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YEDITEPE UNIVERSITY

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DEPARTMENT OF MOLECULAR MEDICINE

INVESTIGATION OF CATECHOL-O-METHYLTRANSFERASE (COMT) GENE VAL158MET POLYMORPHISM IN OVARIAN CANCER

MASTER OF SCIENCES THESIS

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 28.06.2019 and numbered 2019/11-13

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DECLARATION

I have gained all the information for this thesis within the bounds of academic and ethical rules, I have cited all the information and interpretations not obtained by this thesis, and I have listed these sources in the list of references again, I have pledge that this thesis work is my own work, from the planning of the thesis to the writing, I declare that there is no violation of the patent and copyrights during the study and writing of this thesis.

28.06.2019

İpek Yağmur ABAOĞLU

DEDICATION

To my whole family

To my invaluable teacher Prof. Dr.Turgay İSBİR

To my friends who encourage and support me.

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LIST OF SYMBOLS AND ABBREVIATIONS

BMI	:Body Mass Index
CI	:Confidence Interval
CT	:Computed Tomography
COMT	:Catechol-O-Methyltransferase
CST®	:ChargeSwitch® technology
CYP1A1/1A2	:P 450 1A1, 1A2
CYP1B1	:Cytochrome P 4501B1
dsDNA	:Double-Stranded DNA
FIGO	:International Federation of Gynecology and Obstetrics
E1	:Estrone
E2	:Estradiol
E3	:Estriol
EDTA	:Ethylenediaminetetraacetic acid
GLOBACAN	:Global Cancer Observatory
GSTP1	:Glutathione-S-Transferase
MB	:Membrane Bound
MB-COMT	:Membrane-Bound Shape
MRI	:Magnetic Resonance Imaging
NAC	:N-Acetylcysteine
NAT2	:N-acetyltransferase 2
NEOC	:Non-Epithelial Ovarian Cancers
OR	:Odd Ratio
PET	:Positron Emission Tomography
PARPs	:Poly (ADP-ribose) polymerases
Res	:Resveratrol
S	:Cytosolic
SAM	:S-adenosyl-L-methionine

SAH	:S-adenosyl-L-homocysteine
S-COMT	:Soluble Shape
SNPs	:Single Nucleotide Polymorphisms
ssDNA	:Single-Stranded DNA
SPSS	:Statistical Package for Social Science
WHO	:World Health Organization
2/4-MeOE1/E2	:2/4-methoxy-estrone/estradiol

ABSTRACT

Abaoğlu İ.Y. (2019). Investigation of Catechol-O-Methyltransferase (COMT) Gene Val158Met Polymorphism in Ovarian Cancer. Yeditepe University, Health Sciences Institude, Department of Molecular Medicine. Master Thesis. İstanbul.

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. The COMT enzyme synthesized from the Catechol-O-Methyltransferase (COMT) gene detoxifies the carcinogenic catechol estrogens. This enzyme catalyzes the methoxylation reaction to produce 2-methoxy estradiol. This metabolite induces apoptosis and suppresses carcinogenesis. 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. This thesis 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. The two groups were seperated as a patient group with ovarian cancer (n = 47) and control group (n = 47). Genotyping of both groups was determined by Real-Time PCR, and the statistical analysis of the data was performed by SPSS program. According to our results, GG genotype (homozygote wild type) was found in 12 (25.5%), GA genotype (heterozygote type) in 22 (46.8%), AA genotype (homozygote variant type) in 13 (27.7%) in the control group statistically. In the patient group, genotype distributions were determined 17 (37.8%), 19 (42.2%) and 9 (20%) respectively. There was no significant relationship in comparison with genotypes between patient and control groups (p=0.413). This study could provide a novel approach for the clinical treatment of ovarian cancer.

Key words: Polymorphism, Catechol-O-Methyltransferase, Catechol Estrogen, Ovarian Cancer.

ABSTRACT (Turkish)

Abaoğlu İ.Y. (2019). Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Gen Val158Met Polimorfizminin Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Yüksek Lisans Tezi. İstanbul.

Yumurtalık kanseri dünyada yedinci önde gelen ölüm nedenidir ve kadınlar arasında en yaygın sekizinci kanserdir. Bütün jinekolojik kanserler arasında en yüksek ölüm oranına sahiptir. Yumurtalık kanseri olan hastalar için özellikle de hastalık ileri safhada teşhis edildiğinde prognozu kötüdür. Katekol-O-Metiltransferaz geninden sentezlenen COMT enzimi karsinojenik olan katekol östrojenlerini detoksifiye eder. Bu enzim metoksilasyon reaksiyonunu katalizleyerek 2-metoksi östradiol üretir. Bu metabolit, apoptozisi uyararak karsinojenezi baskılar. COMT geninde Val158Met polimorfizmi bu enzimin aktivitesinin azalmasına yol açarak karsinojenik katekol östrojenlerinin birikimine neden olur. Bu tez çalışmasında, yumurtalık kanseri riskini etkilediği düşünülen COMT Val158Met polimorfizmi ile hastalığın ilişkisinin incelenmesi amaçlanmıştır. Yumurtalık kanseri olan hasta grubu (n=47) ile kontrol grubu (n=47) olmak üzere iki gruba ayrıldı. Her iki grubun genotiplemesi gerçek zamanlı PZR ile belirlenmiş ve verilerin istatistiksel analizi SPSS programı kullanılarak gerçekleştirildi. Sonuçlarımıza göre, kontrol grubunda GG genotipi (homozigot yabani tip) 12 (% 25.5), GA genotipi (heterozigot tip) 22 (% 46.8), AA genotipi (homozigot varyant tip) 13 (% 27.7) bulundu. Hasta grubunda genotip dağılımı sırasıyla 17 (% 37.8), 19 (% 42.2) ve 9 (% 20) olarak tespit edildi. Hasta ve kontrol grubu arasında genotiplerle karşılaştırıldığında anlamlı bir ilişki bulunmadı ($p = 0.413$). Bu çalışma, yumurtalık kanserinin klinik tedavisi için yeni bir yaklaşım sağlayabilir.

Anahtar Kelimeler: Polimorfizm, Katekol-O-Metiltransferaz, Katekol Östrojeni, Yumurtalık Kanseri.

1. INTRODUCTION and PURPOSE

In clinical medicine, cancer is a multifactorial disease that is one of the very widespread diseases in the world and the main cause of morbidity globally (1,2).

The most frequent type of female malignancies is ovarian cancer which has the highest death rate and causes many new cases each year (3,4). Global Cancer Observatory (GLOBACAN) stated that there were 239.000 cancer cases which resulted in 152.000 deaths with ovarian cancer. Ovarian cancer is the seventh most frequent type of cancer (3.6 % of cases), also it is the eighth (4.3 %) in terms of cancer-related deaths in women.

In developed countries, the rate of occurence is highest, at more than 7.5 per 100.000. The risk of ovarian cancer mortality in people under 75 years old is two times more in developed countries than underdeveloped countries (5). According to findings by World Health Organization (WHO) in Turkey, there were 3.729 incidences and 2.191 mortality cases in 2018. Furthermore, it's expected that incidence (3.939 cases) and mortality cases (2.336) will increase among females in 2020 (6).

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 (7,8).

The Catechol-O-Methyltransferase (COMT) gene is located on chromosome 22, which consists of six exons and its protein is synthesised as two different types called membrane-bound (MB) and cytosolic (S) forms. The molecular weight of the MB-COMT protein is 30kd, whereas S-COMT protein is 24kd (9). There is a large quantity of COMT in different human tissues such as liver, breast, ovarian and kidney tissues (10). COMT is a significant functional enzyme which metabolizes estrogens, and it is included in the conjugating and deactivation of estrogen catechol metabolite (11). The functional polymorphism in the COMT gene occurs with the base pair change from G to A.This polymorphism called Val158Met (rs4680) results in the replacement of the

valine with methionine (12). It is assumed that this exchange plays an important role in leading to three to four times reduction in COMT activity (13,14).

It has been shown that COMT is associated with the risk of hormonally affected cancers (12,15). **C**onsequentially, the altered COMT gene expression with inactivating catechol estrogens has reduced via generating mediate products catechol estrogen metabolism (15,16). Owing to the production and accumulation of catechol estrogens that lead to have oncogenic activity, the people with COMT-Met polymorphism could have higher risk of ovarian cancer (17).

The objective of this study is to determine to possible relation between Val158Met polymorphism of COMT gene and ovarian cancer in a Turkish population. The COMT gene Val158Met polymorphism was analyzed by using the Real-Time Polymerase Chain Reaction method and we investigated genotypic and allelic differences between the healthy controls and patients with ovarian cancer. Moreover, our results could present scientific data for population variations as a factor. At the same time, this study could provide a novel approach for the clinical treatment of ovarian cancer.

2. LITERATURE REVIEW

2.1. Cancer

Cancer is considered a genetic disease because of its relation to the genetic material of the cell. The hereditary or environmental factors may cause cancer. The basic mechanism of carcinogenesis is a non-lethal genetic damage in the cell. These damages are caused by inactivation and activation in genes that activate or suppress cell proliferation respectively, it occurs as a result of the change in DNA repair genes associated with the programmed death of the cell (apoptosis) (18).

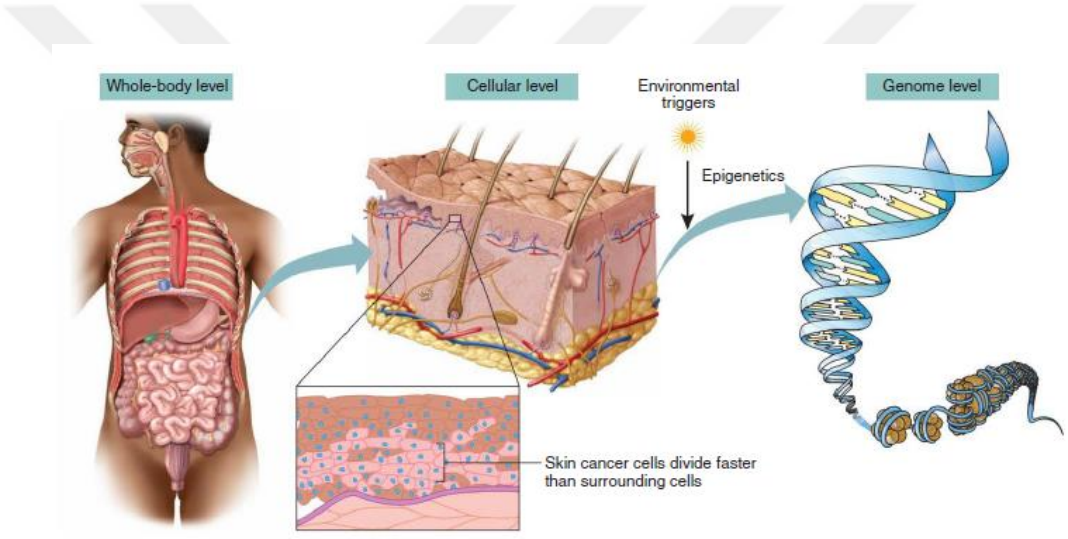


Figure 2.1. The representation of cancer levels (19).

Cancer can be seen various at levels, such as whole-body level, cellular level and genome level in the body (Figure 2.1.) (19).

Through the statistical analysis it is shown that the incidence of all kinds of cancers is 210.2 per hundred thousand between the years of 2010-2014, according to the standardized age and for both sexes in Turkey. The incidence at the standardized age is 173.6 per hundred thousand for females, and 246.8 per hundred thousand for males in 2014. In 2014, 163,417 individuals were diagnosed with carcinoma in Turkey. As the data of the last five years displayed, there is no increase or decrease in the frequency of cancer in both males and females (20).

On the one hand, it is estimated that there are 1,762,450 new cancer cases and 606,880 cancer deaths according to the American Cancer Society' studies in the United States in 2019 (21).

2.2. Gynaecological Cancers

The three most prevalent gynecological malignancies are ovarian, cervical and uterine cancer. Gynecologic malignancies are commonly fatal in the current gynecologic cancer's treatment. The treatment options have provided extensions women's in life expectancy. New therapy strategies have accelerated survival rates due to novel molecular therapies (22).

In 2018, it was estimated that 110,070 women were diagnosed with gynecological cancer in the United States and this resulted 32.120 deaths. Molecular and genetic bases of the ovarian cancer which is the main subject of our study, accounts as the fifth most common cause of cancer-related death among women in the United States, while uterine cancer is the 6th most common cause of cancer-related deaths in women (23).

2.3. Structure, Histological and Physiological Characteristics of Ovaries

The ovary-originated various cancer types are better clarified by understanding embryogenic development and the anatomy of the ovary (24). In the female, the pelvic reproductive organs are ovaries which produce sex hormones such as progesterone and estrogens (25). It is ovoid shaped and there are two ovaries located on both sides of the uterus (26). According to the examinations ovarian length is on average 13 mm, thickness is 4 mm, width is 5.7 mm, and volume is 125.88 mm^3 (27).

Structure of ovaries is included two types of components: cortex located outer and medulla located inner. In the connective tissue embedded part of the cortex is the follicles, that contain the female gametes called oocytes (28), and the cortex includes interstitial cells and corpora lutea (29). As for medulla, it is made up loose connective

tissue and contains blood vein, lymphatic vessels, spiral arteries, hilus cells and neural network (Figure 2.2.) (25,29).

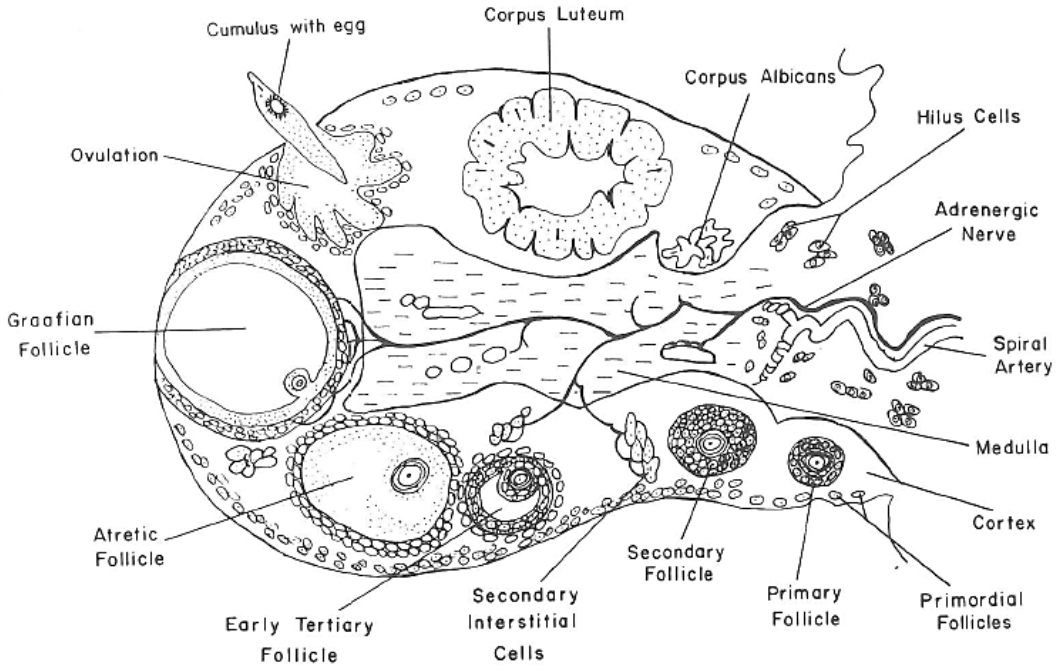


Figure 2.2. The scheme of the human ovary during years of reproduction (29).

2.4. Ovarian Cancer

Ovarian cancer is the most common type among gynecological cancers and accounts for the highest rate of deaths related to gynecological cancers (30,31).

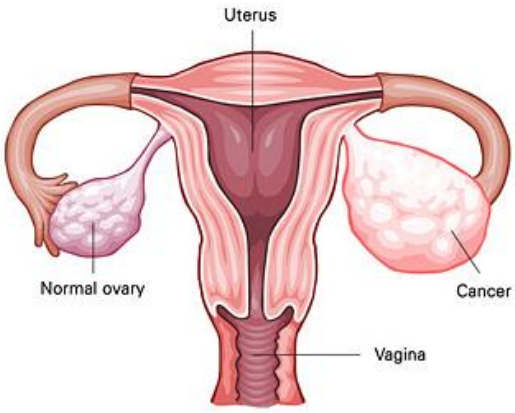


Figure 2.3. The structure of the normal and cancerous ovary (32).

Cancerous tumors stem from one non-normal cell, that cell causes the cancer disease, and spread all ovary (Figure 2.3.) (32).

2.4.1. Incidence and Epidemiology of Ovarian Cancer

Approximately 23,000 new cases are diagnosed with the ovarian cancer in the worldwide every year. The deaths caused by ovarian cancer are estimated to be around 14.000 annually. Incidences of ovarian cancer have been reduced by 1% per year since the mid 1970s (33).

The occurence of ovarian cancer varies by geographical area and age (35). Low rates of ovarian cancer have been determined in Africa and Asia while the highest rates have been revealed in the USA, Northern and Western Europe, but Japan has the lowest rate compared with other developed countries (36).

Five year survival rates are viewed in Table 2.1.,and almost 22,530 new cases of ovarian cancer are determined by the National Cancer Institute in 2019 (34,37).

Table 2.1. The comparison of various cancers diagnosed by National Cancer Institute between 2008 and 2014 (37).

	All stages	Local	Regional	Distant		All stages	Local	Regional	Distant
Breast (female)	90	99	85	27	Oral cavity & pharynx	65	84	65	39
Colon & rectum	65	90	71	14	Ovary	47	92	75	29
Colon	64	90	71	14	Pancreas	9	34	12	3
Rectum	67	89	70	15	Prostate	98	>99	>99	30
Esophagus	19	45	24	5	Stomach	31	68	31	5
Kidney†	75	93	69	12	Testis	95	99	96	74
Larynx	61	78	46	34	Thyroid	98	>99	98	56
Liver‡	18	31	11	2	Urinary bladder§	77	69	35	5
Lung & bronchus	19	56	30	5	Uterine cervix	66	92	56	17
Melanoma of the skin	92	98	64	23	Uterine corpus	81	95	69	16

According to the findings of the Republic of Turkey's Ministry of Health, there were approximately 2,361 women diagnosed with ovarian cancer in 2016 (38).

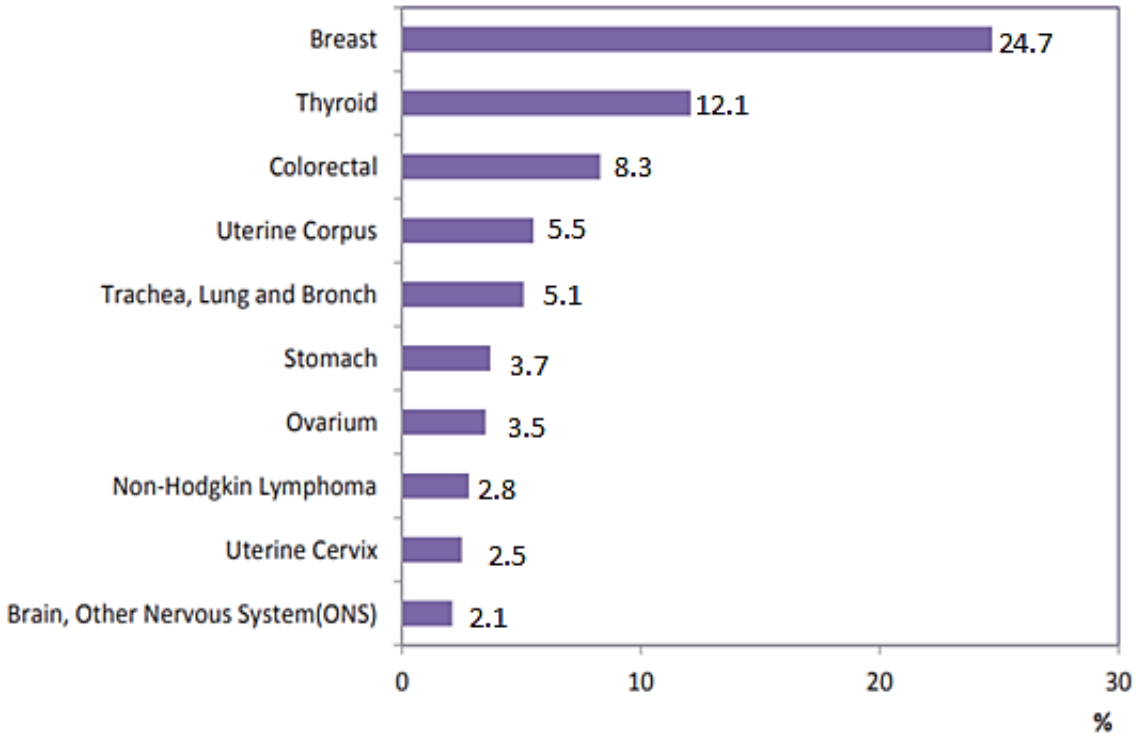


Figure 2.4. The percentage (%) distribution of the most common female cancer types in overall cancers, in 2015 (38).

Figure 2.4. shows that the percentage of ovarian cancer is at 3.5 % in overall cancer among females during 2015 (38).

2.4.2. The Stages of Ovarian Cancer

The clinical condition of malign tumors can be estimated by staging and classification of cancer and so that the appropriate treatment can be determined (39).

In 2014, the International Federation of Gynecology and Obstetrics (FIGO) reorganized the system for doctors to use for the staging of ovarian cancer. In the FIGO system, ovarian cancer consists of four stages, called Stage I, Stage II, Stage III and Stage IV, the first three stages have the sub-stages a, b and c. The stages are determined by whether or not cancer cells spread around the ovaries as well as the size of the cancer. In stage I, cancer cells are seen only in the ovaries and their spread is slow. In stage II and stage III, cells spread out of the ovaries. In stage IV, which is the last stage, cells are seen in the liver or lungs (Figure 2.5.) (40).

70% of the patients are diagnosed during the advanced stages, such as stage III and IV because ovarian cancer does not show any long-term symptoms especially in the early stages of the disease (36).

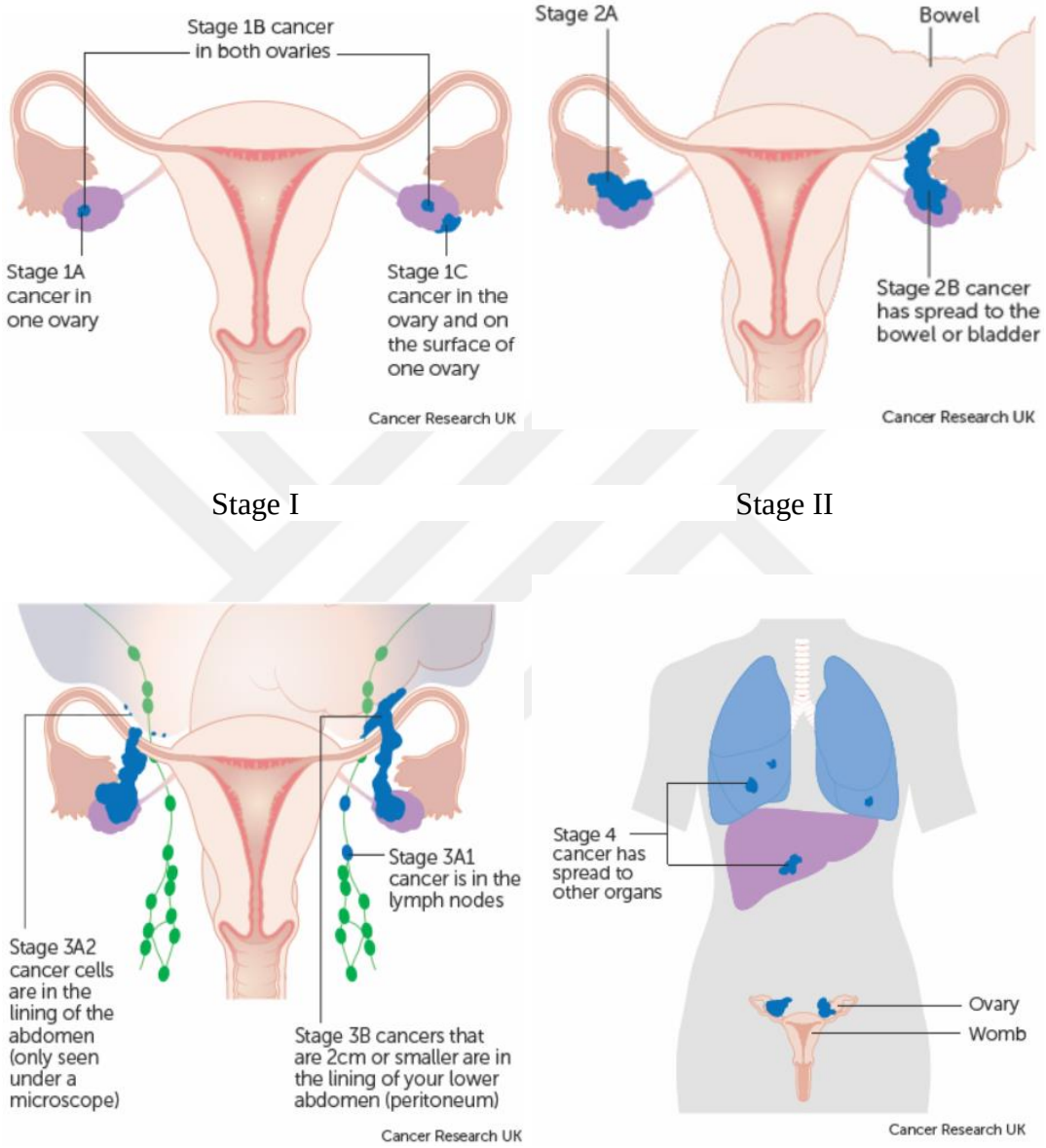


Figure 2.5. The stages of ovarian cancer according to FIGO (40).

2.4.3. The Classification of Ovarian Cancer

The ovarian tumors are categorized according to the different morphological and genetic characteristics. Ovarian cancers are divided into two groups: epithelial and non-epithelial cancers. Non-epithelial ovarian tumors are rare and define as germ cell tumors, sex cord-stromal cell tumours. Non-epithelial ovarian cancers (NEOC) are seen in all age groups, which formed part of 10-15% of ovarian cancers (41).

In addition, the epithelial ovarian tumors were reclassified by the World Health Organization (WHO) after International Federation of Gynecology and Obstetrics (FIGO)'s staging procedures for ovarian cancer in 2014. The new classification is based on current histopathological findings. The FIGO classification is dependant on the tumor grade, while the WHO classification is dependant on the tumor type (42).

The classification of the ovarian tumor by FIGO indicated that the surface epithelium and epithelial ovarian tumors were derived from the ovary, before 2014. According to FIGO, in the more consistent classification it has been shown that a new class called seromucinous tumors are added, but the previous class of the transitional cell tumors were erased from the classification. Thanks to the new classification, it was determined that high grade serous carcinomas were associated with tubal carcinogenesis. In accordance with WHO, classification emphasized that it is not possible to detect the origin of high-grade serous cancers (42,43). The current WHO classification of epithelial ovarian tumors includes six subgroups:

Serous tumors (Serous low-grade carcinoma and Serous high-grade carcinoma)

Mucinous tumors

Endometrioid tumors

Clear cell tumors

Transitional cell tumors

Seromucinous tumors (42).

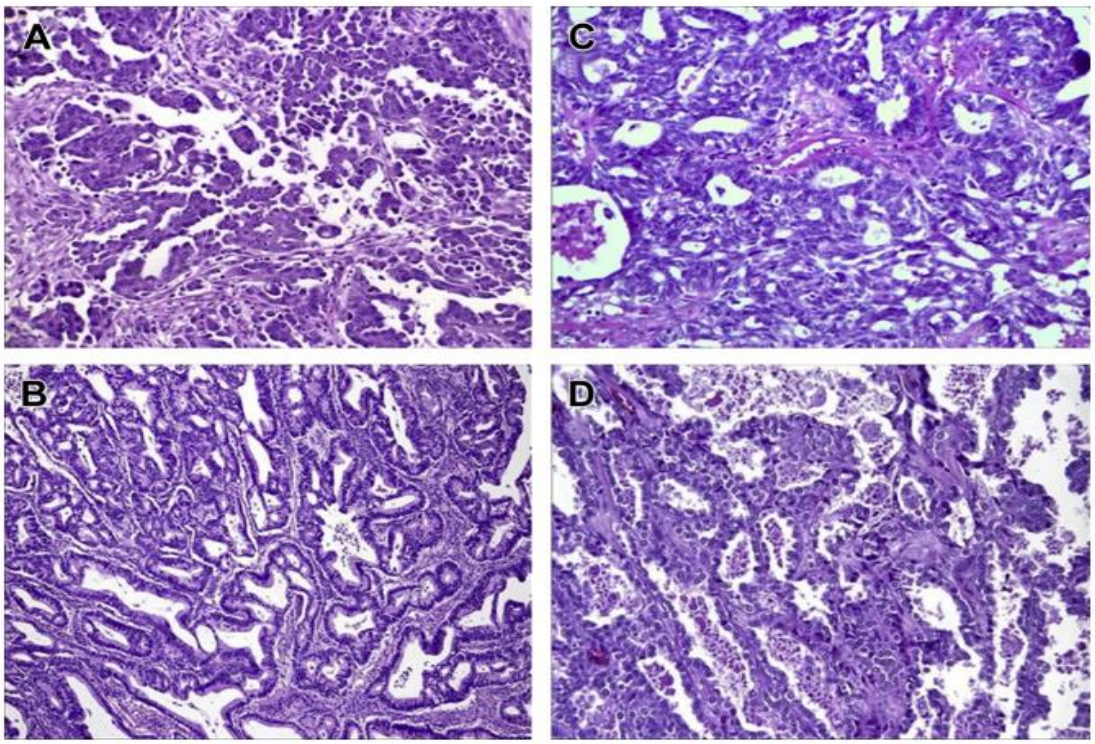


Figure 2.6. The presentation of types of ovarian carcinoma (A) Serous tumor (B) Mucinous tumor (C) Endometrioid tumor (D) Clear Cell tumor. (Stain: hematoxylin and eosin) (44).

The FIGO classification ensures precision treatment for patients with ovarian cancer via the molecular classification of gynecologic malignancies. Moreover, these classifications are able to clarify to the patients' clinical risk accurately (45).

2.4.4. Risk Factors of Ovarian Cancer

There are a number of risk factors for ovarian cancer. The first one is the family history, especially the first and second degree family members diagnosed with ovarian cancer. However, ovarian cancers are mostly detected as sporadic, although ovarian cancers are about 5% to 10%. On the other hand the age is also a potentially risk factor on ovarian cancer (46,47,48).

It was indicated that development of ovarian and breast malignancy which were diagnosed at an early stage were significantly associated with family history, by Whittemore et al. and Kauff et al. (49,50).

The other risk factors are nulliparity and infertility, while the multiparity, oral contraceptives, and hysterectomy are protective factors. It has been shown that the risk of ovarian cancer is decreased by half for five years by using oral contraceptives (47).

Table 2.2. The factors affecting Ovarian cancer (51).

Increased Risk	Decreased Risk
Delayed childbearing	Breastfeeding for 18 months or more
Early menarche	Early menopause
Endometriosis	Multiparity (risk decreases with each additional pregnacy)
Estrogen replacement therapy for more than five years	Hysterctomy
Family History suggesting genetic predisposition	Late menarche
Genetic syndromes	Low fat diet
Hight fat diet	Tubal Ligation
Late menopause	
Low parity	

In Table 2.2. factors that increase and decrease the development of ovarian cancer are presented. The factors such as delayed childbearing, early menarche, low parity endometriosis and high-fat diet decrease the risk, while some factors such as early menopause, hysterectomy and low-fat diet increase the risk for ovarian cancer (51).

2.4.5. The Diagnosis and Treatment of Ovarian Cancer

2.4.5.1. Diagnosis of Ovarian Cancer

There are many tests such as imaging tests and blood tests used for the diagnosis of ovarian cancer. After a physical exam is performed by doctors, they may perform some tests for the accurate diagnosis of ovarian cancer. Imaging tests include Ultrasound, Computed Tomography (CT) scans, Barium Enema X-ray, Magnetic

Resonance Imaging (MRI) scans, Chest x-ray, and Positron emission tomography (PET) scans, whereas other tests include Laparoscopy, Colonoscopy, and Biopsy (52).

The undetermined symptoms such as weight loss, abdominal distension and thamuria are seen at an early stage in individuals with ovarian cancer. Due to delays in ovarian cancer diagnosis, it can be diagnosed at an advanced-stage. Unfortunately, in this case the treatment of the disease is not possible (34,53). Early cancer diagnosis improves survival rates, thus new diagnosis strategies are needed for the early diagnosis of ovarian cancer and effective treatment (54).

The routine pelvic and transvaginal ultrasound examination and blood test, which includes the tumor marker cancer antigen 125, is recommended for individuals with a high risk for ovarian cancer. However, there are no effective results with ultrasound examination and blood test (55).

No effective results have been obtained from performing with ultrasound and CA125 in clinical trials. The studies in recent years imply that diagnostic criteria are inadequate to contribute to ovarian cancer screening, thus recent research continues to determine new candidate biomarkers for this disease (56).

2.4.5.2. Treatment of Ovarian Cancer

The treatment options used for ovarian cancer are chemotherapy and surgery over the years. The effected tissue is staged, surgically debulked and chemotherapy is started (57,58). The emergency and more aggressive treatments are applied to the patients with advanced stage ovarian cancer, but it generally results in poor prognosis (59, 60).

Targeted therapy is one of the new treatment modalities, using some substances such as drugs that lead to minimal damage to noncancerous cells, while only cancerous cells are affected. To give an example, Catumaxomab and Poly (ADP-ribose) polymerases (PARPs) have different functions. Catumaxomab targeting tumor cells is a drug, specifically used for people who carry malignant ascites. Poly (ADP-ribose) polymerases (PARPs) are enzymes, that regulate cell survival and death. In ovarian

cancer with BRCA1 and BRCA2 mutation this treatment is used, however, new approaches have determined that ovarian cancers may be resistent to PARP inhibitors (61,62).

2.6. Catechol-O-Methyltransferase (COMT)

2.6.1. General Characteristics and Chromosome Localization

The structural organization of the Catechol-O-Methyltransferase (COMT) gene was described by Lundström et al. (63). Lundstrom et al. used synthetic oligonucleotides to isolate cDNA clones. It was shown that the clones have an open reading frame, a 24.4-kD polypeptide is coded by the clones. The results obtained by DNA analysis considered that many organisms such as humans and monkeys may have 1 gene for COMT (64).

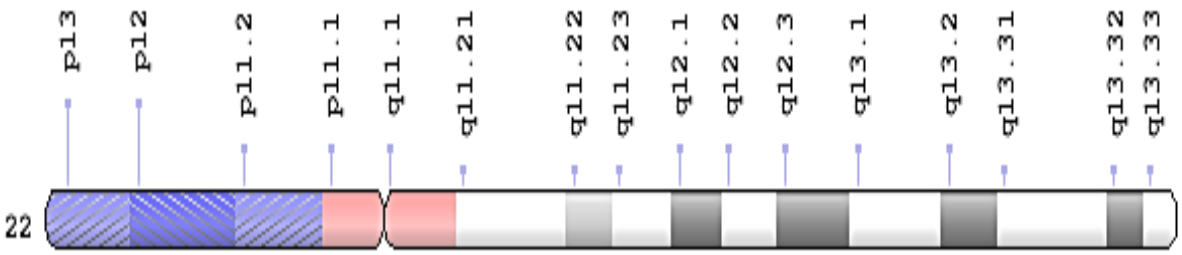


Figure 2.7. The chromosomal location of the COMT gene (65).

In Figure 2.7, the chromosomal location is shown as dark and light bands belonging to the chromosome in the gene. The dark and light bands are obtained by using with a chemical solution microscopically (65).

According to the southern blot analysis, 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 (66,67). COMT gene products are expressed in many tissues like bone marrow, brain, bladder, heart, kidney, liver, lung, ovary and so on (68). The synthesized COMT enzyme participates in the DNA repair mechanism (69).

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 (Figure 2.8.) (70,71).

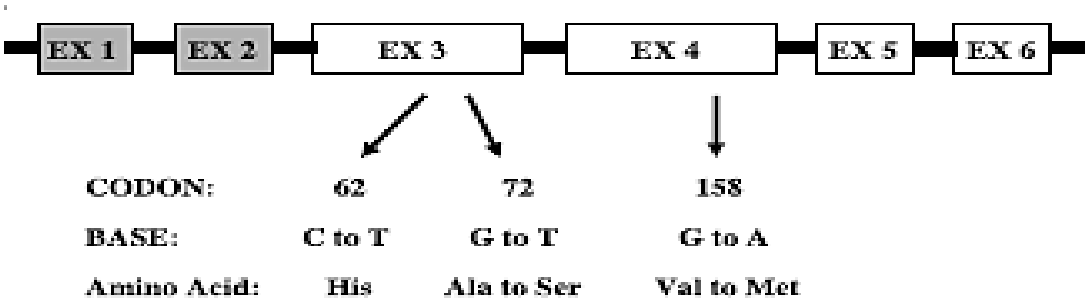
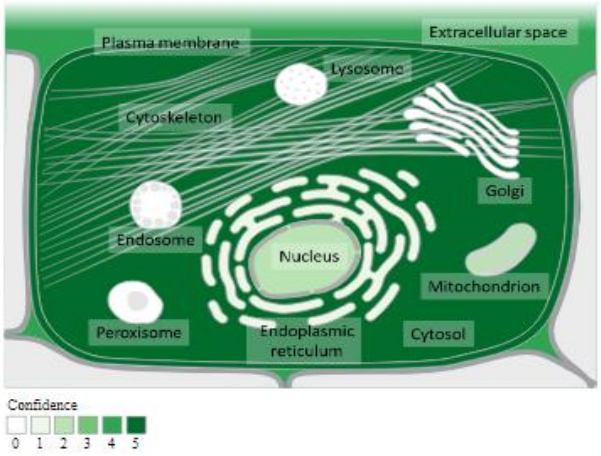


Figure 2.8. Schematic structure of COMT gene (72).

COMT proteins are synthesized from the COMT gene. COMT proteins have been shown to have two groups, these are a soluble shape (S-COMT), which is a shorter form containing 221 amino acids and a membrane-bound shape (MB-COMT) that is a longer form containing 271 amino acids (64,73). S-COMT and MB-COMT proteins differ from each other because they have different N-termini regions (68). The two distinct ATG (which are called start codons) are for promotors P1 and P2; these codons are located on exon 3 of the COMT gene (74).



Compartment	Confidence
plasma membrane	5
cytosol	5
extracellular	4
mitochondrion	2
nucleus	2
endoplasmic reticulum	1

Figure 2.9. Density of COMT protein in the human cell (75).

COMT protein is present in large amounts within the plasma membrane in contrast with the other compartments of the cell (75).

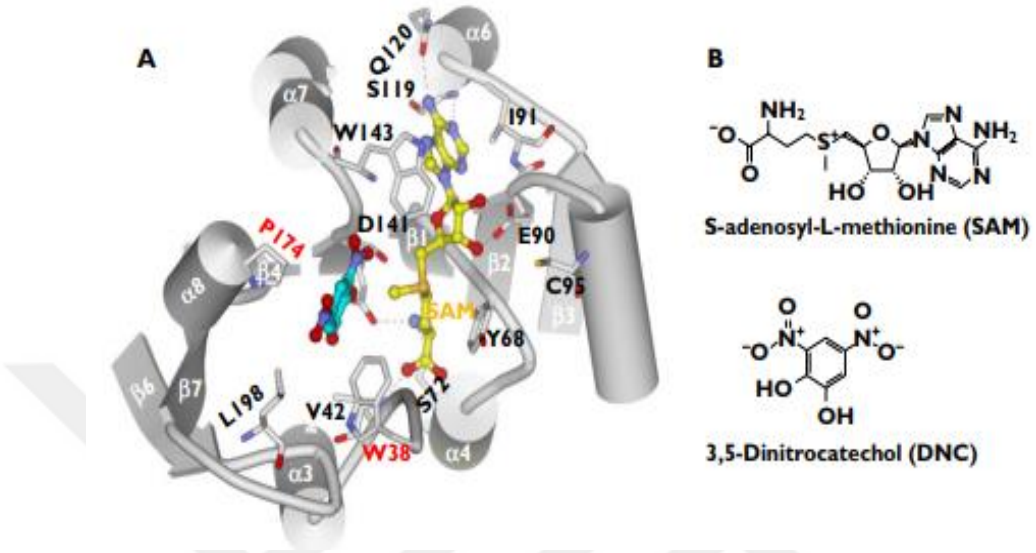


Figure 2.10. A)- Schematical representation of the tertiary structure of the COMT enzyme in the human cell, B)- The structures of S-Adenosylmethionine and 3,5-Dinitrocatechol (76,77).

In the tertiary structure of the COMT enzyme magnesium ion, chloride ion, S-Adenosylmethionine, and 3,5-Dinitrocatechol are present (77). The COMT protein has both the substrate-binding and SAM-binding sites (Figure 2.10.A) (76).

For the first time, Axelrod et al. explained the features of the COMT enzyme by using the soluble part of organ tissues such as the liver in 1958 (78,79).

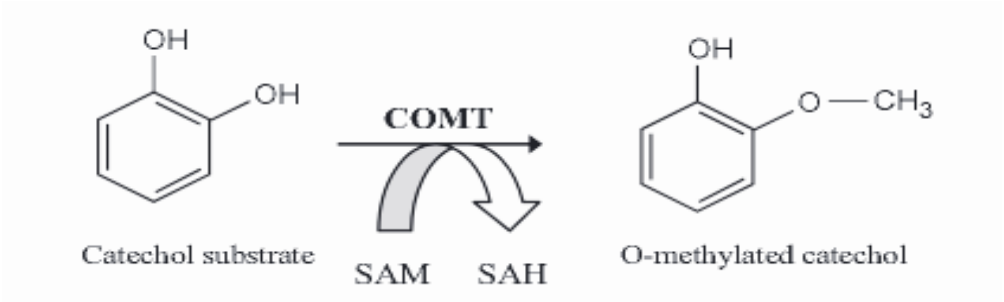


Figure 2.11. Representation of the COMT catalyzed reaction (SAM is explained as S-adenosyl-L-methionine and SAH is described as S-adenosyl-L-homocysteine) (80).

2.6.2. Role of Catechol-O-Methyltransferase Enzyme in Estrogen Metabolism

2.6.2.1. Estrogen Metabolism and Formation of Catechol Estrogens

Cholesterol has a 27-carbon and transforms steroid hormones which have 21-, 19-, and 18-carbons. Cholesterol moves into the mitochondrion in a cell to form pregnenolone or androstenedione as a precursor, after that it goes out from the mitochondria; pregnenolone produces and converts to estradiol step-by-step. Several enzymes such as CYP11A1, P450, CYP17A1, HSD3B2, CYP19A1 and HSD17B1 catalyze steroid hormone synthesis. Estrogens have 18-carbon, a phenol group at 17-carbon, two hydroxylic groups and an aromatic ring such as a phenol group at the third carbon (Figure 2.12.) (81,82,83).

In vivo, the estrogens estrone (E1), estradiol (E2), and estriol (E3) play a crucial role (84). The proliferation of ovarian cells is stimulated by these compounds secreted in ovaries at ovulation (85).

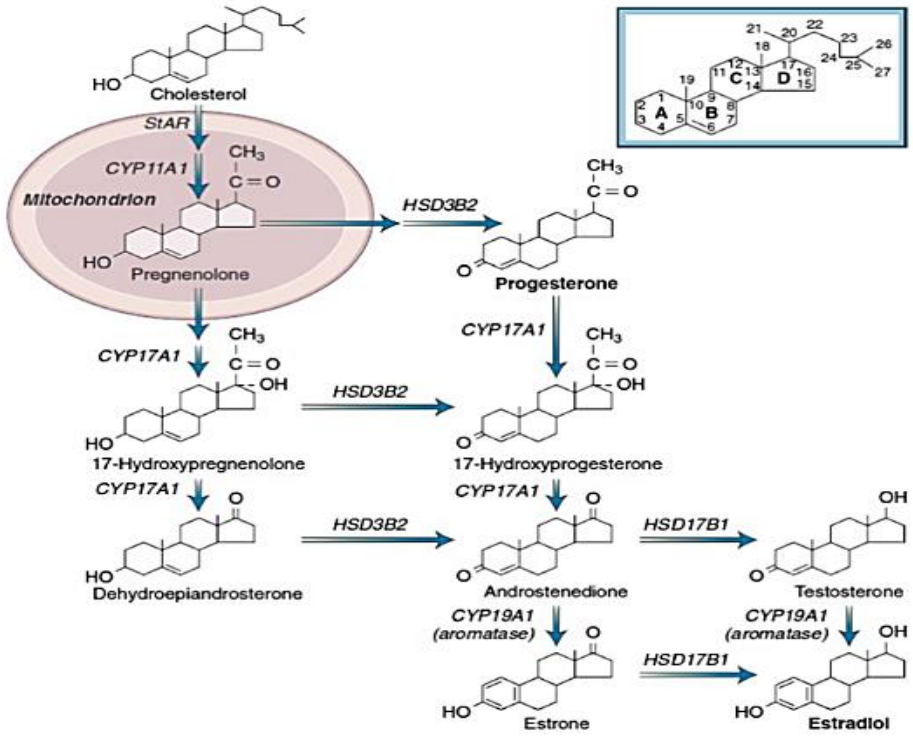


Figure 2.12. The representation of formation of steroid hormone in the human cells (83).

The catechols can stem from endogenous or exogenous substances, therefore the catecholamine and catechol estrogen are both found in endogenous and exogenous substances (86). 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 (87).

Catechol estrogens have reactive and carcinogenic effects. Various enzymes can detoxify these chemical compounds in cells. One of those enzymes is COMT, which formes a methoxy compound. This enzyme catalyzes the transfer of the methyl group in the coenzyme SAM to the hydroxyl group in the catechols (11).

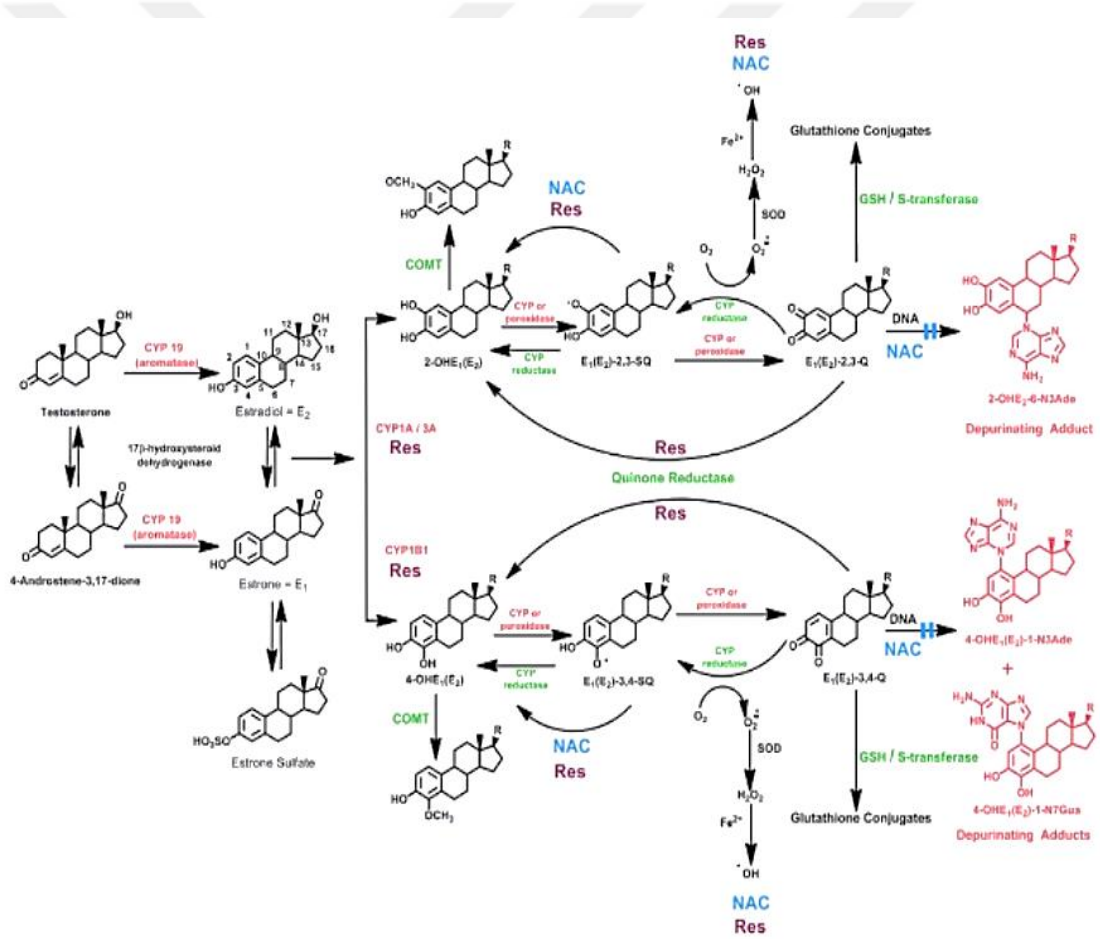


Figure 2.13. The formation of catechol estrogens (88).

In Figure 2.13., protective enzymes are shown in the green color and the enzymes that have a stimulatory effect for the formation of catechol estrogens are shown in red; enzymes and the others shown in red are products of the

apurinic/apyrimidinic site by depurinating DNA adducts in estrogen metabolism. N-Acetylcysteine (NAC) and Resveratrol (Res) affect estrogen metabolism in a positive way by blocking formation of DNA adducts with estrogen (88).

According to research regarding the estrogen metabolism (89,90), it has been implied that estrogen related cancers is initiated by formation carcinogenic catechol estrogens (91,92). The formation of these reactive catechol compounds can damage DNA (84) and have toxic effects against cells (93,94). But, these methoxy estrogens show no estrogenic effects and also lack a strong affinity to estrogen receptors, this reason why these estrogens are inactive (95). In-vitro, 2-methoxy-estrogen have effects on cell lines in cancer against angiogenic and proliferative (96-98) and it has been inhibited DNA synthesis and also mitosis (97).

2.6.2.2. Role of Catechol-O-Methyltransferase Val158Met Polymorphism in Ovarian Cancer

COMT, which is a phase II enzyme (78), is one of the estrogen-metabolizing enzymes (99) catalyzes the methylation of catechol estrogen (14). The other phase II enzymes are Glutathione-S-transferase 1 (GSTP1) and N-acetyltransferase 2 (NAT2). The active estrogen metabolites such as catechol estrogen are inactivated by using these enzymes (100,101).

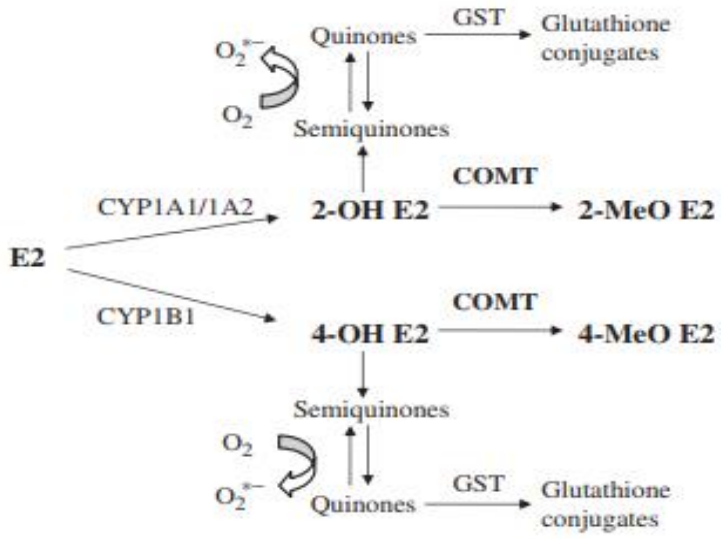


Figure 2.14. The diagram of COMT in estradiol biologically transformation (80).

The polymorphism derived from G to A transition, known as Val158Met in the COMT gene decreases its function. By that transition, valine amino acid converts to methionine amino acid in exon 4. It is shown that Met/Met genotype, which is the homozygous mutant, decreases the COMT enzyme activity by 3-4 times more than the Val/Val genotype called wild-type. The activity of the heterozygous genotype (known as Val/ Met) is intermediate. As a result of COMT, Val158Met polymorphism causes a decrease in enzymatic activity (14).

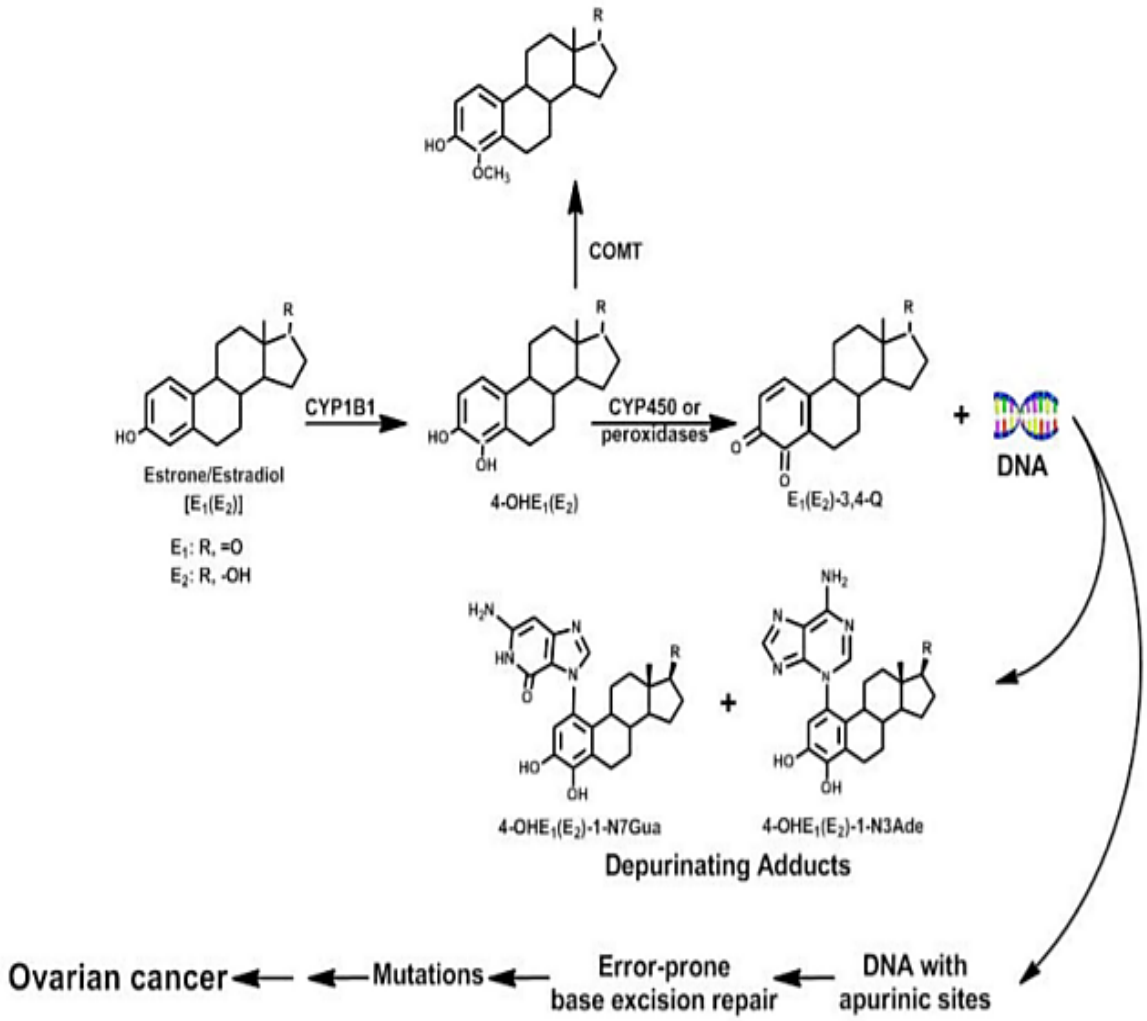


Figure 2.15. The role of the carcinogenic estrogens in cancer initiation (102).

The phase I enzymes involved in estrogen metabolism are cytochrome P450 1A1, 1A2 (CYP1A1/1A2) and cytochrome P 450 1B1 (CYP1B1) (100,101).

By these enzymes (CYP1A1/1A2 and CYP1B1) estrogens are converted to 2-hydroxylated and 4-hydroxylated metabolites (the 2/4-OH catechol estrogens) via hydroxylation (103,104). The 2/4-OH catechol estrogens are converted 2/4-methoxy-estrone/estradiol (2/4-MeOE1/E2) by the COMT enzyme, which catalyzes methylation reactions. Consequently, the 2/4-OH catechol estrogens are inactivated (104). The semiquinones and quinones are produced by oxidization reactions unless 2/4-methoxy-estrone/estradiol are observed. If it is the case, then reactive oxygen species are obtained. These reactive molecules damage the DNA and initiate tumor (105).

In conclusion, COMT detoxifies catechol estrogens, which are carcinogenic metabolites. Furthermore, the metabolite 2-methoxy-estradiol is produced, which causes the suppression of angiogenesis and migration of endothelial cells and cytotoxin; it also stimulates apoptosis, which results in the suppression of carcinogenesis (106).

The aim of this study was to reveal the relationship between the COMT gene polymorphism and ovarian cancer in the Turkish population.

3. MATERIALS AND METHODS

3.1. Sample Selection and Definition

As part of the study, patients diagnosed with ovarian cancer (n=47) and a control group (n=47) were included. The patient group was composed of patients who were diagnosed by the department of Obstetrics and Gynecology, and the healthy control group consisted of healthy individuals. Ethical approval was obtained from Ethics Committee of Yeditepe University for the study (Ethics committee decision no: 915).

Control Group: The control group consisted of healthy individuals aged 18-85 years who were not diagnosed with ovarian cancer following the clinical examination.

Patient Group: The patients were composed of individuals within the Yeditepe University Hospital. They were diagnosed with ovarian cancer and their age range was between 18-85 years old.

3.2. Materials and Devices Used in the Experiment

3.2.1. Materials Used in the DNA Isolation from the Peripheric Blood

The peripheral venous blood samples were set in tubes at -80 °C. The tubes contained Ethylenediaminetetraacetic acid (EDTA), which prevents blood clotting. DNA İsolation Robot (IPrep pure link, Invitrogen and the Thermo Fisher Scientific Inc) system was used for DNA isolation.

The mixture used in the DNA isolation composed of 1.12mg/ml Proteinase K, 8M Guanidine hydrochloride, 10.5 nM EDTA, 10.5 nM NaCl and 10.5 nM Tris-Cl of pH 8,8.

3.2.2. The Equipment Used in the Experiment

DNA Isolation Robot (Iprep Purelink, Invitrogen, Thermo Fisher Scientific Inc), Nanodrop 2000 (Thermofisher Scientific Inc), Real-Time PCR (LIGHT CYCLE 480 II Instrument, Roche Diagnostics, Fast Real Time 7500, Applied Biosystems), 7500 Fast Real-Time PCR Instrument, Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R-Beckman Coulter), +4 C° Refrigerator (Haier), -20 C° Refrigerator (Haier), Ultra-Pure Water (Pure Lab Option Q,Elga), Vortex (V.I. Plus Biosan) and a Pipette Kit (Thermo Fisher Scientific Inc.) were used in this experiment.

3.3. Methods

3.3.1. Genomic DNA Isolation from Blood

All venous blood samples of the patient and control groups were taken into the tubes with EDTA in a volume of 5 ml. Blood samples were stored in a refrigerator at + 4 ° C until DNA isolation. DNA isolation was performed by using a robot of iPrep DNA extraction (Invitrogen), and from the blood genomic DNA isolation Kit with iPrep. DNA can be isolated from 350 µl peripheric blood by this system, so 13 blood samples could be studied at the same time. One cartridge is used for each of samples, the cartridges are agitated for a period of time to bond the magnetic beads to the DNA efficiently before putting the samples to own cartridge.

The robot of iPrep works according to ChargeSwitch® technology (CST®), as an automated extraction method. A high amount of DNA can be prepared from samples with this method. In this method, amounts of genomic DNA are prepared from samples purely by using paramagnetic particles. These particles are surrounded by a DNA-binding surface. CST® (Charge Switch® Technology) extraction method has a unique principle when compared to the silica-based DNA extraction method. The charge of beads can be changed by the pH of its surrounding buffer. In the event of low pH conditions, the backbone of the DNA is negatively charged, then it binds to the positively charged beads. These charged beads are neutralized by using a low salt buffer

that has a higher pH in order to allow for the elution of DNA. Purified nucleic acids pass into the wash buffer, then DNA samples are ready to use (107,108).

At the end of the experiment aqueous DNA samples were obtained and stored at +4 ° C in the refrigerator.

3.3.2. Measurement of DNA Purity

UV absorbance of nucleic acids is measured at 260 nm by UV spectroscopy that called NanoDrop. In this spectrophotometer cuvettes or capillaries are not required. DNA concentrations of both OD260/OD280 and OD260/OD230 proportions are determined by NanoDrop. Thanks to the NanoDrop device, the purity as well as the concentration of nucleic acid molecules such as DNA were able to observed. On the other hand, it has not been shown to distinguish several molecules like RNA, nucleotides, double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) because this device quantifies the absorbance of nucleic acids (109).

In this experiment, we quantified DNA using the NanoDrop 2000 (Thermofisher Scientific Inc). 1,5 µl of DNA samples were used. The DNA samples before measurement were diluted in the ratio of 1/100. The sample was put into place for measurement by opening the arm then the device's arm was turned off. After each measurement, the surface was cleaned by distilled water, and thus it could be safe for the next measurement.

50 µg / ml of double-stranded DNA at a wavelength of 260 nm is equal to one Optical Density (OD) Unit. The purity of DNA samples was measured by analyzing the OD260 / OD280 ratio. The suitable ratio of OD260 / OD280 is between 1.7-1.9 when performing genotyping (110).

The following formula was used to calculate the concentration of DNA at 260 nm.

dsDNA concentration = 50 µg/mL × OD260 × dilution factor

3.3.3. Real-Time PCR Conditions for COMT Polymorphism

Genotyping analysis was performed by using the 7500 Fast-Real-Time Polymerase chain reaction (Applied Biosystems) device with Real-Time PCR.

By Real-Time PCR, fluorescence dyes of probes are utilized to determine the single nucleotide polymorphisms (SNPs). It is a system that allows the genotyping by reading the fluorescence radiations. There are two TaqMan probes, one prob is labeled a FAM dye and the other prob is labeled a VIC dye. The fluorescent dye-bound DNA probes bind to the amplified region. The probes are hydrolyzed by Taq polymerase. Fluorescent signals can be easily detected. 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 sequence of COMT reported by Lundström et al. in 1991 is indicated below. This primer was determined according to the sequence of COMT gene in human cells and it was used in this experiment is used (64). In this method, the region containing this polymorphism was increased by using 5’ –GGA GCT GGG GGC CTA CTG TG- 3’ (Forward) and 5’ –GCC CTT TTT CCA GGT CTG ACA- 3’ (Reverse) 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. Region specific primer and probe sets are used 'TaqMan Genotyping Assays' and fluorescence dyes of probes are given below. Allelic discrimination has been shown using the software of the 7500 Fast Real Time PCR tool.

Forward 5’ –GGA GCT GGG GGC CTA CTG TG- 3’

Reverse 5’ –GCC CTT TTT CCA GGT CTG ACA- 3’ (64).

3.3.3.1. Real Time Protocol

Real-time PCR reagents and the mixture of reaction have been recorded in Table 3.1. Total volume for each sample was determined by the protocol.

Table 3.1. The reaction mixture for the Real-Time PCR

The Material Used	Quantity
Master Mix	5 µl
TaqMan Genotyping Assay	0.5 µl
DNAse, RNAse Free water	3.5 µl
Template DNA	1 µl

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. As illustrated in Table 3.2., denaturation and connecting/elongation were completed for 40 cycles.

Table 3.2. The Real-Time PCR conditions

		40 Cycles	
	Waiting	**Denaturation**	**Connecting/Elongation**
Temperature	95° C	92° C	60° C
Duration	10 minutes	15 seconds	1 minutes

3.3.4. Statistical Analysis

Student’s t-test was used to analyze numeric values. The data obtained from genotyping was evaluated using Chi-square and Fisher’s Exact Tests via the SPSS 25.0 Program for statistical analysis. Chi-square and Fisher’s Exact Tests were used for evaluating the distributions of genotypes and alleles among groups. p-values less than 0,05 were considered statistically significant.

The possible risk factors in ovarian cancer were evaluated by Logistic Regression Analysis.

4- RESULTS

4.1. The Obtained Findings Following Statistical Analysis

Demographic data belonging to the patient and healthy control groups were statistically analyzed as shown in Table 4.1.

Table 4.1. Demographic data related to the patients with ovarian cancer and healthy controls.(n: number of sample, $\bar{x}$ ± SD**:** mean value ± Standard deviation, *(S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$)).

		Control Group (n=47) %	Patient Group (n=47) %	p-value
Body Mass Index, $\bar{x}$ ± SD (kg/m2)		23.04± 3.62	29.31± 5.37	< 0.0001* (S)
Age, $\bar{x}$ ± SD (years)		51.11±12.86	54.87±12.55	0.154 (NS)
Body Surface Area, $\bar{x}$ ± SD (m2)		1.66±0.14	1.77±0.15	< 0.0001* (S)
Fasting Blood Glucose, $\bar{x}$ ± SD (mg/dl)		86.45±7.60	108.52±33.28	< 0.0001* (S)
Alcohol consumption	Yes %	(n=19) 40.4%	(n=1) 2.2%	< 0.0001* (S)
	No %	(n=28) 59.6%	(n=44) 97.8%	
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%	

As a result of the 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 (Table 4.1). There were no differences between the patients and controls regarding mean age (p= 0.154). Other demographic data mentioned in Table 4.1 are diabetes (p<0.001), alcohol consumption (p<0.001), and smoking (p<0.001) and these risk factors were found to be statistically meaninful among groups. Smoking (p <0.001) and diabetes mellitus (p <0.001) were significantly higher in patients with ovarian cancer, while alcohol consumption (p <0.001) was significantly higher in the control group.

Table 4.1 shows that 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%) (Table 4.1). 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.

The demographic and histopathological parameters belonging to the patient group are given in Table 4.2. While the average value of age was determined as 29.31 in the patient group, the value of the BMI was obtained as 54.87. 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. In Table 4.2. it can be observed that the rates of being

pregnant more than once (77.8%) and a number of births (> 1) were high (73.3%) in the patient.

Table 4.2. Demographic and histopathological parameters belonging to the patient group with ovarian cancer

Characteristic features	**Average value ($\bar{x}$ ± SD)**
Age	29.31± 5.37
Body Mass Index (kg/m2)	54.87±12.55
Characteristic features	**Percentage (%) (n=47)**
Menopause	
Postmenopause	20%
Premenopause	80%
Metastasis	
Yes	75%
No	25%
Relapse	
Yes	45.5%
No	54.5%
Adjuvant chemotherapy	
Yes	72.1%
No	27.9%
Neoadjuvant chemotherapy	
Yes	36.4%
No	63.6%
Surgery	
Staging	25%
Debulking	47.5%
Staging+Debulking	32.5%
Pregnant state	
≤1	22.2%
>1	77.8%
Parity	
≤1	26.7%
>1	73.3%

n: number of sample, $\bar{x}$ ± SD: mean value ± Standard deviation

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%) (Table 4.3).

Table 4.3. The percentage distributions of stages, cell types in the patient group

Characteristic features	**Percentage (%) (n=47)**
Stages	
Stage I	23.8%
Stage II	23.8%
Stage III	42.9%
Stage IV	14.3%
Cell Types	
Epithelial tumors	92.3%
Serous tumors	56.4%
Mucinous tumors	10.3%
Endometrioid tumors	5.1%
Clear cell tumors	5.1%
Mixed epithelial tumors	15.4%
Germ cell tumors	2.6%
Sex-cord stromal tumors	5.1%
Germ cell tumors	2.6%
Sex-cord stromal tumors	5.3%

n: number of sample, $\overline{x} \pm$ SD: mean value ± Standard deviation

The ratio of patients with different genotypes in comparison with BMI values are given in Table 4.4. Body mass index ratio (GG, GA, AA, respectively, 27.37, 24.25, 27.10) was found to be nearly the same among all genotypes of COMT gene. Therefore, BMI ratios are statistically meaningful in the patient group. CA125, CEA, CA19_9 and CA15 levels from metabolic parameters were not found to be statistically significant (0.186, 0.161, 0.829, 0.906, respectively) (Table 4.4).

Table 4.4. The distributions of BMI, CA125, CEA and CA19_9 levels according to genotypes of COMT gene polymorphism in the patient group

	GG ($\bar{x}$ ± SD)	**GA ($\bar{x}$ ± SD)**	**AA ($\bar{x}$ ± SD)**	**p-value**
Body Mass Index (kg/m2)	27.37±5.73	24.25±4.74	27.10±5.91	0.041* (S)
CA125	486.88±548	1502.96±1985	1042 ±1951	0.186 (NS)
CEA	16.42±49.62	1.20±0.64	48.15±98.23	0.161 (NS)
CA19_9	265.01±886.51	175.69±469.07	45.34±45.52	0.829 (NS)
CA15	72.43±75.85	106.92±254.94	86.07±93.39	0.906 (NS)

n: number of sample, $\bar{x}$ ± SD: mean value ± Standard deviation, * (S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

In Table 4.5., when we evaluated in terms of metastasis and relapse of ovarian cancer, there was no statistically significant data found (p=0.959, p=0.289 respectively). In the patients with GA and AA genotypes, the ratio of metastasis and relapse of disease was analyzed further in comparison with the GG genotype (Table 4.5.).

Table 4.5. The distribution of metastasis and relapse of ovarian cancer according to genotypes of COMT gene polymorphism in the patient group

	GG % **(n=29)**	**GA %** **(n=41)**	**AA %** **(n=22)**	**p-value**
Metastasis				
Yes	76.5% n=13	72.5% n=13	75% n=6	0.959 (NS)
No	23.5% n=4	27.8% n=5	25% n=2	
Relapse				
Yes	58.8% n=10	66.7% n=6	62.5% n=3	0.289 (NS)
No	41.2% n=7	66.7% n=12	62.5% n=5	

n: number of sample, $\bar{x}$ ± SD**:** mean value ± Standard deviation, * (S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

Table 4.6. shows a comparison between COMT genotypes and tumor stages within the patient group. Following analysis, there were no statistically meaningful findings (p=0.788). The patients' carrier A allele was found to be higher in comparison with carrier G allele (Table 4.6.).

Table 4.6. The percentage distributions of COMT genotypes in comparison with stage I, II, III and IV in the patient group.

Genotypes / Stages	GG % (n=29)	GA % (n=41)	AA % (n=22)	p-value
Stage I	23.5% n=4	23.5% n=4	25% n=2	0.788 (NS)
Stage II	11.8% n=2	17.6% n=3	37.5% n=3	
Stage III	52.9% n=9	41.2% n=7	25% n=2	
Stage IV	11.8% n=2	17.6% n=3	12.5% n=1	

n: number of sample, $\bar{x}$ ± SD**:** mean value ± Standard deviation, * (S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

Table 4.7. shows a comparison between COMT genotypes and various tumor types within the patient group. There were no statistically significant findings (p=0.788, p=0.478). In epithelial tumors, the homozygote wild type (GG), heterozygote (GA) and homozygote mutant type (AA) distributions were calculated respectively as 93.3%, 93.3% and 100% (Table 4.7.).

Table 4.7. The percentage distributions of COMT genotypes in comparison with tumor types in the patient group.

Genotypes / Cell Types	GG % (n=29)	GA % (n=41)	AA % (n=22)	p-value
Epithelial tumors	93.3% n=14	93.3% n=14	100% n=8	0.788 (NS)
Sex cord stromal tumors	6.7% n=1	6.7% n=1	0% n=0	
Mixed epithelial tumors	6.7% n=1	26.7% n=4	12.5% n=1	0.478 (NS)
Serous tumors	66.7% n=10	33.3% n=5	87.5% n=7	
Mucinous tumors	13.3% n=2	13.3% n=2	0% n=0	
Endometrioid tumors	6.7% n=1	6.7% n=1	0% n=0	
Clear cell tumors	0% n=0	13.3% n=2	0% n=0	
Sex cord stromal tumors	6.7% n=1	6.7% n=1	6.7% n=0	

n: number of sample, $\bar{x}$ ± SD: mean value ± Standard deviation, * (S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

4.2. Statistical Evaluation of Real-Time PCR Results

Allelic discriminations were analyzed automatically by the software of the 7500 Fast-Real Time PCR instrument. The readings and interpretations of the fluorescence irradiation are performed by dyes found in the probes. However, some samples could not be discriminated.

FAM dye shows blue color, while VIC dye shows green color. ROX is a reference color for comparing FAM and VIC dyes. Allelic discrimination was analyzed by examining and interpreting the radiance curves (Figure 4.1.).

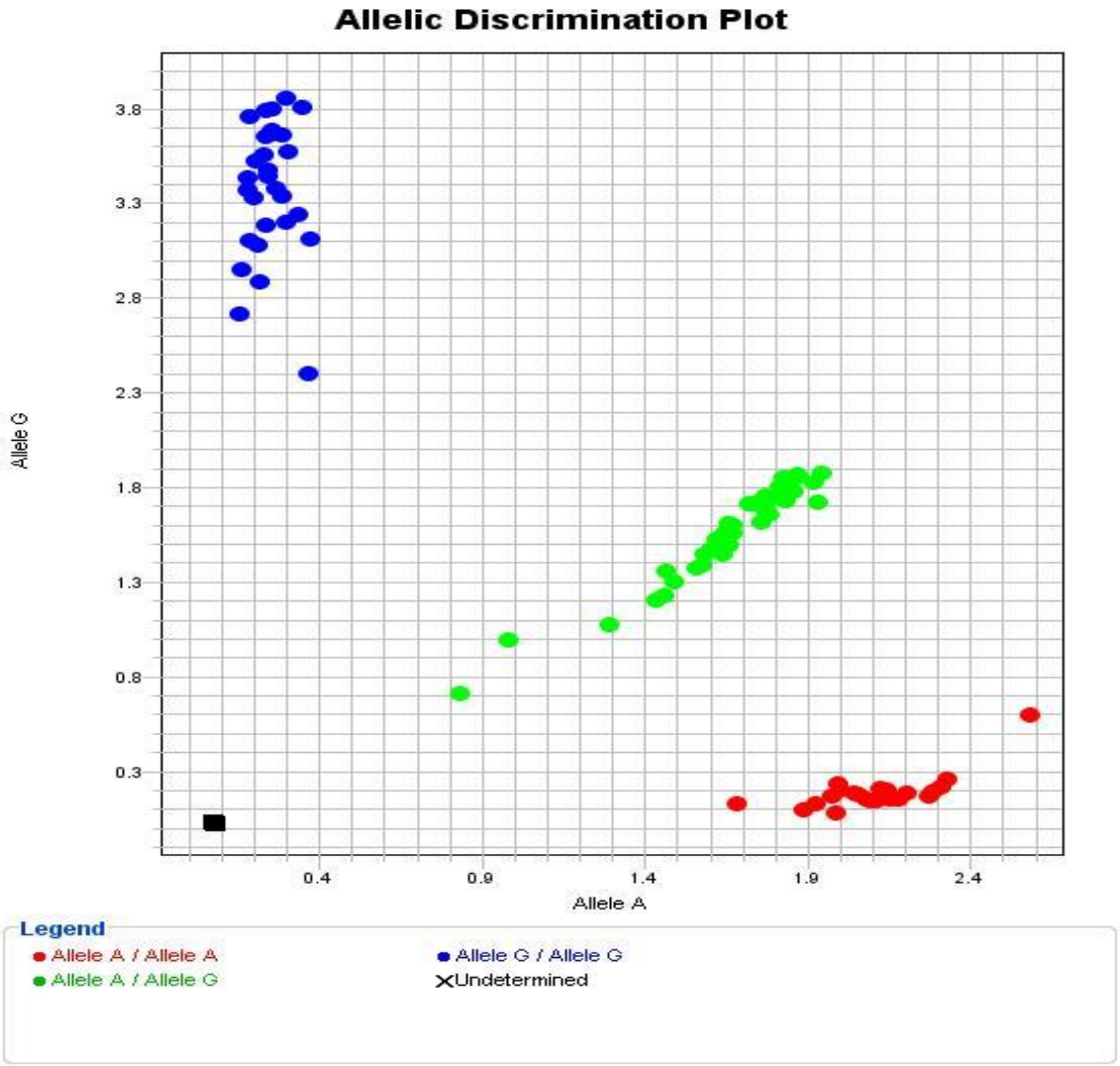


Figure 4.1. Allelic Discrimination Analysis of COMT genotype

GG : Homozygote Wild Type **GA :** Heterozygote **AA :** Homozygote Mutant Type

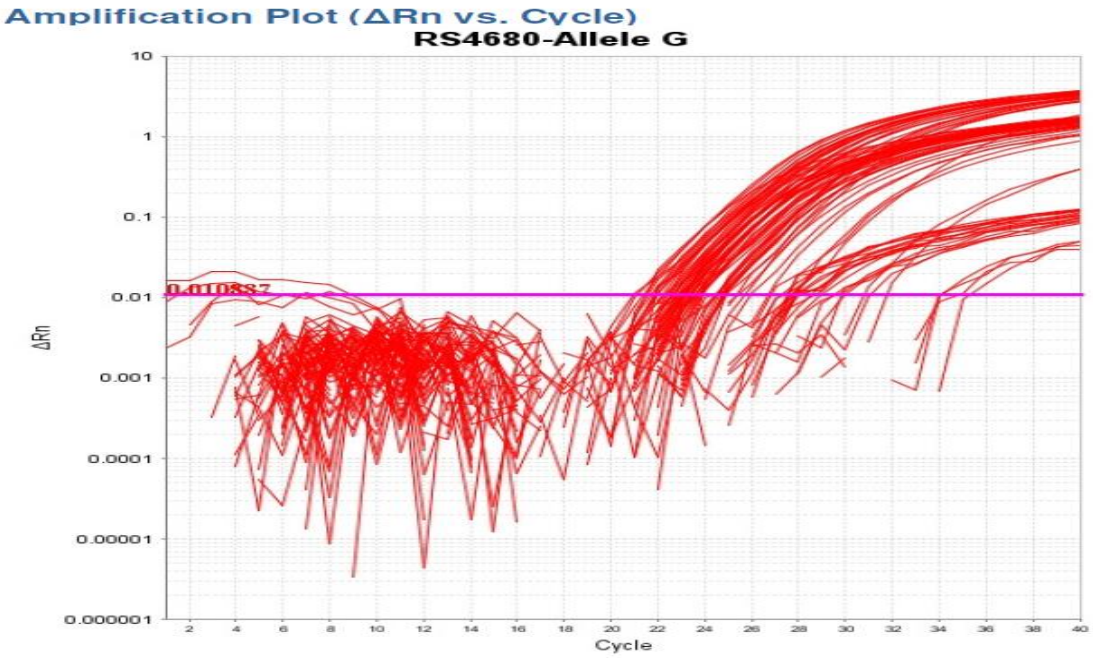


Figure 4.2. Amplification plot display of Allele G

The figure 4.2. shows amplification plots of Allele A. Threshold value (0.0108) is shown as a pink line.

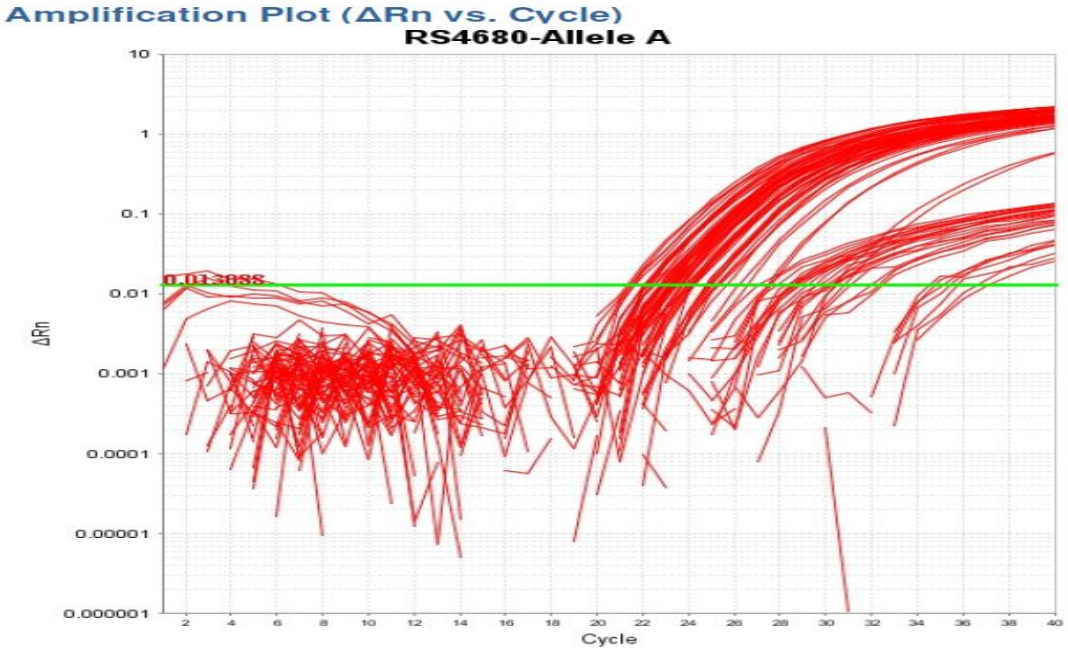


Figure 4.3. Amplification plot display of Allele A

The figure 4.3. shows amplification plots of Allele A. The fluorescent signal pass over the threshold to obtain any data. The threshold value (0.013) is shown as a green line.

In Figure 4.4.,4.5. and 4.6., Allele 1 (Allele A) shows as a blue color, while Allele 2 (Allele G) shows as a red color. Graphs are plotted depending on the quantity of radiation in the samples per cycle (Figure 4.5, Figure 4.6 and Figure 4.7).

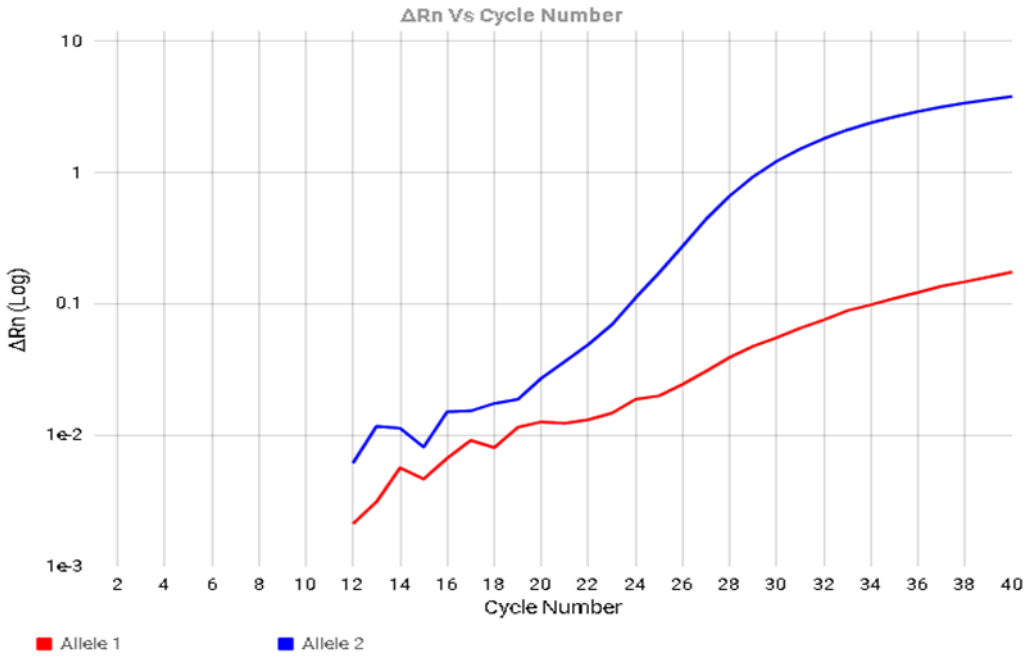


Figure 4.4. The emission graph of the homozygote wildtype genotype

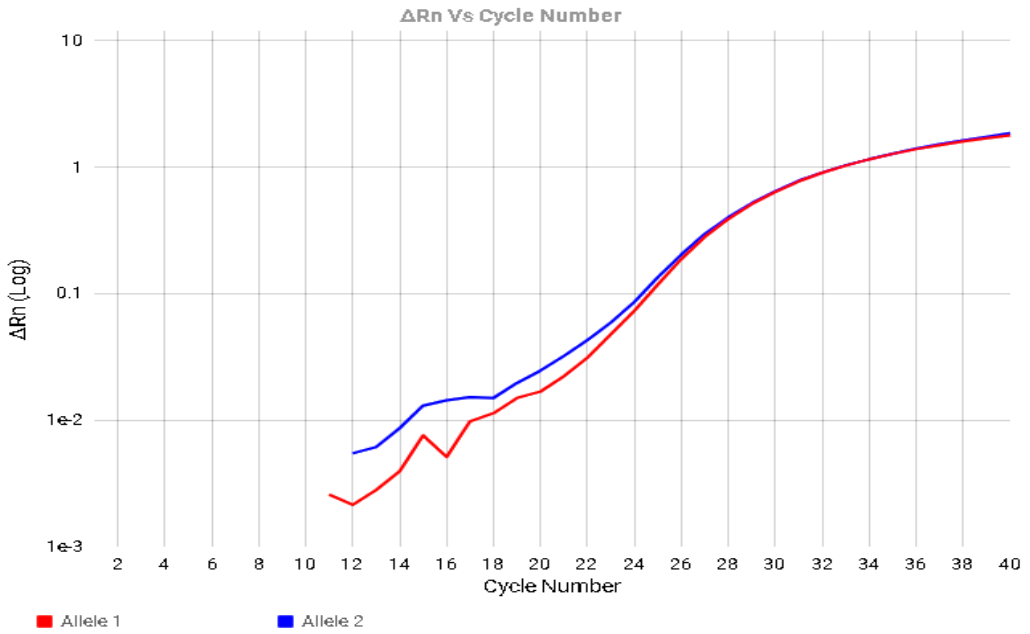


Figure 4.5. The emission graph of the heterozygote genotype

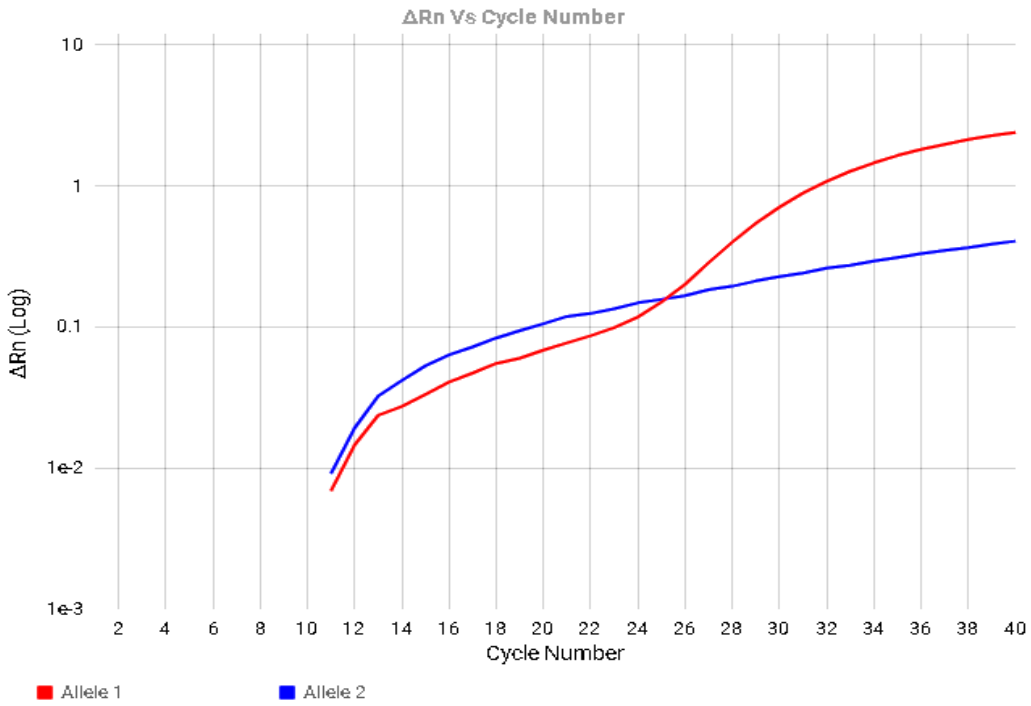


Figure 4.6. The emission graph of the homozygote mutant genotype

4.3. Analysis of Genotype and Allele Related to the Patient and Control Groups

In this experiment COMT Val158Met polymorphism in patients with ovarian cancer and healthy control groups. This study found no statistically significant findings among the two groups (p=0.413).

In Table 4.8., shows that there is no significant relation between the patient and control groups. The value of the homozygote mutant genotype is 0.216, that of heterozygote genotype is 0.658, while homozygote wild type genotype's value is 0.547.

According to our findings, homozygote wild type (GG), heterozygote (GA) and homozygote mutant type (AA) rates are calculated respectively as 37.8%, 42.2% and 20.0%. On the other hand, homozygote wild type (GG), heterozygote (GA) and homozygote mutant type (AA) rates are determined respectively as 25.5%, 46.8%, and 27.7% in the control group (Table 4.8.).

Table 4.8 shows that the value of G allele (56.4%) in patient group was found to be higher than the control group (48.9%). Accordingly, the value of A allele is also not statistically significant distinctly among groups like in G allele (p=0.301) (Table 4.8.).

Table 4.8. The genotype and allele distributions for the COMT Gene between the patient and control groups.

COMT Genotypes	Control Group (n=47)	Patient Group (n=47)	p value	Odd Ratio (OR)	Confidence interval 95%
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
Alleles Distributions					
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, *(S)= significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

To sum up, when COMT gene Val158Met polymorphism is compared in terms of genotype and allele frequencies, no significant relation is obtained among groups ($p=0.413$) (Table 4.9.).

Table 4.9. shows that there is no statistically significant difference between individuals with homozygote variant type genotype (AA) in comparison to individuals with homozygote wild type genotype (GG) and heterozygote genotype (GA) (p=0.547). Also, there is no statistically significant difference between individuals with homozygote wild type genotype (GG) in comparison to individuals with homozygote variant type genotype (AA) and heterozygote genotype (GA) (p=0.206) (Table 4.9).

Table 4.9. Genotypes of COMT gene polymorphism according to logistic regression analysis

COMT Genotypes	Control Group (n=47)		Patient Group (n=47)		p-value	Odd Ratio OR	Confidence interval 95%
	n	%	n	%			
GG	12	25.5	17	37.8	0.206 (NS)	0.565	0.232-1.376
GA+AA	35	74.5	28	62.2			
AA	13	27.7	10	22.2	0.547 (NS)	1.33	0.518-360
GA+GG	34	72.3	35	77.8			

n: number of sample, *(S)= significantly different (p< 0.05), NS= non significant (p>0.05).

5- DISCUSSION AND CONCLUSION

Several studies have shown that cancer has a wide spread all around the world, and the death rate of cancer is increasing in males and females dramatically (111).

Ovarian cancer is one of the most common cancer types in women and is a heterogeneous disease, and morbidity rate of this disease is highest among gynecologic malignancies. There are many risk factors for ovarian cancer such as obesity, diabetes mellitus, aging, smoking and alcohol intake, inherited ovarian cancer. Several molecular signaling pathways play roles in ovarian cancer such as apoptosis, angiogenesis and oxidative stress; these mechanisms affect progression of this disease. In the United Kingdom, almost 7500 women are diagnosed annually. However, there is standard treatment including surgery and chemotherapy for ovarian cancer (7,112).

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 (113).

Ovarian cancer can not be diagnosed at the early stage due to a lack of a specific biomarker to detect this disease. To date, CA125 as a serum biomarker is the most commonly used. But, knowledge of CA125 of levels is not sufficient to diagnose ovarian cancer (114). In our study, we analyzed distributions of CA125 level according to genotypes of COMT gene polymorphism in the patient group. As a result, the homozygote wild type (GG), heterozygote (GA) and homozygote mutant type (AA) distributions were calculated according to CA125 levels respectively as 486.88±548, 1502.96±1985 and 1042 ±1951 in the patients. No association was found between the COMT gene and levels of CA125 ($p=0.186$).

It is considered that Catechol-O-Methyltransferase enzyme plays a significant role in estrogen metabolism based on many different studies (115-119). 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 (120). In estrogen metabolism, COMT prevents DNA damage, therefore, it is called the gate-keeper. Wu et al. reported that COMT transcription is decreased related to epigenetic changes such as DNA methylation (9). In light of these developments, our study will contribute to understanding molecular mechanisms for ovarian cancer.

There are many case-control studies associated with ovarian cancer and COMT Val158Met polymorphisms, but most of these results have been inconclusive.

For example, Goodman et al. reported that COMT Val158Met polymorphism was not related to ovarian cancer risk due to a limited sample size. The lack of relation does not differ according to the individual's age, ovarian cancer histology or family history (117).

Holt et al. and Sellers et al. studied several enzymes such as CYP1A1, CYP1B1, COMT and SULT1A1 related to estrogen metabolism. The polymorphisms regarding these genes were performed by genotyping. The obtained data analyzed by using logistic regression tests. As a result of these studies, they found that there was no strong relationship between COMT genotypes or the other gene polymorphisms (CYP1A1, CYP1B1, SULT1A1) and ovarian cancer development in the American population (121,122).

Goodman et al. indicated that no meaningful association was seen between the COMT genotype and ovarian cancer risk. 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 (116).

Similarly, Delort et al. stated that the ratio for ovarian cancer was 2.02 in French subgroups. In this study seven genes that play a role in estrogen metabolism were included. They proposed that advanced studies were required to determine risk factors for ovarian cancer. The homozygote wild type (GG), heterozygote (GA) and homozygote mutant type (AA) distributions were determined as 23.7%, 48%, and 28.3% in the control group, respectively. In the patient group, genotype distributions of GG, GA and AA were found to be 21.6%, 43.1% and 35.3%, respectively. In this study it has been worked with limited number of sample and this situtation was prevent our results (123).

Pan et al. showed relations between COMT Val158Met polymorphism and the development of ovarian carcinogenesis. As a result of meta-analysis, there was no significant correlation related to an increased risk for ovarian cancer. Similar to our results, it was found by the meta analysis that the ratio of the GG genotype was not different in comparison with the ratio of GA+AA genotypes (p=0.76) (124).

It was indicated by Du et al. that there are relations between COMT Val158Met polymorphism and susceptibility of ovarian carcinogenesis. 1,293 cases and 2,647 controls were included regarding Caucasians and other populations in this meta-analysis. They performed a subgroup analysis in regards to ethnicity. In consequence of genotype analysis, there were no meaninful associations for developing ovarian cancer ($p>0.05$) (17).

In addition to these studies, it is indicated by Garner et al. that a decreased risk for mucinous ovarian tumors is associated with American females who carry variant COMT alleles (A) (OR 0.28, 95% CI 0.13–0.61) (125). Our findings showed that GG genotype was found in 2 (13.3%), GA genotype in 2 (13.3%), and AA genotype in 0 (0%) of the patients with mucinous tumors. Consequently, our results support the findings accomplished Garner et al.

Smoking affects on tumor formation in ovarian cancer especially, and it is the known as the first risk factor. In our study, smoking was found statistically meaningful ($p<0.001$). Smoking was significantly higher in patients with ovarian cancer (82.2%) than in the control group (55.3%). These results show that smoking may be an important risk factor for developing ovarian cancer (126).

Moreover, it was reported by Goodman et al. that the risk of ovarian cancer increases 2.2 fold in Hawaiian smokers with a low-activity COMT allele. They showed that is no association between individuals with homozygotes wild type genotype (Val/Val) and ovarian cancer risk ($p= 0.17$) (117).

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.

Our findings correspond with the studies accomplished by Delort et al., Holt et al., Goodman et al., Du et al., Sellers et al. and Pan et al. In our study, we examined the presence of the COMT gene Val158Met polymorphism among patients with ovarian cancer and a control group in the Turkish population. Patients with ovarian cancer (n=47) and healthy controls (n=47) were included in this study. First of all, DNA isolation was performed by using a robot of iPrep DNA extraction and with the blood genomic DNA isolation Kit by iPrep. UV absorbance and the purity of nucleic acids was measured at 260 nm by the NanoDrop device. Genotyping analysis was performed by using a 7500 Fast-Real Time Polymerase chain reaction device utilizing Real-Time PCR method. The demographic and histological data were evaluated statistically. Student T-test, Chi-square and Fisher's Exact Tests were used for analyzing the distributions of genotypes and alleles among groups.

The distributions of genotypes and alleles were analyzed for ovarian cancer among the patient and control groups. In our study, the GG genotype was found in 12 (25.5%), GA genotype in 22 (46.8%), and AA genotype in 13 (27.7%) regarding the control group. In the patient group, GG, GA and AA genotypes were determined 17 (37.8%), 19 (42.2%) and 9 (20%) respectively. GA heterozygote genotype carriers were observed in 42.2% of the patient group compared to other genotypes (GG and AA). There was no significant variation in comparison with genotypes between patient and controlgroups(p=0.413).

In consequence of this research;

This study is the first and only study showing the relationship between ovarian cancer and the COMT gene Val158Met polymorphism within the Turkish population. It is represented that there are no statistically meaningful relationships among genoytpes belonging to the patient and control groups in this study.

It is proposed that this study should be performed further in the future due to some limitations such as patients'medical history. COMT may be proposed as a new biomarker for ovarian cancer. This study should be further carried out by increasing the sample size of the study groups to determine a more exact knowledge for this disease.

Although COMT polymorphism does not have a direct relationship with ovarian cancer patients, and so this polymorphism does not carry any risk for the Turkish population.

6. REFERENCES

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7. APPENDICES

7.1. Forms

7.1.1. Biological Materials Transfer Form

KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER ANLAŞMASI

Araştırmanın Açık Adı : **Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Araştırılması**

Protokol Numarası :

Araştırmanın Özeti :

Yumurtalık kanseri en yaygın kadın kanserlerinden biridir ve jinekolojik kanserler arasında önde gelen ölüm nedenidir. 205.000'in üzerinde yeni vaka ve 125.000 ölüm, yumurtalık kanserinden kaynaklanmaktadır. Etiyoloji açısından, östrojen katabolizmasından sorumlu genlerdeki polimorfizmlerin, hücresel genotoksik 4-hidroksile edilmiş katekol östrojenleri ve antianjiyojenik 2-metoksestradiol seviyelerini değiştirdiği ve böylece yumurtalık kanseri gelişme riskini etkilediği bilinmektedir. Katekol-O-metiltransferaz (COMT), östrojenin katekol metabolitinin bağlanması ve inaktivasyonunda yer alan önemli bir östrojen katalizleyen enzimdir. COMT; yumurtalık, göğüs, karaciğer ve böbrek dokuları dahil olmak üzere çeşitli insan dokularında yüksek miktarlarda bulunur. Katekol-O-metiltransferaz (COMT) geni 22q11.2 kromozomunda yer alır ve kodlanmayan ekson 1 ve 2 olmak üzere altı ekzondan oluşur. rs4680 polimorfizmi, ekson 4'te kodon 158'de bir G'den A'ya dönüşümüdür ve bu da valin yerine bir metiyonin değişimine yol açar; bu, azalan aktiviteye sahip bir termolabil enzim ile sonuçlanır. rs4680 ile yumurtalık kanserine karşı yatkınlık arasındaki ilişki üzerine birçok vaka kontrol çalışması yayınlanmıştır. Bununla birlikte, bu çalışmalar araştırma kalitesindeki varyasyonlar, küçük örnek boyutu veya deneklerin farklı bölgesel ve etnik altyapısı da dahil olmak üzere bazı sınırlamalara sahiptir. Bu nedenle, bu çalışmalardan elde edilen sonuçlar çok sağlam değildir ve sınırlı güvenilirliğe sahiptir. Ancak bu çalışmanın sonuçları, bu yıkıcı hastalığa yönelik temel araştırma ve klinik tedavi için daha güvenilir kanıtlar sağlayacaktır.

Bu çalışmanın amacı, katekol-O-metiltransferaz (COMT) genindeki tek nükleotid polimorfizm rs4680 ile yumurtalık kanserine yatkınlık arasındaki korelasyonların sistematik incelemesinin yapılmasıdır. Yayımlanan ilgili vaka-kontrol çalışmaları için bir bilgisayar araştırması gerçekleştirilecektir.

İşbu anlaşma ile biyolojik materyali gönderen araştırmacı ve kurum "**Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Araştırılması**" isimli araştırmada kullanılmak üzere gönderilecek "**5ml ve araştırma**" amacı ile kullanılması üzere **26 Ağustos Yerleşkesi Yeditepe Üniversitesi Kayışdağı / İSTANBUL** adresindeki **Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı ' ndaki** merkezine göndermeden önce GÖNDERİCİ ve ALICI'dan aşağıdaki koşulları kabul etmesi istenmektedir;

1. Gönderilen biyolojik materyaller yalnızca yukarıda yazılı amaç için kullanılabilir. ALICI biyolojik materyallerin alınma amacından dışında ikincil amaç için kullanılma isteklerini GÖNDERİCİ'ye yazılı olarak bildirecektir.
2. Biyolojik materyaller ALICI'ya gönderilmeden önce biyolojik materyalin sağlandığı gönüllülere ait Türkiye İlaç ve Tıbbi Cihaz Kurumu ve Etik Kurul'un onayladığı tüm kullanım amaçlarına yönelik olarak düzenlenmiş bilgilendirilmiş gönüllü olur formunun her bir gönüllüden alınmış olması gerekmektedir.
3. ALICI biyolojik materyali GÖNDERİCİ'nin yazılı izni olmadan üçüncü kişi/kurumlara vermeyecektir.

4. Biyolojik materyaller GÖNDERİCİ tarafından bireyin kimlik ve tanımlayıcı bilgileri olmaksızın ALICI'ya gönderilecektir.
5. ALICI biyolojik materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel Beyannamesine uygun olarak kullanacaktır.
6. Bu anlaşma ile gönderilecek biyolojik materyalin araştırma için kullanılacak olduğu ve biyolojik materyal kullanımına ait risklerin var olduğu ALICI tarafından kabul edilmektedir. Söz konusu risklere karşı uygun önlemlerin alınması gerekmektedir.
7. GÖNDERİCİ ve ALICI gönderilen biyolojik materyalin herhangi bir şekilde ticari kazanç kaynağı olarak kullanılamayacağı ancak elde edilebilecek fikri mülkiyet ve patent haklarının bu durumdan istisna olduğu kabul etmektedir. GÖNDERİCİ ve ALICI söz konusu haklarını araştırma başlangıcında karşılıklı olarak belirleyecektir.
8. ALICI bu anlaşmanın sonlanması veya biyolojik materyalin sağlandığı gönüllülere ait anlaşmanın 2. maddesinde belirtilen olurun geri çekilmesi halinde bütün materyalleri geri vermeyi veya ortadan kaldırmayı ve bunu belgelemeyi kabul eder.
9. Bu anlaşma, araştırmanın sonlanması, ilgili mevzuat hükümlerine uyulmaması veya ilgili tarafların anlaşma hükümlerine uymaması durumlarında son bulacaktır.
10. Bu anlaşmanın yürütülmesinde ALICI ve GÖNDERİCİ yetkilileri sorumludur. Anlaşmazlık halinde ihtilafın çözümü için her iki ülke mahkemeleri de yetkilidir.

BİYOLOJİK MATERYALİ GÖNDEREN ARAŞTIRMACI BİLGİSİ

Adı Soyadı ve Unvanı	**:** Prof. Dr. Rukset Attar
Uzmanlık Alanı	**:** Kadın Hastalıkları ve Doğum
Kurumu	**:** Yeditepe Üniversitesi Hastanesi
Adresi	**:** İçerenköy Mah. Hastane Yolu Sok. 4,4/1 34718 Ataşehir / İstanbul
Telefon	: 05378401900
Faks	**:**
E-posta	: rattar@yeditepe.edu.tr

BİYOLOJİK MATERYALİ ALAN ALICI BİLGİSİ

Adı Soyadı ve Unvanı	: Prof. Dr. Turgay İsbir
Uzmanlık Alanı	: Moleküler Tıp
Kurumu	: Yeditepe Üniversitesi
Adresi	: Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı 26 Ağustos Yerleşkesi Kayışdağı
Telefon	: 0533 282 37 26
Faks	:
E-posta	: turgay.isbir@yeditepe.edu.tr

Bu anlaşmada belirtilen koşulları okudum ve anladım. Gönderilen materyalde bu anlaşmada belirtilen koşullara uyacağımı taahhüt ederim.

	GÖNDERİCİ				**ALICI**
	Gönderen Araştırmacı	Gönderen Destekleyici Firma Yetkilisi veya Yasal Temsilcisi	Eğitim Görevlisi / Ana Bilim Dalı Başkanı	Kurum Amiri / Rektör veya Yetkilendirdiği Makam	Alıcı Kurum Yetkilisi
El Yazısı ile Adı Soyadı Unvanı					
Tarih					

İmza					

Not: Bu anlaşmada yer alan alıcı kurum yetkilisinin imzası yerine alıcı kurum tarafından verilecek olan ve içerik olarak bu anlaşmadaki hükümlere benzer hükümleri içeren imzalı “end use certificate” “son kullanım sertifikası” de kabul edilebilir.

AGREEMENT FOR TRANSFER OF BIOLOGICAL MATERIAL TO BE USED IN CLINICAL TRIALS

Full name of the clinical trial:

Protocol code :

Summary of the clinical trial:

Click here on enter text

By this agreement, the investigator and the institution who send the biological material requires the CONSIGNEE and CONSIGNOR to agree on the below terms before sending *(Specify biological material type and amount)* which shall be used for to be dispatched to the address of .

1. Delivered biological materials shall be used only for the above-mentioned purposes. CONSIGNEE shall use those materials only for secondary purposes, which are initially approved by the CONSIGNOR in written.
2. Prior to the dispatch of the biological materials to the CONSIGNEE, Turkish Medicines and Medical Devices Agency and Ethics Committee approved informed consent forms, which belong to the persons for whom the biological material is provided, should be obtained. This consent form should explain all the purposes of use of the biological samples.
3. CONSIGNEE cannot provide the biological material to the third parties without prior written approval of the CONSIGNOR.
4. Biological materials shall be dispatched by the CONSIGNOR to the CONSIGNEE without the identity or any descriptive information of the individuals.
5. CONSIGNEE shall use the biological materials in accordance with as the United Nations Human Genome and Universal Declaration of Human Rights.
6. CONSIGNEE acknowledges and agrees that the biological materials to be dispatched under this agreement shall be utilized for research purposes and have some risks associated with their usage. Appropriate preventive actions should be taken for those risks.
7. CONSIGNOR and CONSIGNEE shall mutually agree that the biological materials cannot be used as a source for any commercial profit and the rights relating to a joint publication or a patent right that may arise may be the only exception for that. CONSIGNOR and CONSIGNEE shall mutually agree on those rights prior to trial initiation.
8. CONSIGNEE agrees to return or dispose of all materials and to evidence such acts accordingly in the event of termination of the agreement or withdrawal of written consent of the volunteer referred in Item 2.
9. This agreement shall be terminated in the event of, termination of the trial, violation on the terms of related regulations or noncompliance with agreement clauses of either of the parties.
10. CONSIGNEE and CONSIGNOR shall be responsible from the execution of this Agreement and performances hereunder. In case of conflict, both countries of the parties’ courts are authorized.

INFORMATION REGARDING THE INVESTIGATOR SENDING THE BIOLOGICAL MATERIAL

Name Surname and Title :
Specialization :
Institution :
Address :
Telephone :
Fax :
E-mail :

INFORMATION REGARDING THE CONSIGNEE RECEIVING THE BIOLOGICAL MATERIAL

Name Surname and Title :
Specialization :

Institution :
Address :
Telephone :
Fax :
E-mail :

I read and understood the terms under this agreement. I hereby agree and undertake that I will act in accordance with the terms of this agreement with respect to the dispatched materials.

	CONSIGNOR				**CONSIGNEE**
	Consignor Investigator	Consignor Sponsor Company Official or Legal Representative	Chief / Head of the Department	Chief Officer of the Institution / Rector or Assigned Person	Consignee Institution Official
Name Surname and Title in Handwriting					
Date					
Signature					

Note: Instead of the signature of the consignee representative, a signed “end use certificate” including clauses similar to this agreement’s to be issued by the consignee institution may also be accepted.

7.1.2. Volunteer Form

Asgari Bilgilendirilmiş Gönüllü Olur Formu

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz**. Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da k**arar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz**.
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşürü**nden edinebilirsiniz. Ayrıca Hastane personeli hakkında http://www.yeditepe.edu.tr web sayfasından daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

TANIMLAMA

1. ***Araştırmanın Adı:*** **Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Araştırılması**

Araştırmaya Katılımcı Sayısı: 100

Bu araştırmanın Amacı:

Yumurtalık kanseri en yaygın kadın kanserlerinden biridir ve jinekolojik kanserler arasında önde gelen ölüm nedenidir. 205.000'in üzerinde yeni vaka ve 125.000 ölüm, yumurtalık kanserinden kaynaklanmaktadır. Etiyoloji açısından, östrojen katabolizmasından sorumlu genlerdeki polimorfizmlerin, hücresel genotoksik 4-hidroksile edilmiş katekol östrojenleri ve antianjiyojenik 2-metoksestradiol seviyelerini değiştirdiği ve böylece yumurtalık kanseri gelişme riskini etkilediği bilinmektedir. Katekol-O-metiltransferaz (COMT), östrojenin katekol metabolitinin bağlanması ve inaktivasyonunda yer alan önemli bir östrojen katalizleyen enzimdir. COMT; yumurtalık, göğüs, karaciğer ve böbrek dokuları dahil olmak üzere çeşitli insan dokularında yüksek miktarlarda bulunur. Katekol-O-metiltransferaz (COMT) geni 22q11.2 kromozomunda yer alır ve kodlanmayan ekson 1 ve 2 olmak üzere altı

ekzondan oluşur. rs4680 polimorfizmi, ekson 4'te kodon 158'de bir G'den A'ya dönüşümüdür ve bu da valin yerine bir metiyonin değişimine yol açar; bu, azalan aktiviteye sahip bir termolabil enzim ile sonuçlanır. rs4680 ile yumurtalık kanserine karşı yatkınlık arasındaki ilişki üzerine birçok vaka kontrol çalışması yayınlanmıştır. Bununla birlikte, bu çalışmalar araştırma kalitesindeki varyasyonlar, küçük örnek boyutu veya deneklerin farklı bölgesel ve etnik altyapısı da dahil olmak üzere bazı sınırlamalara sahiptir. Bu nedenle, bu çalışmalardan elde edilen sonuçlar çok sağlam değildir ve sınırlı güvenilirliğe sahiptir. Ancak bu çalışmanın sonuçları, bu yıkıcı hastalığa yönelik temel araştırma ve klinik tedavi için daha güvenilir kanıtlar sağlayacaktır. Bu çalışmanın amacı, katekol-O-metiltransferaz (COMT) genindeki tek nükleotid polimorfizm rs4680 ile yumurtalık kanserine yatkınlık arasındaki korelasyonların sistematik incelemesinin yapılmasıdır. İlgili vaka-kontrol çalışmaları için bir bilgisayar araştırması gerçekleştirilecektir.

Süresi: 1 Yıl

İzlenecek Yöntem/Yöntemler:

Hasta (n=) ve kontrol (n=) grubu olmak üzere iki gruptan oluşması planlanmıştır.

DNA İzolasyonu:

Yumurtalık kanseri olduğu tespit edilen hastalardan 5ml EDTA ' lı tüplere yaklaşık 350 ml ' lik periferik kan alınacaktır.

DNA izolasyonu için iPrep DNA (iPrep Pure link , invitrogen) izolasyon robotu kullanılacaktır.

Elde edilen DNA ' ların konsantrasyonları nanodrop (Thermoscientific) cihazı ile belirlenecektir.

Saflık ölçümleri için NanoDrop cihazı ile spektrofotometrik olarak ölçüm yapılacaktır. DNA ' lar çalışma gününe kadar +4^{o}C ' de bekletilecektir.

Genotip Analizi :

COMT genotiplemesi Applied bioscience 7500 Fast Real Time cihazı kullanılarak

Gerçek zamanlı PZR ile yapılacak ve hedef varyasyonlarındaki olası mutasyonların

belirlenebilmesi için özel primer dizileri kullanılacaktır.

Çalışmanın istatistiksel analizi için SPSS 24.0 sürümü kullanılacaktır.

Hasta grubu ve kontrol grubu katılımcıların karşılaştırılmalarında allel fraksiyonlarının ve genotiplerin karşılaştırılması için ki kare testi kullanılacaktır.

Hasta ve kontrol grubu anlamlılık ölçümü için student 's t test kullanılacaktır. Anlamlılık seviyesi $p < 0.05$ kabul edilecektir.

Araştırma Sonunda Beklenen Fayda

Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Etkisinin Belirlenmesi

Bu Çalışmada Herhangi Bir Alternatif Tedavi ya da Girişimde Bulunulmayacaktır.

Bu Araştırma Gönüllüler İçin Hiçbir Risk Teşkil Etmemekte ve Hiçbir Rahatsızlığa Sebep olmamaktadır.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim. Tarafımdan alınan kan ve doku örneklerinin daha sonra başka araştırma çalışmalarında kullanılmasında herhangi bir sakınca yoktur.

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün:

Adı Soyadı:

İmzası:

Tarih:

Açıklamaları Yapan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Yasal Temsilcinin :

Adı Soyadı:

İmzası:

Tarih:

Klinik Araştırma Proje Koordinatörü İletişim Bilgileri:

Adı Soyadı:

Uzmanlık alanı:

Kurumu:

E-posta adresi:

Telefon numarası:

7.1.3. Case Report Form

1- ÇALIŞMANI ADI: **Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Araştırılması**

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Grupları için;

- Gönüllü Olma
- Yumurtalık Kanseri Hastası Olma
- 18 – 85 yaş aralığında olma
- Yukarıda belirtilenler haricinde bir hastalığa sahip olmama

Kontrol Grubu için ;

- Gönüllü olma
- Sağlıklı olma (yukarıda belirtilenlerde dahil olmak üzere hiçbir hastalığa sahip olmama)
- 18 – 85 yaş aralığında olma

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü olmama
- 18 – 85 yaş aralığı dışında olma
- Yukarıda belirtilenler dışında bir hastalığa sahip olma

Sorumlu Araştırmacı

7.2. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1560 **25/10/2018**
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makama (İpek Yağmur Abaoğlu)

Yeditepe Üniversitesi Biyokimya, Moleküler Tıp Anabilim Dalı Prof. Dr. Turgay İsbir'in koordinatör, Yeditepe Üniversitesi Hastanesi Kadın Hastalıkları ve Doğum Anabilim Dalı Prof. Dr. Rukset Attar'ın sorumlu olduğu **"Yumurtalık Kanserinde Katekol-O-Metiltransferaz (COMT) Geni Val158Met Polimorfizminin Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1532** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **24.10.2018** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 915**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

Yeditepe Üniversitesi 26 Ağustos Yerleşimi, İnönü Mahallesi Kayışdağı Caddesi 34755 Ataşehir / İstanbul
T. 0216 578 00 00 www.yeditepe.edu.tr F. 0216 578 02 99

8. RAW DATA

COMTgeneotype * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTgeneotype	GG	Count	12	17	29
		% within COMTgeneotype	41,4%	58,6%	100,0%
		% within hasta_kontrol_grubu	25,5%	37,8%	31,5%
		% of Total	13,0%	18,5%	31,5%
	GA	Count	22	19	41
		% within COMTgeneotype	53,7%	46,3%	100,0%
		% within hasta_kontrol_grubu	46,8%	42,2%	44,6%
		% of Total	23,9%	20,7%	44,6%
	AA	Count	13	9	22
		% within COMTgeneotype	59,1%	40,9%	100,0%
		% within hasta_kontrol_grubu	27,7%	20,0%	23,9%
		% of Total	14,1%	9,8%	23,9%
Total		Count	47	45	92
		% within COMTgeneotype	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,597[a]	1	,206		
Continuity Correction[b]	1,080	1	,299		
Likelihood Ratio	1,602	1	,206		
Fisher's Exact Test				,263	,149
Linear-by-Linear Association	1,580	1	,209		
N of Valid Cases	92				

COMTgeneotypeGG * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTgentypeGG	yok	Count	35	28	63
		% within COMTgentypeGG	55,6%	44,4%	100,0%
		% within hasta_kontrol_grubu	74,5%	62,2%	68,5%
		% of Total	38,0%	30,4%	68,5%
	var	Count	12	17	29
		% within COMTgentypeGG	41,4%	58,6%	100,0%
		% within hasta_kontrol_grubu	25,5%	37,8%	31,5%
		% of Total	13,0%	18,5%	31,5%
Total		Count	47	45	92
		% within COMTgentypeGG	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	**1,597[a]**	**1**	**,206**		
Continuity Correction[b]	**1,080**	**1**	**,299**		
Likelihood Ratio	**1,602**	**1**	**,206**		
Fisher's Exact Test				**,263**	**,149**
Linear-by-Linear Association	**1,580**	**1**	**,209**		
N of Valid Cases	**92**				

COMTgenotypeGA * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTgenotypeGA	yok	Count	25	26	51
		% within COMTgenotypeGA	49,0%	51,0%	100,0%
		% within hasta_kontrol_grubu	53,2%	57,8%	55,4%
		% of Total	27,2%	28,3%	55,4%
	var	Count	22	19	41
		% within COMTgenotypeGA	53,7%	46,3%	100,0%
		% within hasta_kontrol_grubu	46,8%	42,2%	44,6%
		% of Total	23,9%	20,7%	44,6%
Total		Count	47	45	92
		% within COMTgenotypeGA	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,196[a]	1	,658		
Continuity Correction[b]	,054	1	,816		
Likelihood Ratio	,196	1	,658		
Fisher's Exact Test				,680	,408
Linear-by-Linear Association	,194	1	,660		
N of Valid Cases	92				

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTgenotypeAA	yok	Count	34	35	69
		% within COMTgenotypeAA	49,3%	50,7%	100,0%
		% within hasta_kontrol_grubu	72,3%	77,8%	75,0%
		% of Total	37,0%	38,0%	75,0%
	var	Count	13	10	23
		% within COMTgenotypeAA	56,5%	43,5%	100,0%
		% within hasta_kontrol_grubu	27,7%	22,2%	25,0%
		% of Total	14,1%	10,9%	25,0%
Total		Count	47	45	92
		% within COMTgenotypeAA	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

COMTgenotypeAA * patient_control_group

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,362[a]	1	,547		
Continuity Correction[b]	,130	1	,718		
Likelihood Ratio	,363	1	,547		
Fisher's Exact Test				,633	,360
Linear-by-Linear Association	,359	1	,549		
N of Valid Cases	92				

COMTalleleG * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTallelG	yok	Count	13	9	22
		% within COMTallelG	59,1%	40,9%	100,0%
		% within hasta_kontrol_grubu	27,7%	20,0%	23,9%
		% of Total	14,1%	9,8%	23,9%
	var	Count	34	36	70
		% within COMTallelG	48,6%	51,4%	100,0%
		% within hasta_kontrol_grubu	72,3%	80,0%	76,1%
		% of Total	37,0%	39,1%	76,1%
Total		Count	47	45	92
		% within COMTallelG	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,741[a]	1	,389		
Continuity Correction[b]	,380	1	,538		
Likelihood Ratio	,745	1	,388		
Fisher's Exact Test				,467	,269
Linear-by-Linear Association	,733	1	,392		
N of Valid Cases	92				

COMTalleleA * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
COMTallelA	yok	Count	13	17	30
		% within COMTallelA	43,3%	56,7%	100,0%
		% within hasta_kontrol_grubu	27,7%	37,8%	32,6%
		% of Total	14,1%	18,5%	32,6%
	var	Count	34	28	62
		% within COMTallelA	54,8%	45,2%	100,0%
		% within hasta_kontrol_grubu	72,3%	62,2%	67,4%
		% of Total	37,0%	30,4%	67,4%
Total		Count	47	45	92
		% within COMTallelA	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,741[a]	1	,389		
Continuity Correction[b]	,380	1	,538		
Likelihood Ratio	,745	1	,388		
Fisher's Exact Test				,467	,269
Linear-by-Linear Association	,733	1	,392		
N of Valid Cases	92				

Group Statistics

	hasta_kontrol_grubu	N	Mean	Std. Deviation	Std. Error Mean
yıl	over ca	47	54,87	12,555	1,831
	kontrol	47	51,11	12,863	1,876
kg/m2	over ca	39	29,3128	5,37255	,86030
	kontrol	47	23,0468	3,62233	,52837
m2	over ca	37	1,7727	,15493	,02547
	kontrol	47	1,6645	,14643	,02136
mg/dl akş	over ca	42	108,52	33,288	5,136
	kontrol	47	86,45	7,604	1,109

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
yıl	Equal variances assumed	,125	,724	1,436	92
	Equal variances not assumed			1,436	91,946
kg/m2	Equal variances assumed	5,945	,017	6,430	84
	Equal variances not assumed			6,206	64,494
m2	Equal variances assumed	,093	,761	3,278	82
	Equal variances not assumed			3,256	75,295
mg/dl akş	Equal variances assumed	15,691	,000	4,422	87
	Equal variances not assumed			4,201	44,826

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
yıl	Equal variances assumed	,154	3,766	2,622
	Equal variances not assumed	,154	3,766	2,622
kg/m2	Equal variances assumed	,000	6,26601	,97456
	Equal variances not assumed	,000	6,26601	1,00960
m2	Equal variances assumed	,002	,10823	,03302
	Equal variances not assumed	,002	,10823	,03324
mg/dl akş	Equal variances assumed	,000	22,077	4,992
	Equal variances not assumed	,000	22,077	5,255

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
yıl	Equal variances assumed	-1,441	8,973
	Equal variances not assumed	-1,441	8,973
kg/m2	Equal variances assumed	4,32800	8,20402
	Equal variances not assumed	4,24941	8,28262
m2	Equal variances assumed	,04255	,17391
	Equal variances not assumed	,04202	,17445
mg/dl akş	Equal variances assumed	12,155	31,999
	Equal variances not assumed	11,492	32,662

changingmenopause * patient_control_group Crosstabulation

			hasta_kontrol_grubu		
			kontrol	over ca	Total
değişenmenapoz	premenapoz	Count	28	9	37
		% within değişenmenapoz	75,7%	24,3%	100,0%
		% within hasta_kontrol_grubu	59,6%	19,1%	39,4%
		% of Total	29,8%	9,6%	39,4%
	postmenapoz	Count	19	38	57
		% within değişenmenapoz	33,3%	66,7%	100,0%
		% within hasta_kontrol_grubu	40,4%	80,9%	60,6%
		% of Total	20,2%	40,4%	60,6%
Total		Count	47	47	94
		% within değişenmenapoz	50,0%	50,0%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	50,0%	50,0%	100,0%

diabetes * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
diyabet	yok	Count	47	33	80
		% within diyabet	58,8%	41,3%	100,0%
		% within hasta_kontrol_grubu	100,0%	73,3%	87,0%
		% of Total	51,1%	35,9%	87,0%
	var	Count	0	12	12
		% within diyabet	0,0%	100,0%	100,0%
		% within hasta_kontrol_grubu	0,0%	26,7%	13,0%
		% of Total	0,0%	13,0%	13,0%
Total		Count	47	45	92
		% within diyabet	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

smoking * patient_control_group

Crosstab

			hasta_kontrol_grubu		
			kontrol	over ca	Total
sigara_kullanımı	kullanmıyor	Count	26	37	63
		% within sigara_kullanımı	41,3%	58,7%	100,0%
		% within hasta_kontrol_grubu	55,3%	82,2%	68,5%
		% of Total	28,3%	40,2%	68,5%
	kullanıyor	Count	21	8	29
		% within sigara_kullanımı	72,4%	27,6%	100,0%
		% within hasta_kontrol_grubu	44,7%	17,8%	31,5%
		% of Total	22,8%	8,7%	31,5%
Total		Count	47	45	92
		% within sigara_kullanımı	51,1%	48,9%	100,0%
		% within hasta_kontrol_grubu	100,0%	100,0%	100,0%
		% of Total	51,1%	48,9%	100,0%

9. CURRICULUM VITAE

Personal Information

Name	Ipek Yagmur	**Surname**	ABAOGLU
Birth Place	Istanbul	**Date of Birth**	23/01/1995
Nationality	Turkish	**Identity Number**	
E-mail	yagmurabaoglu@gmail.com	**Phone**	

Education

Degree	**Department**	**Institution**	**Graduation Year**
Doctorate			
Master			
University	Molecular Biology and Genetics	Yıldız Technical University	2017
High School	Science-Math	Hasan Sabriye Gümüş Anatolian High School	2013

Scientific Work

Additional Education & Certificates

Thermofisher Scientific Days 2018 Workshop, September 2018

Computer Knowledge

Program	**Usage**
Office Programs	Very good
Statistical Package for the Social Sciences (SPSS)	Good