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Prevalence of MMP-3 E45K polymorphism in Turkish patients with endometrial carcinoma

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Abstract

Endometrial carcinoma is a metastatic and recurrent disease has a worse prognosis. We aim to investigate the MMP-3 gene polymorphism in Turkish patients with endometrial carcinoma. In recent years, number of genetic studies have been increased to find new prognostic and theraupeutic markers. Matrix metalloproteinases [MMPs] are proteolytic enzymes that degrade all components of the extracellular matrix which are play an important role of cancer metastasis and invasion. MMP-3, also known as stromelysin-1, to lyse basal membrane collagen and induce the synthesis of some other MMPs. 97 patients with endometrial carcinoma and 100 healthy controls were included in this study. MMP-3 E45K polymorphism was determined using polymerase chain reaction/ restriction fragment length polymorphism in study groups. There were no significant differences between any genotypes or allele in control and patient groups for MMP-3 E45K polymorphism. Besides no significant correlation was found between MMP-3 E45K polymorphisms and clinical characteristics, such as histology, grade, metastasis and family history. ur study suggests that the MMP-3 E45K polymorphism is not associated with endometrial carcinoma process in Turkish patients.

Keywords: MMP-3, polymorphism, endometrial carcinoma, prevalence

Introduction

Endometrial cancer is the most important female disease in genital tract [1]. The global morbidity rate is 6.5% and the global mortality rate is about 1.6% [2].

The extracellular matrix (ECM) hosts some structural molecules which may have roles to create cellular microenvironments or niches [3,4]. In case the regulation of ECM remodeling is failure to leading to development of some pathological processes such as cancer, and metastasis [5,6].

Matrix metalloproteinases (MMPs), an enzyme family that contributes the ECM remodeling, are responsible from degradation and turnover of ECM elements [7,8]. The type-IV collagenase group of MMP-2 and MMP-9 has been linked to endometrial cancer progression and invasion [9].

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MMP3, also known as stromelysin-1, to lyse basal membrane collagen and induce the synthesis of some other MMPs [10]. MMP-3 gene expressions and polymorphisms vary on different types of cancer pathogenesis. There are some evidence that MMP-3 has an important role in pathogenesis of astrocytomas. Mercapide et al reported that MMP-3 may play a crucial role in the molecular events that account for the invasive process in astrocytoma cell lines [11,12]. Blons et al suggested that a significant relation between the MMP-3 -1612insA polymorphism and response to chemotherapy in head and neck cancer patients [13]. Besides, there are some studies which is shown that no relation between MMP-3 and cancer. It is shown that the 5A/6A polymorphism of MMP-3 gene may not be linked with appearance and development to ovarian cancer [14].

There is an A/G transition at nucleotide position 28 within exon 2 resulting lysine/glutamate for E45K polymorphism. This changes may cause reduction of MMP-3 production [15]. The findings of Ricketts et al demonstrated that an association between MMP-1/ MMP-3 (rs1799750/rs679620) variants and sporadic renal cell carcinoma [16]. In another study, no association is found between MMP-1, -3, and -7 SNPs and endometrial cancer risk [17]. Another study have been pointed out that no relationship between MMP-3 E45K polymorphism and skin cancer risk [18].

Present study evaluates the correlation between MMP-3 E45K polymorphism and endometrial carcinoma.

Material and Methods

Study Design

Blood samples were collected from 97 patients diagnosed with endometrial cancer in Gynecology Clinic of Medical Faculty of Istanbul University. Endometrial biopsy was performed and on the basis of diagnosis and histological examination by Gynecology Clinic of Medical Faculty. The control group consisted of 100 healthy individuals with a negative family history of cancer. The specimens were taken after obtaining informed consent and the study was conducted prospectively. Local Ethical Committee approval was obtained for the study and the samples were collected after obtaining written informed consent from the participants and approval from Istanbul University's Ethics Committee based on World Medical Association Declaration of Helsinki.

MMP-3 E45K [rs679620] Polymorphism

DNA was extracted from peripheral blood lymphocytes using the salting-out procedure [19]. Polymorphism was genotyped using polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) methods. PCR was used to amplify the region of MMP-3 E45K polymorphism. PCR Primers were designed by using Primer 3 Input (version 0.4.0). Reaction were performed with 10 pmol of each primer: F:5'-AAATTTGCCATTATTTCAGCAAG-3',R:5'-CCCCTCTGAACCATTACCTG-3'. Template DNA [0.5-1.0 µg] was used in a PCR under stringent conditions to avoid the possibility of false positives for genotyping. Reactions were performed with 10 pmol of each primer in final volume of 50 µL containing 25 mM MgCl2, 10 mM Tris-HCl (pH 8.4), 2mM of each dNTP and 5 unit Taq Polymerase (Invitrogen, Carlsbad, USA). Amplification was carried out in a DNA Thermal for 35 cycles with denaturation steps at 94°C for 30 seconds, annealing at 59 °C for 30 seconds and extension at 72°C for 30 seconds for E45K polymorphism. Restriction enzyme was determined by using NEBcutter V2.0 program. For E45K genotypes, presence of the polymorphisms was determined by enzymatic digestion of the initial PCR product with TaqI at 60 °C for 3h. Three genotypes could be determined after electrophoresis: genotype AA (399 bp), GG (236, 163 bp) and AG (399, 236, 163 bp).

Statistical analysis

Categorical variables such as genotypes and alleles were compared using Chi-Square ($\chi 2$) test. Allele and genotype frequencies were determined by direct counting. Differences in continuous variables between carriers and control subjects were tested using Student's t test. Statistical analyses were performed with SPSS 16.0 software (SPSS Inc, Chicago, USA).

Results

We did not observe any differences between study groups according to ages (p>0,05; as mean age ± SD; patient = 54.86 ± 9.72; control = 56.90 ± 6.99). Table 1 summerizes the characteristics of patients with endometrial carcinoma. Table 2 shows that distribution of E45K genotypes in study groups. There were no significant differences between any genotypes or allele in control and patient groups for MMP-3 E45K polymorphism. Frequency of heterozygote genotype was increased in controls at the limit of statistically significant (p=0,061). Besides no significant correlation was found between MMP-3 E45K polymorphisms and clinical characteristics, such as histology, grade, metastasis and family history (Table 3). Some histological groups had less number of patients, therefore it was not be able to evaluate as statistically.

Table 1. Characteristics of patients with endometrial cancer

Parameters	Patient (n = 97)	Control (n=100)
Age, years ± SD	54.86 ± 9.72	56.90 ± 6.99
Oral contraceptive use (%)	20.7	-
Family history (%)	41.7	0
Histology (%)		-
Endometrioid	89.7	
Adenocarcinoma	2.6	
Seros	2.6	
Clear cell	5.1	
Grade (%)		-
1/2/3	64/23/12	
CA 125 levels	30.98 ± 6.63	-
CA 19.9 levels	41.57 ± 18.37	-
CA 15.3 levels	223.52 ± 145.80	-
CEA levels	4.09 ± 1.65	-

n=number of subjects

Table 2. Distribution of MMP-3 E45K genotype and allele frequencies in patient and control groups

MMP-3 E45K polymorphism	Patient (n = 97)	Control (n = 100)	p value
Genotypes			
AA	54(55.7%)	46(46%)	0.175
GG	25(25.8%)	24(24%)	0.773
AG	18(18.6%)	30(30%)	0.061
Alleles			
A	126(64.9%)	122(61%)	0.773
G	68(35.1%)	78 (39%)	0.175

n=number of subjects

Table 3. Distribution of MMP-3 genotypes and alleles according to clinical parameters in patients

MMP-3 E45K polymorphism		AA (n = 54)	GG (n = 25)	AG (n = 18)	A (n = 126)	G (n = 68)
Histology						
Endometrioid		50 (92%)	22 (88%)	12 (66%)	112 (89%)	56 (82%)
Adenocarcinoma		2 (4%)	0	0	4 (3%)	0
Serous		0	3 (12%)	0	0	6 (9%)
Clear cell		2 (4%)	0	6 (34%)	10 (8%)	6 (9%)
Grade						
	1	39 (72%)	9 (36%)	12 (66%)	90 (71%)	30 (44%)
	2	12 (22%)	9 (36%)	0	24 (19%)	18 (26%)
	3	3 (6%)	7 (28%)	6 (34%)	12 (10%)	20 (30%)

n=number of subjects

Discussion

ECM degradation is an important factor for invasion of malignant tumors and metalloproteinases play a crucial role on this process. It has been needed the new studies to consolidate the effects of the metalloproteinases on this process. Our aim was to investigate the MMP-3 gene polymorphism in Turkish patients with endometrial carcinoma.

Recently, several studies have exam¬ined the MMP-3 polymorphisms in different cancers [20,21]. Motovali-Bashi et al suggest that the presence of 5A polymorphism at the MMP-3 promoter region could be looked at as a risk factor for a worse prognosis in colorectal cancer patients [22]. In another study, Guan et al implicate this MMP3 −1612 5A/6A polymorphism as a contributor to ESCC susceptibility [20].

We studied MMP-3 E45K polymorphism which may cause to changes of MMP-3 production levels. There is less number of studies about E45K polymorphism and diseases. Ling et al demonstrated that E45K polymorphism is significantly associated with increased susceptibility and severity of post-operative stiffness [23]. Ma et al suggested that there may be an association of MMP-3 polymorphisms (rs3025058, rs522616, rs679620) with susceptibility to stoke in northern Han Chinese population [24].

We did not find any statistically differences between genotypes and study groups. We observed A allele 64,9% and G allele 35,1% in patients with endometrial carcinoma. A similar result was observed by Nan et al. They studied different SNPs of MMP-3 including E45K, on skin cancers. They did not show any relationship between skin cancer and E45K polymorphism [18]. Beegly-Fadiel et al was investigated MMP-1, -3, and -7 polymorphisms in endometrial carcinoma. They found G allele frequency as 32.9% in patients. Unfortunately, none of these polymorphisms were found to be significantly associated with endometrial cancer risk [17].

However, it is been known that MMP-3 is a risk factor for metastasis and invasion in cancer process [25], we did not show and association between clinical characteristics of patients such as histology, grade, metastasis.

Conclusion

Present study suggests that the MMP-3 E45K polymorphism is not associated with endometrial carcinoma process in Turkish patients.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
The financial support for this study was provided by the investigators themselves.

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