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Investigation of PON1 192 and PON1 55 Polymorphisms in Ovarian Cancer Patients in Turkish Population

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Abstract. *Background: Ovarian cancer is the leading cause of death due to gynecological malignancies among women. Oxidative stress is potentially harmful to cells and reactive oxygen species are known to be involved in the initiation and progression of cancer. Paraoxonase (PON1) is an antioxidative enzyme, which eliminates lipid peroxides. PON1 has two common polymorphisms (M/L55 and A/B192) that influence PON1 concentration and activity. Patients and Methods: Whether or not the M/L55 or A/B192 genotype relates with ovarian cancer was studied in 51 patients and 54 controls. Polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine these polymorphisms. Results: The proportion of smokers was significantly higher in the patients than the controls (26.9% vs. 7%; Chi-square: 7.81, p:0.005; Odds ratio (OR): 4.88 95% CI: 1.49-15.99). The frequencies of the PON1 192 AA, BB and AB genotypes among the patients were 0.76, 0.12 and 0.12 and among the control subjects, 0.33, 0.11 and 0.56, respectively. The AA genotype frequency was significantly higher in the patients than the controls (Chi-square: 19.242, p=0.000; OR: 2.80 95% CI:1.653-4.757). The frequencies of the PON1 55 LL, MM and LM genotypes among the patients were 0.53, 0.10 and 0.37 and among the control subjects there were 0.46, 0.04 and 0.50, respectively. The MM genotype frequency was higher in the patients than the controls, but not statistically significantly (p>0.05). Conclusion: The two polymorphisms were associated with the age of onset of ovarian cancer, which increased in the genotype order AB<AA<BB in the PON1 192 polymorphism and LM<LL<MM in the PON1 55 polymorphism. The PON1 192 AA genotype may play an important role as a risk factor for ovarian cancer in the Turkish population and the A and L alleles may be associated with early onset of disease.*

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Ovarian cancer is one of the leading causes of death for women worldwide (1). Chemical, dietary, viral, hormonal and genetic factors are thought to contribute to the development of this cancer (1). It had previously been determined that an imbalance between oxidant-antioxidant metabolites caused a tendency to tumor development and it has been suggested that activated oxygen radicals, reactive aldehyde and peroxidases might cause defects in the genes related to the proliferation and differentiation proteins (2-4). It has been shown that high levels of steroid hormones and gonadotrophin exposure increased the risk of ovarian cancer development by increasing lipid peroxidation (5).

Human paraoxanase (PON1), a Ca^{++}-dependent esterase, synthesized in liver, is related to high density lipoprotein (HDL) (6). PON1 has two main roles: detoxifying organophosphate compounds such as paraoxone and protecting LDL by hydrolysis of lipid peroxides (7, 8). Lipid peroxidation has really important roles in the control of the cell cycle (9), in particular polyunsaturated fatty acids (PUFA) are known to be vulnerable to free oxygen radical interaction creating lipid peroxidation (10). The peroxidation products can interact with DNA bases to form exocyclic DNA base products, the most common forms of which are exocyclic pyrimido-purinones which are known to be cytotoxic and mutagenic (11). In tumor cells, there is additional oxidative stress because of rapid metabolism and this is an additional factor causing insufficiency of the antioxidant enzymes (12). As in several other cancer types, the end products of lipid peroxidation have been found to increase and antioxidant enzyme levels to decrease in ovarian cancer patients. It has previously been shown that PON1 gene polymorphisms causing a change from glutamine to arginine at the 192 position and leucine to methionine at the 55 position changed both the level and activity of the enzyme (13-16). Those people with the PON1 192 BB genotype had higher enzyme levels than the PON1 192 AA individuals and the PON1 A allele hydrolysed paraoxone less than the PON1 192 B allele (17).

Table I. *Age and body mass index of the study groups (x±SD).*

	Control (n=54)	Patient (n=51)
Age (years)	45.41±16.94	46.38±11.03
Body Mass Index (BMI) (kg/m^2)	24.387±3.92	25.761±5.78

The most common genotypes are in the order AA(QQ), AB(QR), BB(RR) (15,18). It has also been shown that the PON1 55 M allele increased the tendency of proteolysis of this enzyme therefore changing the serum levels (19).

The aim of this study was to determine the PON1 192 and PON1 55 gene polymorphisms in a Turkish population and whether there was any tendency to ovarian cancer created by this polymorphism.

Patients and Methods

Fifty histologically confirmed ovarian cancer patients were diagnosed and followed up in Istanbul University, Department of Obstetrics and Gynecology. Fifty two healthy control subjects were carefully chosen not to have first degree relatives having such a cancer diagnosis. The patient and control groups were matched for age and body mass index (BMI) (Table I). Blood specimens protected with EDTA were taken from all the study subjects.

Polymorphism analysis. A previously reported PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method was used to determine the PON1 192 and PON1 55 gene polymorphisms (20).

PCR was used to amplify the gene with 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and 5'-CAC GCT AAA CCC AAA TAC ATC TC-3' primers for determination of the PON1 192 gene polymorphism and 5'-GAA GAG TGA TGT ATA GCC CCA G-3' and 5'-TTT AAT CCA GAG CTA ATG AAA GCC-3' for the PON1 55 polymorphism.

The PCR mixture (total, 25 μl) contained 1-2 μl DNA, 1 μl of each primer, 5 μl *dNTP*, 1.5 μl of $MgCl_2$ and 0.3 μl Taq polymerase. For the PON1 192 gene polymorphism the mixture was incubated at 95˚C for 2 minutes, then 35 cycles of 94˚C for 1 minute to denature, 61˚C for 1 minute to anneal the primers and 72˚C for 1 minute to elongate the strand. After the PCR process, the *Alw1* (*BspI*) restriction enzyme was used and 2% agarose gel electrophoresis was performed to identify the possible polymorphism. *Alw1* (*BspI*) digestion generated the following fragments: PON1 192 B allele, fragments of 66 bp and 33 bp; PON1 192 A allele, a single fragment of 99 bp.

For the determination of the PON1 55 locus polymorphism, the PCR reactions started with incubation of 95˚C for 5 minutes and 30 cycles of denaturation for 1 minute at 92˚C, followed by annealing for 45 seconds at 52˚C and elongation for 45 seconds at 72˚C. The restriction enzyme was *N1a111* (*Hsp192II*) for determination of the PON1 55 polymorphism and 2% agarose gel electrophoresis was performed to determine possible polymorphisms. *N1a111* (*Hsp192II*) digestion generated the following fragments: for PON1 55 M allele, fragments of 126 bp and 44 bp; for PON1 55 L allele, a single fragment of 170 bp.

Table II. *PON1 192 genotype and allele distributions in study groups.*

	Control group (n:54)	Patient group (n:51)
PON 192 genotypes		
AA	17 (32.7%)	38 (76%)
BB	6 (11.5%)	6 (12%)
AB	29 (55.8%)	6 (12%)
Alleles		
A	63 (60.57%)	82 (82%)
B	41 (39.42%)	18 (18%)
B (–)	17 (32.7%)	38 (76%)
B (+)	35 (67.3%)	12 (24%)

Table III. *PON1 55 genotype and allele distributions in study groups.*

	Control group (n:54)	Patient group (n:51)
PON55 genotypes		
LL	25 (46.30%)	27 (52.90%)
MM	2 (3.70%)	5 (9.80%)
LM	27 (50%)	19 (37.30%)
Alleles		
L	77 (71.29%)	73 (71.56%)
M	31 (28.70%)	29 (28.43%)
L (–)	2 (3.70%)	5 (9.80%)
L (+)	52 (96.30%)	46 (90.20%)

Statistical analysis. The statistical analyses were conducted using the SPSS 11.0 programme. Chi-square and Fisher's Exact tests were used to determine the differences of the genotypes and alleles between groups.

Results

The PON1 genotype and allele distributions are shown in Table II. The risk of ovarian cancer was 2.8 times higher in the subjects that were not carrying any B allele (subjects with AA genotype) (Chi-square: 19.242, p=0,000; Odds ratio (OD): 2.80 95% CI:1.653-4.757).

Table IV. *PON1 genotype distributions in relation to the starting age of the disease.*

	Starting age of the disease (year)
PONI 192 genotype	
AA	43.68±11.46
BB	50.50±12.81
AB	37.33±8.11
PON1 55 genotype	
LL	44.59±11.51
MM	45.80±11.18
LM	43.26±12.98

The PON1 55 genotype and allele frequencies are given in Table III.

The L (-) subjects were more numerous in the patient group than in the control group but this result was not statistically significant (Chi-square: 1.569, p=0.261 Fisher's exact test).

The relationship of the disease starting age and the polymorphisms was also investigated and the patients with the BB genotype were significantly older than those with the AB genotype (p.0.049). For the PON1 55 genotypes, the starting age of the disease was, in order, MM>LL>LM, but this was not statistically significant (p>0.05) (Table IV).

There were 4 (7%) smoking and 50 (92.6%) non-smoking subjects in control group and 14 (27.4%) smoking and 37 (72.5%) non-smoking subjects in patient group and the risk of developing ovarian cancer in the smokers was 4.8 times higher than in the non-smokers (Chi-square:7.81, p.0.005; OR: 4.88 95% CI: 1.49-15.99).

Discussion

Conflicting results have been reported in previous studies of PON1 polymorphisms and their relationship to cancer. PON1 192 and PON1 55 genotype distributions were found to be similar in colorectal patients group and controls (21). In another study, it was found that PON1 192 AB and PON1 55 LM/MM genotypes had a higher risk of developing prostate cancer in Italian people (22).

In the present study the individuals with the PON1 192 AB genotype developed ovarian cancer earlier than those with the PON1 BB genotype which might be due to a protective effect of the PON1 BB genotype.

In several studies, smoking was found to be related to several kinds of cancer by inhibiting PON1 activity (23-25). In parallel, smoking increased the risk of ovarian cancer development by 4.8 times, in the present study. In a previous study, it was found that the PON1 55 M genotype increased the risk of ovarian cancer development in smokers (25), but a similar result was not found in the present study.

This study was important being the first to assess the relationship of PON1 polymorphisms and ovarian cancer in a Turkish population. The determination of the PON1 activity levels and enlargement of the study is planned groups for future investigations.

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