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Original Research

Relation of MPO, MnSOD, NQO1 gene variants in endometrial carcinoma in the line of PCR-RFLP methods

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Abstract: Reactive oxygen species (ROS) have been shown to be responsible for inducing DNA damage leading to mutagenesis, carcinogenesis, and cell death if the capacity of the protective antioxidant system is impaired. Endometrial carcinoma is the primary cancer type in the female genital system. The enhanced cell lipid peroxidation and impaired antioxidant enzyme activities observed in patients with endometrial cancer indicate the potential for oxidative injury to cells and cell membranes in such patients. The aim of the study was to investigate the possible association between gene variants of superoxide dismutase (SOD), myeloperoxidase (MPO), and NADPH quinone oxido reductase (NQO1), and their possible role in endometrial cancer in Turkish patients. According to results, MPO G+ genotype and AG genotype were significantly increased in patients compared with controls (P<0.001). We suggest that the MPO polymorphism might be a risk for endometrial cancer.

Key words: MnSOD; NQO1; MPO; Polymorphism; Endometrial carcinoma.

Introduction

Endometrial cancer is one of the most important diseases for women that occurs frequently, which is caused by various factors including aging, obesity, hypertension, diabetes, genital flora, and estrogens (1,2). These factors lead to cancer by causing hypoxia in the endometrium (2). In the hypoxia process, inflammation occurs due to reactive oxygen species (ROS). According to the activation of ROS, the cellular environment and DNA structure become damaged (3). To protect the cell structure, the antioxidant defense system, which contains enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), myeloperoxidase (MPO), NAD(P)H: quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), and non-enzymatic antioxidants (e.g., vitamin C, vitamin E, vitamin A, flavinoids) act against oxidative damage that occurs due to ROS. Carcinogenesis caused by the increase in oxidant derivatives and by failure in the antioxidant system is the reason why the antioxidant system becomes a big title to investigate of cancer progression (4).

It is well known that if any imbalance occurs in the antioxidant system, the process of mutations and oncogenesis begins. When there are deviations from the regular system, diseases including cancer may be initiated (5).

Consequently, because of the potential for the development of cancer due to the relationship between ROS and antioxidant genes in several cancer types, we investigated the role of antioxidant gene variants superoxide dismutase (SOD), myeloperoxidase (MPO), NADPH quinone oxido reductase (NQO1) in patients with endometrial cancer.

Materials and Methods

Study groups

Two hundred sixty-seven women who were admitted to the Gynecology Clinic of Istanbul University Istanbul Medical School and Sisli Etfal Government Hospital, Department of Obstetrics and Gynecology, for gynecologic evaluation within routine examinations or for abnormal uterine bleeding were included in our study. Endometrial biopsy was performed and on the basis of diagnosis and histologic examination, women were divided into two groups; a control group (n=112) and an endometrial cancer group (n=155). Specimens were taken after obtaining informed consent and the study was conducted prospectively. Local Ethics Committee approval was obtained for the study. The study protocol was conducted in accordance with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

Table 1. Primer sequences, conditions for amplification, restriction pattern, and restriction enzymes used.

Reagents and Conditions	MnSod	NQO1	MPO
Forward Primer	5'ACCAGCAGGCAGCTGGCGCCGG-3	5-ATTCTCTAGTGTGCCTGAG-3	5-CGGTATAGGCACACAAATGGTGAG-3
Reverse Primer	5- GCGTTGATGTGAGGTTCCAG -3'	5-AATCCTGCCTGGAAGTTTAG-3	5-CGGTATAGGCACACAAATGGTGAG-3
Annealing temperature (°C)	61	60	62
PCR product	107bp	318bp CC	289bp, 61bp AA
Restriction enzyme	NaeI	AciI	HinfI
Restriction pattern (bp)	VV: 107bp AA: 89bp, 18bp VA: 107bp, 89bp,18bp	CC: 318bp TT: 164bp, 154 bp CT: 318bp,164bp,154bp	AA: 289bp, 61bp GG: 169bp,120bp AG: 289bp,169bp,120bp, 61bp

Polymorphism analysis

Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood according to a salting-out technique (6). Genotyping was performed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP); the procedures of PCR-RFLP are given in Table 1 (7,8). The PCR products were visualized using electrophoresis through a 3% agarose gel. The relative size of the PCR products was determined by comparison of the migration of a 50-1000 bp DNA molecular weight ladder. A permanent visual image was obtained using an ultraviolet (UV) illuminator.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software package (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.). Clinical laboratory data are expressed as means+SD. The mean values were compared between patients and controls using Student's unpaired t-test. Chi-square tests were used to differentiate the genotype and allele frequencies between groups. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Values of $p<0.05$ were considered statistically significant.

Results

The characteristics of the patients with endometrial cancer and the control group are given in Table 2. Table 3 summarizes the distributions of genotypes and alleles of MnSOD, NQO1, and MPO genes in the patients and controls. There were no findings that were statistically important in MnSOD and NQO1genotypes frequencies, only MPO genotype distribution was statistically significant between the patients and controls. MPO G+ genotype was significantly increased in patients (83.2%) compared with the controls (63.4%) and carriers of G+ genotype had a 2.8-fold increased risk for endometrial cancer (χ^2=13.6; OR=2.86; 95% CI:[1.62-5.06]; P<0.001). The MPO AG genotype was increased in patients compared with controls (Table 3). The individuals who had MPO AG genotype had a 3.1-fold increased risk for endometrial cancer (χ^2=19.30; OR=3.10; 95% CI:[1.85-5.18]; p<0.001). The frequencies of MPO AA genotypes in controls were higher than in patients (Table 3) and these genotypes seem to be protective aganist endometrial cancer (χ^2=13.60; OR=0.34; 95% CI:[0.19-0.61], P<0.001).

Table 2. Characteristics of patients with endometrial cancer and the control group.

Parameters	Controls (n=112)	Patients (n=155)	P value
Mean age, years±SD	50.84±14.87	54.67±12.89	0.052
Menarche age, years±SD	13.50±1.71	13.41±1.51	ns
MDA	7.71±0.72	5.99±0.93	ns
MPO	465.63±38.42	520.48±33.97	ns
MNSOD	150.58±35.06	203.55±29.86	ns
Oral contraceptive use (%)			
Yes	15.8	20.0	ns
No	84.2	80.0	
Family history (%)			
Yes		43.6	
No		56.4	
Diabetes (%)			
Yes		71.9	
No		28.1	
Hypertension (%)			
Yes		44.9	
No		55.1	
Histology (%) **Endometrioid/Adenocarcinoma/Serous/Clear cell/Undifferentiated**		86.7/2.2/4.4/4.4/2.2	
Grade (%) **1/2/3**		61.4/20.5/18.2	

Table 3. Distribution of MnSOD, NQ1, and MPO genotype frequencies in patients with endometrial carcinoma and the control group.

Polymorphism	Controls n	%	Patients n	%	P value	χ^2
MNSOD						
VV	36	32.1	62	40.0	0.419	1.73
AA	7	6.3	9	5.8		
AV	69	61.6	84	54.2		
V	141	62.94	208	67.09	0.319	0.98
A	83	37.05	102	32.90		
NQO1						
CC	69	61.6	109	70.3	0.208	3.14
TT	3	2.7	6	3.9		
CT	40	35.7	40	25.8		
C	178	79.46	258	83.22	0.26	1.22
T	46	20.53	52	16.77		
MPO						
AA	41	36.6	26	16.8	<0.001	19.75
GG	19	17.0	16	10.3		
AG	52	46.4	113	72.9		
A	134	59.82	165	53.22	0.129	2.29
G	90	40.17	145	46.77		

Discussion

The present study is the first to investigate the relationship between endometrial cancer and the antioxidant genes MPO, MnSOD, and NQO1 in Turkish patients. We found that MPO G+ genotype was significantly increased in patients (83.2%) compared with controls (63.4%) and carriers of G+ genotype and had a 2.8-fold increased risk for endometrial cancer (P<0.001). It is known that ROS production and detoxification mechanisms can result in disorders including age-related disorders, genetic instability, and cancer.

MnSOD is found in many cells including normal ovarian cells. Despite this, there are studies about increased expression levels of MnSOD in ovarian tumors (9,10). In addition to this, many tissues express NQO1 but normal ovarian cells have low-level expression (11). Accordingly, some studies focused on MPO, MnSOD, and NQO1 gene variants to find a relation with underlying the disease mechanism or for early prediction for different types of cancers. Rosenblum et al. suggested that the MnSOD polymorphism was effective for the transportation of the MnSOD protein into mitochondria, which located free radicals (12). Similarly, Hiroi et al. found that MnSOD had less impact on mature protein (13) in breast cancer-related research associated with MnSOD genotypes and suggested that it had increased risk for the breast cancer (14, 15). In addition, several studies in prostate, bladder, and lung cancer studies showed the role of MnSOD (16-20). In contrast, Purdie et al. found no relation regarding MnSOD genotypes in ovarian cancer between patients and controls (21). Hou et al. showed a positive relation between NQO1 genotype and adenoma risk. Moreover, they suggested that NQO1 polymorphisms were decreased when there was a high-risk potential for malignant adenoma (22). Another study demonstrated that NQO1 and MPO polymorphisms could have a relation through low enzyme activity in patients with lung cancer (23). Even though MnSOD an NQO1 have been found statistically important for some types of cancer, we found no significant distribution in our research.

Owing to genotype studies about MPO and cancer, several correlations have been found. Hung et al. have showed that MPO A/A genotypes carriers have a protection on bladder cancer risk even though MPO G/A genotypes carriers don't have the same protection (24). Olson et al. showed reduced risk with AA genotypes for MPO in patients with ovarian cancer. The authors considered that the region might be protective owing to its weaker binding site (25).

According to some research, although the MPO gene G allele has the activity of transcription than the A allele (26-28), in a research, MPO levels have been found in women and hypothesized that it is related with estrogen levels included MPO levels (29-31). Castela et al. studied the relationship the MPO gene and cervical cancer. They found a statistically significant difference between patients and controls and the GA genotype was found higher level in patients with cervical cancer, respectively (28).

Antioxidants are potent scavengers of free radicals and are of particular importance in the protection against human diseases associated with free radical damage to cellular DNA, lipids, and proteins. Based on MPO's role in biologic systems and the production of free radicals such as DNA damage and carcinogenesis (32), it is hard to explain which genetic differentiations have potential to be important markers; our study focuses on recent studies about women's diseases. MPO is a lysosomal enzyme that catalyzes hypochlorous acid and H_2O_2, which is important in oxidative stress (33-35). Increasing or decreasing expression of MPO genes might initiate cancer through the effect of DNA damage. Studies have shown that decreased MPO in A allele carriers has a protective role in some cancers, whereas the

G allele might be risk factor, similar to our results. In line with our results, statistically significant distribution results have been found between patients with endometrial cancer and controls (33-37). We thought that the MPO G allele might have a relation in genital cancer in women.

The present study has some potential limitations. The small sample size makes our study under-powered. This might account for some of the results where no statistical significance was demonstrated, such as with MnSOD and NQO1. Large-size studies in different races will help us to understand whether MnSOD and NQO1genotypes affect patients and MPO may become a possible biologic marker for endometrial cancer. Our study was a preliminary study and further studies with larger sample groups are necessary to clarify the role of antioxidant enzyme genes and the development of endometrial cancer.

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