

Original Investigations

Investigation of catechol-o-methyltransferase (COMT) Gene *Val158Met* polymorphism in ovarian cancer

Abaoğlu et al. COMT Val158Met polymorphism in ovarian cancer

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Abstract

Objective: The COMT enzyme synthesized from the Catechol-O-Methyltransferase (COMT) gene detoxifies the carcinogenic catechol estrogens. In present study we aimed to examine the relationship of the disease with *COMT Val158Met* polymorphism which is thought to affect the risk of ovarian cancer.

Material and Methods: The study groups consist of 97 individuals as a patient group with ovarian cancer (n=47) and control group (n=47). The allele and genotype frequencies determined according to Hardy-Weinberg equilibrium (HWE). Genetic analysis were performed by Real-Time PCR device, and the statistical analysis were performed by SPSS program.

Results: *COMT* gene *Val158Met* polymorphism is compared in terms of genotype and allele frequencies, there were no significant relation is obtained among groups (p=0.413)

Conclusion: This study is the first and only study determine the relationship between ovarian cancer and the *COMT* gene *Val158Met* polymorphism within the Turkish population. Although there are no statistically meaningful relationships among genotypes belonging to the patient and control groups, this study should be further carried out by increasing the sample size of the study groups.

Keywords: COMT Val158Met Polymorphism, Catechol Estrogen, Ovarian Cancer

Introduction

Ovarian cancer is the seventh leading cause of death and is the eighth most common cancer among women. It has the highest mortality rate among all gynecological cancers. The

prognosis of ovarian cancer is poor, especially when the disease is diagnosed at an advanced stage (1). There are many risk factors which change the genetic predisposition to ovarian cancer, including consumption of alcohol, obesity, aging and hereditary ovarian cancer. In addition, the oxidative stress, inflammation, angiogenesis, and apoptosis could alter the progression of carcinoma. Thus, controlling these factors play a crucial role in cancer prevention (2).

COMT gene is located on chromosome 22 and the chromosomal region of q11.2. *COMT* gene includes six exons, of which exon 1 and 2 are noncoding (3). *COMT* gene products are expressed in many tissues like bone marrow, brain, bladder, heart, kidney, liver, lung, ovary and so on (4). The synthesized COMT enzyme participates in the DNA repair mechanism (5). Many polymorphisms have been identified in the coding region of the *COMT* gene; these are codon 158 (G→A), codon 72 (G→T) and codon 62 (C→T). At codon 158 of the *COMT* gene Valine alters to Methionine. These alterations in the *COMT* gene lead to a decrease in the function of proteins (6,7).

The catechols can stem from endogenous or exogenous substances, therefore the catecholamine and catechol estrogen are both found in endogenous and exogenous substances (8). Some evidence indicates that reactive catechol metabolites cause cancers originating from estrogens. The COMT enzyme, which is synthesized from the *COMT* gene, catalyzes O-Methylation, therefore, this enzyme deactivates catechol estrogens (9). Catechol estrogens have reactive and carcinogenic effects. Various enzymes can detoxify these chemical compounds in cells. One of those enzymes is COMT, which forms a methoxy compound. This enzyme catalyzes the transfer of the methyl group in the coenzyme SAM to the hydroxyl group in the catechols. In the *COMT* gene, the *Val158Met* polymorphism results in a decrease in the activity of this enzyme, resulting in the accumulation of carcinogenic catechol estrogens (10).

In the present study, it is aimed at examining the relationship of the disease with *COMT Val158Met* polymorphism which is thought to affect the risk of ovarian cancer.

Material and Methods

The present study consisted of total 94 individuals. The study group was composed of patients with ovarian cancer (n=47) who were diagnosed by The Obstetrics and Gynecology Department of XXX University, XXX, XX. The aged matched control group (n=47) consisted of healthy females who visited the hospital for gynecologic evaluation as part of routine checkups. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1975 Declaration of Helsinki guidelines and its later amendments. The research on humans study protocol was approved by the XXX University Medical Faculty Ethics Committee (File no: 915/25.10.2018).

Genomic DNA Isolation from Blood: After obtaining informed consent, from the participants, all venous blood samples of the patient and control groups were taken into the tubes with EDTA in a volume of 5 ml. DNA isolation was performed by using a of iPrep DNA Extraction Robot (Invitrogen, Carlsbad, California, USA), DNA purity and concentrations of isolated samples are determined by NanoDrop (Invitrogen, Carlsbad, California, USA).

Genotyping analysis: The allele and genotype frequencies determined according to Hardy-Weinberg equilibrium (HWE). Genetic analysis performed by using the 7500 Fast-Real-Time Polymerase chain reaction device (Applied Biosystems, Foster City California, USA). By Real-Time PCR, fluorescence dyes of probes are utilized to determine the single nucleotide polymorphisms (SNPs). There are two TaqMan probes, one prob is labeled a FAM dye and the other prob is labeled a VIC dye. The probes with fluorescence dye used in the Real-Time PCR have two different wavelengths for allele specific, which are wildtype and mutant

alleles, detection. The primer sequences of *COMT* determined by Forward 5' –GGA GCT GGG GGC CTA CTG TG- 3' and Reverse 5' –GCC CTT TTT CCA GGT CTG ACA- 3' primers. A region of the gene was generated by genotyping and *COMT* (rs4680) polymorphism was analyzed. The focused gene region of genotyping was *rs4680* (G>A) for the *COMT* gene. The conditions for Real-Time PCR were arranged by waiting for 10 minutes at 95° C, accomplishing denaturation for 15 seconds at 92° C for each cycle and also connecting/elongation for 1 minute at 60° C for each cycle.

Statistical Analysis

The data obtained from genotyping was evaluated using Chi-square and Fisher's Exact Tests via the SPSS 25.0 Program. Student's t-test was used to analyze numeric values. P-values less than 0,05 were considered statistically significant.

Results

As a result of the Demographic data analysis, body mass index, body surface area and fasting blood glucose values in ovarian cancer patients were found to be significantly higher than the control group. There was no difference between the patients and controls regarding mean age ($p= 0.154$). While significant differences were detected in diabetes ($p<0.001$), and smoking ($p<0.001$) and these risk factors were found to be statistically meaningful among groups. Also menopausal status (premenopausal and postmenopausal), pregnancy status (number of pregnancies ≤ 1 or number of pregnancies >1) and parity (number of births ≤ 1 or number of births > 1) are statistically meaningful. In the patients with ovarian cancer, the rate of postmenopausal women (80.9%) was higher than the control group (40.4%). The controls whose number of births were less than or equal to one (number of births ≤ 1) (54.4%) were found to be statistically higher than the patient group (26.7%). The rate of postmenopausal women (80.9%) in the patients with ovarian cancer was higher than the control group. While the rate of pregnancy status (number of pregnancies ≤ 1) (54.4%) in the control group was higher than the patient group. (Table 1).

In the present study, in the patients with ovarian cancer, the distribution of premenopause (80%) was analyzed to be high in comparison with postmenopause state (20%). When we evaluated in terms of relapse and metastasis of tumors, tumor statements were obtained respectively as 45.5% and 75%. The treatment parameters were resulted that 72.1% of the patients received adjuvant chemotherapy and 27.9 % of the patients did not receive adjuvant chemotherapy. While 36.4 % of the patients received neoadjuvant chemotherapy and 63.6 % of the patients did not receive neoadjuvant chemotherapy. 47.5% of the patients underwent debulking surgery. Also it was observed that the rates of being pregnant more than once (77.8%) and a number of births (> 1) were high (73.3%) in the patients with ovarian cancer. The ratio of patients at stage III (42.9%) were found to be high as compared to stage I (23.8%), stage II (23.8%) and stage IV (14.3%). When we evaluated the tumors in terms of cell types, epithelial tumors were found at a high rate (92.3%) in comparison to the other types. Serous epithelial tumors from epithelial tumor types were calculated in a ratio of 56.4%. The ratio of sex-cord stromal tumors (5.3%) was found to be higher than germ cell tumors (2.6%)

The allele and genotype frequencies for *COMT Val158Met* (rs4860) polymorphism in groups are shown in Table 2. In the present study, found no statistically significant findings among the two groups as patients with ovarian cancer and healthy control groups ($p=0.413$).

Although there were no significant difference, G allele (56.4%) in patient group was found to be higher than the control group (48.9%) ($p=0.389$). Accordingly, the value of A allele is also not statistically significant distinctly among groups like in G allele ($p=0.301$).

Discussion

Several molecular signaling pathways as hormone signaling, apoptosis, angiogenesis and oxidative stress, play crucial roles in ovarian cancer such progression of ovarian cancer (11). When the individuals with ovarian cancer are at a late-stage, 70% of patients are able to be diagnose. On the other hand, symptoms of ovarian cancer are not clear, and survival rate is almost 90% for 5-years during the first stages (12).

It is considered that COMT enzyme plays a significant role in estrogen metabolism based on many different studies (13-17). To give an example, Tolba et al. investigated that COMT might be as a biomarker, which can be important factor in suppressing tumor development and treatment of cancer (18). In estrogen metabolism, COMT prevents DNA damage, therefore, it is called the gate- keeper. Wu et al. reported that *COMT* gene transcription is decreased related to epigenetic changes such as DNA methylation (19). In light of these developments, present study will contribute to understanding molecular mechanisms for ovarian cancer.

Ovarian cancer development is influenced by various risk factors such as BMI, age, tumor histology, family history of patients with ovarian cancer and smoking. Therefore, there are many factors that create a risk for ovarian cancer. In a nutshell, it is not sufficient to diagnose individuals simply because they carry the *Val158Met* polymorphism in the *COMT* gene (14) Goodman et al. reported that *COMT Val158Met* polymorphism was not related to ovarian cancer risk due to a limited sample size. That study contained 108 cases and 106 controls from the German population. The ratio of heterozygote genotype carriers (50%) with ovarian cancer was found to be high in comparison with homozygote wild and variant type genotype carriers (25%, 25%) in the cases. There was no evidence that *COMT Val158Met* polymorphism increases the risk of ovarian cancer ($p=0.73$), and Goodman et al. implied that advance studies are required to explain different combinations of polymorphisms in estrogen metabolizing enzymes (15).

A multigenic model constructed by eleven gene variations that including the *COMT Val158Met* polymorphism, was performed by Delort et al. Although there was no significant difference, they suggested that heterozygote *COMT Val158Met* polymorphism was one of high risk genotype. Heterozygote *COMT Val158Met* genotype could have possible effect on ovarian cancer by reducing activity of phase II enzyme that decrease the elimination of carcinogens. (16). There are two meta-analysis performed in order to determine the role of *COMT Val158Met* polymorphism in over cancer susceptibility and both of them could not find any associations (17, 18). But it should not be forgotten that meta analysis data could be tested in clinical studies by recruiting homogeneous patients and controls. Moreover, in meta-analysis there are population bias caused by homogeneity, and they did not implicate any data for Turkish population. Thus, our study aim to provide reliable data about the role of *COMT Val158Met* polymorphism in ovarian cancer due to these uncertain results.

Conclusion

Owing to *COMT* polymorphism regulates enzyme capacity, *COMT* gene has a significant role in estrogen metabolism on hormone based gynecological cancers. Although, there were no statistically significant difference between the patient and control groups in present study, associations between *COMT* gene variations and ovarian cancer risk should be investigate in large sample size of study groups in a Turkish population. Also, we proposed that this study should be performed further in the future due to some limitations such as patients' medical history.

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Table 1. Demographic data related to the patients with ovarian cancer and healthy controls				
		Control Group (n=47) %	Patient group (n=47) %	p-value
Body Mass Index, $\bar{x} \pm SD$ (kg/m ²)		23.04± 3.62	29.31± 5.37	< 0.0001* (S)
Age, $\bar{x} \pm SD$ (years)		51.11±12.86	54.87±12.55	0.154 (NS)
Body Surface Area, $\bar{x} \pm SD$ (m ²)		1.66±0.14	1.77±0.15	< 0.0001* (S)
Fasting Blood Glucose, $\bar{x} \pm SD$ (mg/dl)		86.45±7.60	108.52±33.28	< 0.0001* (S)
Smoking	Yes %	(n=26) 55.3%	(n=37) 82.2%	< 0.0001* (S)
	No %	(n=21) 44.7%	(n=1) 17.8%	
Menopause	Postmenopause %	(n=19) 40.4%	(n=38) 80.9%	< 0.0001* (S)
	Premenopause %	(n=28) 59.6%	(n=9) 19.1%	
History of Diabetics	Yes %	(n=0) 0%	(n=12) 26.7%	< 0.0001* (S)
	No %	(n=47) 100%	(n=33) 73.3%	
Parity	≤ 1	(n=27) 54.4%	(n=12) 26.7%	< 0.0001* (S)
	>1	(n=20) 42.6%	(n=33) 73.3%	
Pregnant State	≤ 1	(n=27) 54.4%	(n=10) 22.2%	< 0.0001* (S)
	>1	(n=20) 42.6%	(n=35) 77.8%	

(n: number of sample, $\bar{x} \pm SD$: mean value ± Standard deviation, *(S)=significantly different (p< 0.05), NS= non significant (p>0.05)). Method for statistical analysis: The difference between the groups was analyzed by the chi-square test and independent sample student t-test

Table 2. The genotype and allele distributions for the COMT Gene between the patient and control groups

	Control group (n=47)	Patient group (n=47)	p value	Odd Ratio (OR)	Confidence interval 95%
COMT genotype	-	-	0.413	-	-
GG	(25.5%) n=12	(37.8%) n=17	0.216 (NS)	1.77	0.727-4.314
GA	46.8% n=22	(42.2%) n=19	0.658 (NS)	0.83	0.364-1.892
AA	(27.7%) n=13	(20%) n=9	0.547 (NS)	0.747	0.289-1.932
Allele count					
G	(48.9%) 46	(56.4%) 53	0.389 (NS)	1.529	0.579-4.037
A	(51.1%) 48	(39.4%) 37	0.301 (NS)	0.630	0.262-1.516

n: number of sample, NS= non significant ($p>0.05$). Method for statistical analysis: The difference between the groups was analyzed by the chi-square test