjects enrolled in the study were Caucasians. They had a similar socio-economical status and were applied to Bakirkoy State Hospital, Division of Obstetrics or Istanbul Faculty of Medicine, Department of Obstetrics and Gynaecology. They gave informed consent for participation in the study. The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine and the Research Fund of Istanbul University.

Sample collection. Blood samples from preeclamptic and control subjects in the third trimester were obtained after an overnight fast. Ethylene diamine tetra-acetic acid (EDTA) was used as an anticoagulant for DNA extraction and homocysteine measurements.

Genotyping method for paraoxonase 55/192 polymorphisms. DNA samples were extracted from whole blood using the salting-out procedure (Miller et al., 1998). *PON1* genotypes were determined following PCR according to previously published protocols (Adkins et al., 1993; Humbert et al., 1993). The PCR amplification reactions were performed using the GeneAmp PCR Systems 9700 (Applied Biosystem, Foster City, CA).

Homocysteine measurement. Total (free plus protein-bound) homocysteine (tHcy) concentrations were measured with tri-N-butyl-phosphine used as a reducing agent and ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate used as the fluorochromophore (Ubbink et al., 1991), followed by HPLC (Waters 600 controller, GenTech Scientific, New York, NY) with fluorescence detection (Waters 474, GenTech Scientific). DL homocysteine was used as a standard, and N-acetyl-cysteine was used as an internal standard.

Statistical analysis. Statistical analyses were performed using the SPSS software package, version 7.5. Clinical laboratory data are expressed as means ± SD. Mean values were compared between patients with preeclampsia and healthy pregnants by the unpaired Student's *t*-test. Differences in the distribution of *PON* 55 and 192 genotypes or alleles between cases and controls were tested using the χ^2 test. *PON* 55 and 192 allele frequencies were estimated by gene counting methods. $P < 0.05$ was considered statistically significant.

Results

The characteristics of the healthy and preeclamptic pregnants are given in Table 1. All preeclamptic women included in this study had a diastolic blood pressure higher than 110 mmHg, and systolic blood pressure higher than 140 mmHg. A mild but significant increase

Table 1. Characteristics of the study population

	GROUPS		
	Healthy pregnants	**Preeclamptic pregnants**	**P value**
Number of patients	42	64	
Median age (range)	26 (19–35)	28 (18–40)	NS
BMI	24.14 ± 3.11	25.99 ± 4.29	0.009
Gestational week	30–38	30–38	NS
SBP (mmHg)	< 140	> 140	
DBP (mmHg)	70–90	> 110	
Proteinuria	trace	300–1000 mg/l	
Homocysteine	8.12 ± 2.98	9.73 ± 3.83	0.01

BMI: Body mass index, NS: not significant

Table 2. Genotype and allele frequencies of 55/192 polymorphisms of the PON gene in study groups

	GROUPS		
	Healthy pregnant women	**Preeclamptic women**	**Total pregnant women**
PON 55 polymorphism	(N = 39)	(N = 64)	(N = 103)
PON 55 genotypes			
LL	20 (51.3 %)	33 (51.6 %)	53 (51.5%)
MM	4 (10.3 %)	5 (7.8 %)	9 (8.7 %)
LM	15 (38.5 %)	26 (40.6 %)	41 (39.8 %)
PON 55 alleles			
L	55 (70.51 %)	92 (71.87 %)	147 (71.35 %)
M	23 (29.48 %)	36 (28.12 %)	59 (28.64 %)
PON 192 polymorphism	(N = 42)	(N = 63)	(N = 105)
PON 192 genotypes			
QQ	24 (57.1 %)	34 (54.0 %)	58 (55.2 %)
RR	6 (14.3 %)	6 (9.5 %)	12 (11.4 %)
QR	12 (28.6 %)	23 (36.5 %)	35 (33.3 %)
PON 192 alleles			
Q	60 (71.42 %)	91 (72.22 %)	151(71.90 %)
R	24 (28.57 %)	35 (27.77 %)	59 (28.09 %)
PON 55/192 haplotypes			
55L-192R	16 (42.1 %)	28 (45.9 %)	44 (44.4 %)
55L-192Q	30 (78.9 %)	51 (83.6 %)	81 (81.8 %)
55M-192R	9 (23.7 %)	14 (22.6 %)	23 (23.0 %)
55M-192Q	15 (39.5 %)	27 (44.3 %)	42 (42.4 %)

N: number of individuals

in plasma tHcy was seen in preeclamptic patients as compared to their healthy counterparts (9.73 ± 3.83 vs 8.12 ± 2.98 μmol/l, $P < 0.01$).

The *PON* 192 and *PON* 55 genotypes and allele frequencies for preeclamptic patients and control subjects are shown in Table 2. The frequencies of *PON1* 55/192 genotypes were similar between preeclamptic and healthy pregnants, and the observed frequencies were in Hardy-Weinberg equilibrium ($P < 0.05$).

Frequencies of *PON* 192 QQ, RR and QR genotypes among the patients with preeclampsia were 0.54, 0.095 and 0.365, respectively; among the healthy pregnants, they were 0.57, 0.14 and 0.29, respectively. The gene frequency for the *PON1* 192 polymorphisms in study groups was not significantly different.

The frequencies of *PON* 55 LL, MM, and LM genotypes among the patients with preeclampsia were 0.52, 0.08 and 0.41, respectively; among the control subjects, they were 0.51, 0.10 and 0.39, respectively. The gene frequency for the *PON1* 55 polymorphisms in controls and preeclamptic patients was not significantly different. Also, we didn't observe any difference in the study groups regarding *PON1* 55 – *PON1* 192 haplotypes (Table 2).

The association between serum total homocysteine concentration and *PON* 55/192 polymorphisms is shown in Table 3. In the preeclamptic women with *PON1* 192RR genotype, homocysteine levels were observed between 8.8–12.0 μmol/l, while these levels in healthy pregnant women with the same genotype were between 3.9–9.0 μmol/l. Homocysteine levels in preeclamptic and healthy pregnants bearing *PON1* 192RR (mutant type) were significantly different (10.31 ± 1.87 and 6.31 ± 2.30 μmol/l, respectively; $P = 0.03$). A slight, but non-

Table 3. Effects of PON 55/192 polymorphisms on serum homocysteine concentrations in preeclamptic and healthy pregnant women

			Homocysteine concentrations		
			Preeclamptic pregnants	**Normotensive pregnants**	**Total pregnants**
PON 55 Polymorphism	Genotypes	LL	9.65 ± 3.20	7.72 ± 3.42	8.83 ± 3.39
		MM	9.39 ± 3.34	9.11 ± 2.95	9.23 ± 2.84
		LM	8.46 ± 3.37	8.62 ± 3.51	8.55 ± 3.38
	Alleles	L	9.19 ± 3.26	8.17 ± 3.43	8.71 ± 3.36
		M	8.64 ± 3.26	8.73 ± 3.32	8.69 ± 3.24
PON 192 Polymorphism	Genotypes	QQ	9.11 ± 3.33	7.89 ± 3.18	8.52 ± 3.27
		RR	10.31 ± 1.87 *	6.31 ± 2.30	7.64 ± 2.86
		QR	8.86 ± 3.48	9.74 ± 3.83	9.26 ± 3.57
	Alleles	Q	9.02 ± 3.33	8.49 ± 3.44	8.77 ± 3.36
		R	9.17 ± 3.20	8.37 ± 3.65	8.76 ± 3.40

Data are given as means ± SD, * $P = 0.03$

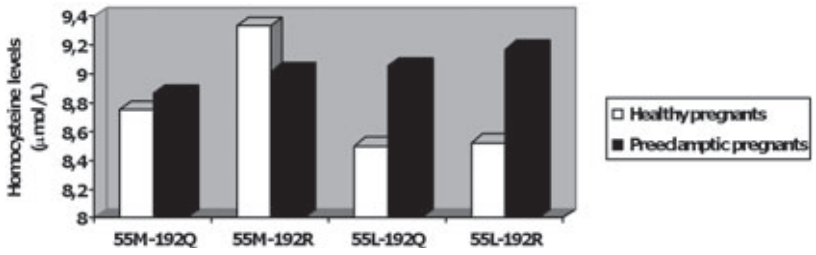

Fig. 1. PON 55/192 haplotypes and serum homocysteine concentrations in preeclamptic and healthy pregnants

significant difference was observed in patients bearing wild-type *PON1* 55LL genotype (9.65 ± 3.20 µmol/l in preeclamptics; 7.72 ± 3.42 µmol/l in healthy pregnants; P = 0.106). However, no effect of *PON* 55/192 haplotypes on serum homocysteine concentrations was observed in the study groups (Fig. 1).

Discussion

Preeclampsia, a frequently seen pregnancy-associated disease, is the leading cause of both maternal and foetal morbidity and mortality (Noris et al., 2005). The aetiology of preeclampsia remains unknown. Endothelial dysfunction is thought to contribute to the clinical features of preeclampsia (Dekker and van Geijn, 1996; Gratacos, 2000).

Increasing clinical and biochemical evidences suggest that a disturbance of normal endothelial cell function may be a primary cause in the pathogenesis of preeclampsia (Roberts and Gammill, 2005). In previous studies, vascular lesions in the placental bed of women with preeclampsia were reported to be similar to atherosclerotic plaques (Hubel, 1999). Increased levels of lipid peroxidation products and altered antioxidant enzyme activities have also been reported in preeclampsia and sustained hypertension (Gratacos et al., 1998). One of the antioxidant enzymes is serum paraoxonase (PON1).

Kumru et al. analysed paraoxonase and arylesterase values in preeclamptic women and they suggested that an abnormal lipid profile and a decrease of the lipophilic antioxidants paraoxonase and arylesterase may play a role in pathogenesis of preeclampsia through increased susceptibility to lipid peroxidation (Kumru et al., 2004). Uzun et al. reported that serum concentrations of MDA and ox-LDL were significantly higher, while PON1 activity was significantly lower in preeclampsia compared with normotensive controls (Uzun et al., 2005). On the contrary, Sarandol et al. have shown that serum paraoxonase and arylesterase activities are similar in both preeclamptic and healthy pregnants (Sarandol et al., 2004).

Paraoxonase activity varies widely among individuals. Part of this variability is due to the polymorphisms of the *PON1* gene (Eckerson et al., 1983; Adkins et al., 1993; Lacinski et al., 2004). The frequency of *PON1* gene polymorphisms has been investigated in various diseases in which endothelial dysfunction is common pathology (Antikainen et al., 1996; Herrmann et al., 1996; Schmidt et al., 1998; Gardemann et al., 2000; Agaçhan et al., 2004, 2005; Bilge et al., 2007). There are several studies about *PON1* polymorphisms and preeclampsia. Lowler et al. suggested that maternal R allele of *PON1* Q192R is associated with preterm births, but not with pregnancy-associated hypertension (Lawlor et al., 2006). Kim et al. have recently reported that the distribution of *PON1* 192 genotypes does not differ between the control and preeclamptic pregnants (Kim et al., 2005). Kim et al. also observed an association between *PON1* 192R allele and serum oxidized LDL levels. In our study, we found no significant differences in the distribution of *PON* 192/55 genotypes between preeclamptic patients and healthy pregnants.

Elevated maternal concentrations of homocysteine have been reported in preeclampsia (Dekker and van Geijn, 1996; Yilmaz et al., 2004; Braekke et al., 2007). It is now evident that homocysteine damages endothelial cells. Hyperhomocysteinaemia seen in preeclamptics was thought to take part in promoting endothelial dysfunction. We observed that preeclamptic women have higher plasma homocysteine concentrations than healthy pregnants in this study. In normal pregnancies, tHcy concentrations are lower in the second trimester than in the first trimester and slightly increase toward pre-pregnancy values in the second half of the third trimester (Holmes et al., 2005). The reason for the elevation of tHcy in preeclampsia is not clear yet. Studies of methylene-tetrahydrofolate reductase (MTHFR) polymorphisms, vitamin B_{12} and folate have failed to prove these to be of significant impact (Mignini et al., 2005). In our previous study, we observed that the preeclamptic patients carrying *MTHFR* CC genotype (wild type) had significantly higher levels of tHcy than those of the healthy pregnants with the same genotype, which rules out the possibility that the presence of a mutated allele (T) is associated with hyperhomocysteinaemia seen in preeclamptics (Yilmaz et al., 2004).

Recently, it was found that the paraoxonase protein, carried on HDL, has a homocysteine thiolactone hydrolase (HTase) activity and protects against protein homocysteinylation *in vitro* (Jakubowski et al., 2001). Jakubowski et al. reported a strong association between the homocysteine thiolactonase/paraoxonase activity and *PON1* genotypes in humans (Jakubowski et al., 2001; Lacinski et al., 2004). They reported that high Hcy thiolactonase activity was associated with the presence of both L55 and R192 alleles. They also claimed

that the low-activity HTase/paraoxonase alleles (Q192 and M55), combined with elevated Hcy, may predispose humans to vascular dysfunction (Lacinski et al., 2004). Contrary to that, we observed that the high-activity *PON1* alleles (R192 and L55) are associated with elevated Hcy in preeclamptic patients. However, all homozygous RR192 didn't exhibit the increase in tHcy (preeclamptic pregnants: 8.8–12.0 μmol/l; healthy pregnants: 3.9–9.0 μmol/l) and the frequency of RR192 genotype was relatively low in our study groups. To our knowledge, this is the first report on *PON1* 55/192 polymorphisms and homocysteine levels in preeclampsia, and it is difficult to compare our results with the previous findings that were carried out in different patient populations. Further studies involving both serum PON and HTase activities in preeclamptic subjects are needed to evaluate the association of *PON* gene polymorphisms and hyperhomocysteinaemia.

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