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YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
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THE ROLE OF CYP24A1 GENE POLYMORPHISMS IN OVARIAN CANCER

MASTER OF SCIENCE THESIS

RIMAN NABIL SHAMALLAKH, BSc

ISTANBUL-2022

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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 ..... and numbered .....

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

11.10.2022

RIMAN NABIL SHAMALLAKH

DEDICATION

To my life’s greatest blessing,

the one I owe him my steadiness in this journey,

My husband Mohsen..

ACKNOWLEDGEMENTS

I would love to express my gratitude to Prof. Dr. Turgay ISBIR, head of the Department of Molecular Medicine for his generosity in welcoming us and providing us with the proper environment to conduct our research.

My patient husband, Mohsen, for his support, carring and for sharing my ambition to accomplish this degree. Our little princess, Asya, who has inspired me with her love, as she bore my busyness and swingy moods.

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LIST OF ABBREVIATIONS AND SYMBOLS

BRCA:	Breast Cancer
CYP24A1:	Cytochrome P450 Enzym Family 24 Sub-family Member 1
EDTA:	Ethylen Diamine TetraAcitic Acid
HBOC:	Hereditory Breast and Ovarian Cancer Syndrome
HNPCC:	Hereditary Non-Polyposis Colon Cancer AKA Lynch Syndrome
IUD:	Intrauterine Device
IVF:	In-Vitro Fertilization
LDH:	Lactate Dehydrogenase
OC:	Ovarian Cancer
OD:	Optical Density
PCR:	Polymerase Chain Reaction
PLAP:	Placental Alkaline Phosphatase
SNP:	Single Nucleotide Polymorphisms
SPSS:	Statistical Package For The Social Sciences
UV:	Ultra Violet
VIC\FAM:	Fluorescent Dye

ABSTRACT

SHAMALLAKH, R.N. The Role of CYP24A1 Gene Polymorphisms in Ovarian Cancer. Yeditepe University Health Sciences Institute, Department of Molecular Medicine. Master of Science Thesis. Istanbul, 2022.

Ovarian cancer (OC) is considered as an autoimmune disease as a result of environmental and hereditary changes. Like any other disorder it has many either genetic or environmental factors that increase or reduce the risk of the tumor susceptibility such as Hypovitaminosis D. Our study aimed to indicate the association of rs2248137 SNP in CYP24A1 gene and ovarian cancer risk. Bone metabolism and calcim are controlled by vitamin D, a prohormone that is fat-soluble. About 3% of the genome of human is regulated by the endocrine system of vitamin D. Previous studies identified that vitamin D to have considerable anti-cancer action and preventative properties. Vitamin D levels are influenced by sun exposure, diet, and medications. Low levels of the vitamin may raise the chance of developing ovarian cancer, according to some research. Genotyping of CYP24A1 SNPs was investigated both in ovarian cancer patients and in healthy controls. Molecular analysis was performed by real-time PCR. The distribution of genotypic and allelic frequencies was not significantly different between patients and controls regarding rs2248137 SNP of CYP24A1 gene. Our research results indicate that rs2248137 CYP24A1 doesn't show any effects in ovarian cancer.

Key words: Ovarian Cancer, CYP24A1, Single Nucleotide Polymorphisms**.**

ABSTRACT (TURKISH)
ÖZET

SHAMALLAKH, R.N. Yumurtalık Kanserinde CYP24A1 Gen Polimorfizmlerinin Rolü. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Moleküler Tıp Anabilim Dalı. Yüksek Lisans Tezi. İstanbul, 2022.

Yumurtalık kanseri (YK), çevresel ve kalıtsal değişikliklerin bir sonucu olarak otoimmün bir hastalık olarak kabul edilir. Diğer herhangi bir hastalık gibi, Hipovitaminozis D gibi tümör duyarlılığı riskini artıran veya azaltan birçok genetik veya çevresel faktöre sahiptir. Çalışmamız, CYP24A1 genindeki rs2248137 SNP ile yumurtalık kanseri riski arasındaki ilişkiyi göstermeyi amaçlamıştır. Kemik metabolizması ve kireçlenme, yağda çözünen bir prohormon olan D vitamini tarafından kontrol edilir. İnsan genomunun yaklaşık %3'ü, D vitamininin endokrin sistemi tarafından düzenlenir. Önceki çalışmalar, D vitamininin önemli bir kanser önleyici etkiye ve önleyici özelliklere sahip olduğunu belirlemiştir. D vitamini seviyeleri güneşe maruz kalma, diyet ve ilaçlardan etkilenir. Bazı araştırmalara göre, düşük vitamin seviyeleri yumurtalık kanseri geliştirme şansını artırabilir. CYP24A1 SNP'lerinin genotiplemesi hem yumurtalık kanseri hastalarında hem de sağlıklı kontrollerde araştırıldı. Moleküler analiz, gerçek zamanlı PCR ile yapıldı. CYP24A1 geninin rs2248137 SNP'si ile ilgili olarak, hastalar ve kontroller arasında genotipik ve alelik frekansların dağılımı önemli ölçüde farklı değildi. Araştırma sonuçlarımız rs2248137 CYP24A1'in yumurtalık kanserinde herhangi bir etki göstermediğini göstermektedir.

Anahtar kelimeler: Yumurtalık Kanseri, CYP24A1, Tek Nükleotid Polimorfizmleri.

1. INTRODUCTION AND PURPOSE

Ovarian Cancer is marked as the most common and deadliest gynecologic cancers. It is an abnormal growth of the epithelial cells of the ovaries. These abnormal cell ’production is rapid resulting in metastasis. A woman has a pair of ovaries each set on a side of the uterus. An ovary has a size of an almond. Ovarian cancer can be treated in either ways surgically or chemically.

CYP24A1 is one of the family cytochrome P450 enzyme, that takes part in vitamin D metabolism by catabolizing and degrading 1,25(OH)2D3 through 24-hydroxylation. This gene is primarily discovered as a candidate oncogene in breast cancer, and lately has been found in different expressions levels in other types as prostate, endometrial, and lung cancer (1). Hence, the CYP24A1 enzyme regulates vitamin D level, it also functions in the vitamin D endocrine system and calcium homeostasis.

Our study is performed on a group of 50 healthy people and 50 patients of ovarian cancer to indicate the link of the (rs2248137) single nucleotide polymorphisms of the CYP24A1 gene with Ovarian Cancer.

2. LITERATURE OVERVIEW

2.1 Normal Ovaries:

A female genital system is composed of two main parts which are the uterus that's responsible of hosting and developing the fetus, vaginal and uterine discharges production and it works as a passage for the male sperm to the fallopian tubes. The other part is the ovaries that work as glands where the egg cells are formed and estrogen and progesterone hormones are synthesized and secreted (2).

2.1.1 Structure, Histological and Physiological Properties:

A normal ovary has a solid structure, an almond size and shape, 2 cm width, and 1 cm thickness and about 3.5 cm long. The ovaries are positioned in the lateral walls of the abdominal region, on either side of the uterus.

A single layer of cuboidal epithelium termed "germinal epithelium" surrounds the whole ovary, followed by the "tunica albuginea," a thick irregular connective tissue capsule. The ovaries are divided into two parts. The cortex is the outer portion, which contains several ovarian follicles at various stages of development, each of which contains a germline cell "oocyte." The central area is the medulla that contains all the blood vessels, nerves and the lymphatics of the ovaries (3).

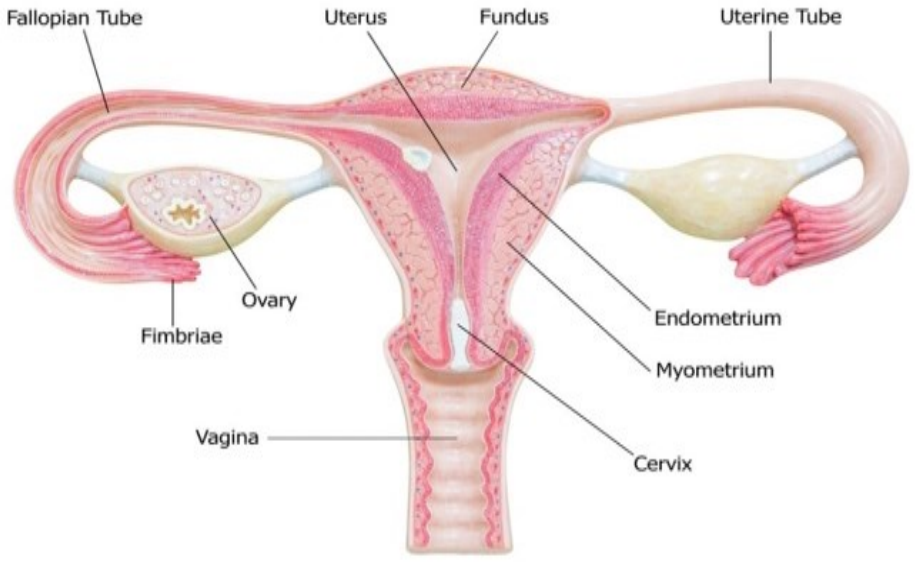

Figure 2.1.1. Female Reproductive System. (4)

2.1.1.1 Ovarian Follicle Development Stages:

A growing oocyte is encased by one or more layers of follicular cells in an ovarian follicle. Follicular cells undergo modifications at the same time that the egg goes through meiosis.

2.1.1.1.A Primordial follicles

Consists of oocyte surrounded by simple squamous follicular cells where they appear in ovaries upon birth and during childhood.

2.1.1.1.B Primary Follicles

Consists of the primary oocyte bounded by multiple layers of cuboidal granulosa cells, resulting in a uni-laminar or a multi-laminar primary follicle.

2.1.1.1.C Secondary Follicle (Antral Follicle)

In this stage of fluid-filled area is developed called “Antrum”, and here the follicle still has the primary oocyte and surrounded by multiple layers of cuboidal granulosa cells.

2.1.1.1.D Mature Follicle (Graafian Follicle)

It has a huge antrum and the ovum which is covered with corona radiata and a collection of granulosa cells called cumulus oophoricus. In the inside it has follicular cells and in the outer side it has theca externa and theca interna. In this stage the follicle releases the ovum and the ovulation process begins.

Once the egg is out it passes through the fallopian tube into the uterus. The egg fertilization by a sperm may occur in the fallopian tube then the egg will be implanted in the uterus wall (5).

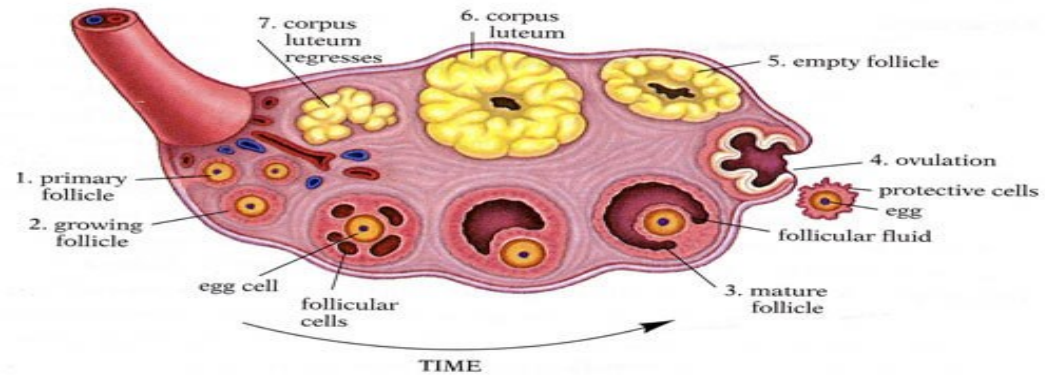

Figure 2.1.1.1. The internal structures of an ovary.

2.2 Ovarian Cancer

2.2.1 Definition

Ovarian cancer is a gynecologic cancer ranking the highest mortality rate and the 5th most common type in females. However, survival rate is increased in early stages and early diagnosis. As of each ovary is consists of 3 sorts of cells, a tumor may develop according to the cell type.

2.2.2 Epidemiology

It's been revealed that ovarian cancer varies greatly across geographical areas. The greatest age-adjusted number of cases are recorded in industrialized regions worldwide, notably Western and Northern Europe, and America with rates above 10 per 100,000 people. In contrast, Japan has recorded (6.4 per 100,000). South America has a median rate of (7.7 per 100,000), while Asia and Africa luckily have the least. Risk increasing when moving through low-risk regions to high-risk regions, emphasizing the relevance of nongenetic variables. However, racial inequalities in risk are visible even inside the United States, mirroring the reported international heterogeneity (7).

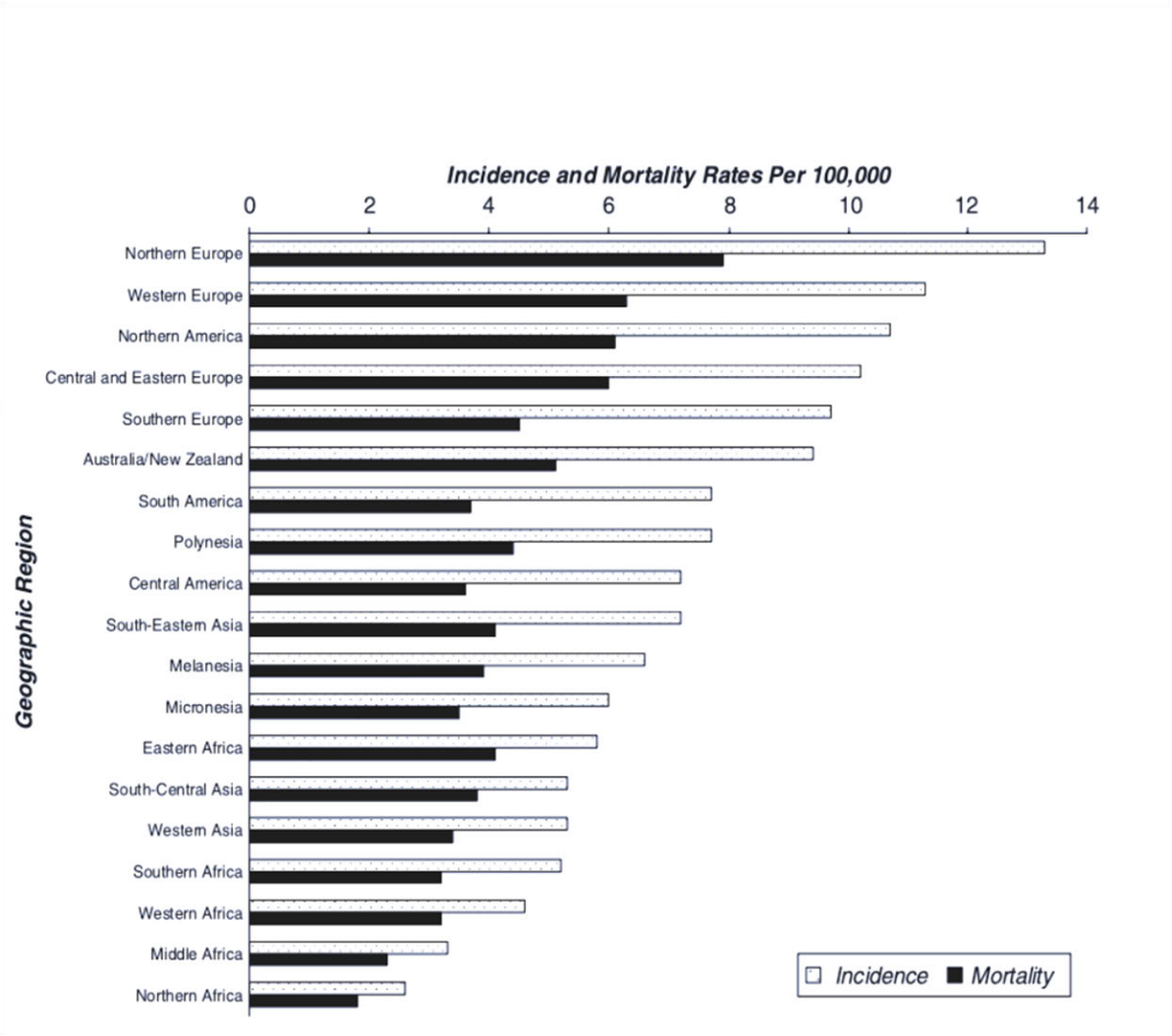

Figure 2.2.2 Incidence and Mortality Rate According to Geographical Regions between Races (7).

2.2.3 Major Risk Factors

Although the exact reason that causes most ovarian cancers is unclear. But there are several factors that indicated to trigger the possibility of ovarian cancer occurrence. Being affected by one or more risk factors, however, doesn't assure that you are getting the disease. In addition, some patients may not have any of the identified risk factors. It is shown that the majority of the identified risk factors are related to epithelial ovarian cancer while it is not indicated the lees common types of the tumor such as stromal tumors and germ cell carcinomas.

The chance of acquiring ovarian cancer increases as getting older. So far all in women under the age of 40, ovarian cancer is infrequent. As most ovarian malignancies are found post-menopause. The majority of ovarian malignancies develop during menopause. 50 % of the diagnosed patients are in average age of 63.

Also, obesity is considered as a thread for many tumors. However, further researches are required to clarify its exact effect on ovarian cancer. As the BMI for an obese female raises the risk of getting ovarian cancer gets higher besides decreasing the survival rate (8).

Ovarian cancer is more likely to occur to who have their first pregnancy when they are over 35 or who have never delivered a baby. In general, following hormonal treatments during post menopause period leads to increase the risk. As taking estrogen or progesterone enhances the risk in contrast to women who never taken before.

Furthermore, Family history may raise the chance of having ovarian cancer since it is an inherited disease. The more cancer patients in relatives the more the risk gets higher especially if the cases arise from the father's side. This is also applying to if any of colorectal or breast cancer run in the family since they as a result of alterations in certain genes (9).

BRCA1 and 2 mutations, perhaps some undiscovered ones, cause (HBOC). Breast, ovarian, primary peritoneal and fallopian tube malignancies have all been associated to this disease. Other malignancies, such as prostate or pancreatic carcinomas, are also at a higher risk. BRCA1 and 2 mutations cause the most of ovarian malignancies by heredity.

In United States, it's been recorded that BRCA 1 and BRCA2 gene mutations are 10 times more frequent in Ashkenazi Jews. Statistically, 35-70 in 100 ovarian cancer case are as a result of BRCA1 gene mutation (10).

Apart from this, it is cleared that in vitro fertilization (IVF) enhances ovarian tumors incidence while other some researches have shown no link 2between fertility treatment and ovarian cancer (11). In general, smoking has not that impact on ovarian cancer, but for mucinous type it's said to be an increased risk factor (12). Androgens are male hormones such as testosterone is an example of androgens which are male hormones. Certain types of ovarian are related to androgens, but there are no enough enlighten researches clearing androgens' role in ovarian cancer (46). According to some studies, vegetarian females have a lower possibility of getting ovarian cancer however other studies reject this hypothesis. Undoubtedly eating healthy keeps human body healthy and minimize the risk of being diseased in general (13). Being pregnant for a full term and breastfeeding have a positive impact in reducing ovarian cancer risk. It is also noted that as the period of using birth control such as oral contraceptives elongate the lower the risk becomes. Thus, other birth control methods including IUD and tubal ligation have the same positive impact regarding ovarian cancer occurrence (14). Also, hysterectomy appears to lessen the risk for an extent (15).

2.2.4 Pathogenesis of Ovarian Cancer

2.2.4.1 CLASSIFICATIONS

Ovarian tumors are classified according to where it can originate in the ovarian cells into 3 subtypes:

2.2.4.1.A Epithelial Tumors

The tumors that are originated from the ovaries epithelial cells are the frequently occurred tumors among ovarian cancer. However, post-menopausal females or younger females with a strong family history of BRCA1 mutation are the targeted category to be tested with it. Basically, Serous carcinomas can be defined as an epithelial lined cyst of clear fluid with psammoma bodies, while Mucinous carcinoma is an epithelial lined cyst of mucous. On the other hand, Brenner tumor as a cyst with bladder epithelium, while Endometroid is an endometrial tissue in the ovary with additional tumor in the uterus. The benign tumor is determined by a single layer of epithelium and the malignant is determined by a papillary projection. In addition, these kinds of tumors secrete a marker known as CA-125 that is used to indicate the responses to treatments and relapse.

2.2.4.1.B Germ Cell Tumors

In contrast to epithelial tumors, germ cell tumors target premenopausal women. It includes Dysgerminoma carcinomas that is considered as the most common malignant tumors of germ cell. It is distinguished having large cells with clear cytoplasm and large nuclei so called “fried egg appearance”. These tumors secrets several markers yet the most distinguishing and tested one is PLAP besides beta-HCG and LDH.

Yolk sac carcinomas “also known as endodermal sinuses” are another kind of germ cell tumors, it is also commonly tested in premenopausal women. As these tumors form Schiller-Duval Bodies, this makes testing easer and takes it step further knowing it secretes marker alpha-fetoprotein.

Teratoma are tumors that contain germ cells that have differentiated down more and can be seen having Multiple Cell Types while Choriocarcinoma has Cyto-Syncytiotrophoblast , and embryonal carcinoma but it is not specific.

2.2.4.1.C Sex/Stromal Cord Tumors

This type involves in Sertoli Leydig and Granulosa cell tumors. Even though these cells are commonly seen in the testicle and testicular cancers they can cause ovarian tumors too (16).

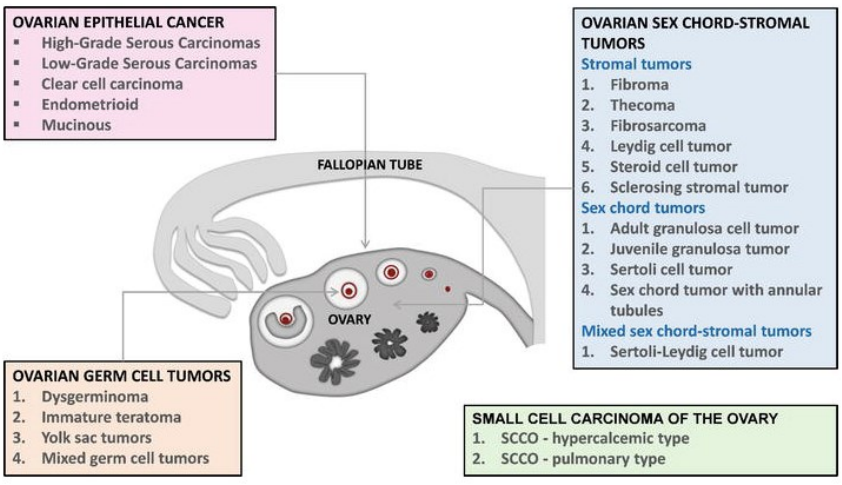

Figure 2.2.4.1 Molecular Classification of ovarian cancer (16).

2.3 The Relationship between VitaminD and Ovarian Cancer

Vitamin D function in ovarian cancer has been extensively studied, with the hypothesis that it regulates cellular proliferation and metabolism, acting as a protective and anti-tumorigenic factor. Vitamin D status has been proven to be precise indicator of outcome in this cancer cases. Cures that include vitamin D increase anticancer effects, allowing for potential therapeutic use. Calcium and vitamin D supplements can be an effective cancer inhibition strategy.

FokI rs10735810 and rs2228570 are found to have impact on cancer risk from VDR gene, They are positioned at the 5 end of the gene. It is commonly researched SNPs in ovarian cancer. Because of this polymorphism, the sequence of VDR is changed, resulted in a shorter protein for F allele carriers than the ressive one carriers. The FokI f allele was originally related to an elevated ovarian cancer in Caucasian women (20). In 1980, The Garland brothers who were first to declare that the relation between vitamin levels and survival of cancer through a conducted study on latitude area population finding that low levels of vitamin D elevate the chance of growing cancer (17).

According to a previous trial on epithelial ovarian cancer, it's indicated that excessive concentrations of 25(OH)D have been linked to prolonged survival rates. Thus, high pre-diagnostic levels of 25(OH)D in people with colorectal cancer led to decreased cancer risk and mortality. Therefore, vitamin D supplements show an effective early-stage cancer therapy (17).

An experiment shown that Vitamin D and progestins together trigger apoptosis and lower viability of the cell of the ovaries. Progestins PR-dependently block CAL-induced CYP24A1, prolonging CAL activity (18).

2.4 CYP24A1

2.4.1 General Characterization

The family of the cytochrome P450 enzyme includes CYP24A1 which takes place in vitamin D metabolism by catabolizing and degrading 1,25(OH)2D3 through 24-hydroxylation. This gene was discovered largely as a potential oncogene in breast cancer, but it has since been reported at various expression levels in many cancers including endometrial and lung cancer (1). Hence, the CYP24A1 enzyme regulates vitamin D level, it also functions in and vitamin D endocrine system and calcium homeostasis.

Furthermore, according to study, CYP24A1 plays a significant role in humans, because CYP24A1 inactivating mutations may contribute to idiopathic infantile hypercalcemia. Studies using knockout and transgenic mice also gave perspectives into the function of vitamin D in tissues of interest and shown the impact of vitamin D's extra skeletal, including cardiovascular effects, immunomodulatory effects in the prevention of cancer developing (22). Because of widespread genetic variations in the vitamin D 25-hydroxylase, vitamin D receptor genes and 24-hydroxylase, the elevation in [25(OH)D] induced by vitamin D3 treatment may differ (25).

A study on CRC samples underwent amplification for mRNA expression for revealed that ZNF217, but not CYP24A1 has correlation between mRNA overexpression and copy number increases. The results clarify that CNVs of certain oncogenes may play a role in CRCs (26).

CYP27B1 mRNA expression was lower in tumor tissues than in neighboring normal tissues, although CYP24A1 mRNA expression was significantly higher in tumor tissues. This large disparity revealed that CYP24A1 was expressed only little in normal breast tissues. These data suggest that vitamin D signaling, and metabolic pathways are disrupted during breast cancer development. Local alterations in active vitamin D anabolism and catabolism by the CYP27B1 and CYP24A1 enzymes in breast cancer may impair its anticancer activity (27).

2.4.2 Chromosome Localization

Chromosome 20 , q arm, band 13 sub-band 2 (21)

Ch20 q13.2

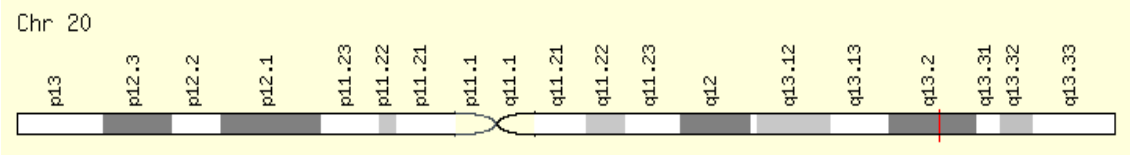

Figure 2.4.2. Chromosome localization of CYP24A1 Gene (21).

3. MATERIALS AND METHODS

3.1. Sample Selection

The work got approved by the ethics committee and the experiment was performed at Yeditepe University. Samples of 50 ovarian cancer patients and 50 controls were collected from yeditepe university included in the work. A form of consent was read and signed by each patient. That blood samples measuring 2 ml were put into tubes including EDTA, to avoid cloting, and kept at 4° C.

3.2. DNA Isolation

According to the (iPrep DNA Blood Kit), bu using automated robot (iPrep Pure Link, Invitrogen, thermo fisher scientific Inc) the genomic DNA was izolated from each case blood sample then placed into the tubes with EDTA. 350 µl of blood samples were placed into the device using a 5 ml tube. Isolation mixture: 8M Guanidine hydrochloride, 1.12 mg/ml proteinase k, 10.5 nM NaCl of pH 8,8, 10.5 nM EDTA, 10.5 nM Tris-CL. The steps have taken 45 min in a closed system, as a last step 100 µl of DNA was acquired and kept at + 4 °C in the refrigerator.

3.2.1 Measurement of the Purity of the Extracted DNA

The putity of the collected DNA samples was measured using NanoDrop (nanodrop 2000, thermo scientific Inc.), That measures the optical density ratios and concentrations of DNA samples. UV absorbance of the DNA was measured at 260/280nm. Samples were measured by taking 1.5 ul from each and diluted in the ratio of 1/100. The ratio of optical densities (OD) taken at wavelength 260nm and 280nm gives the purity of the samples. The acceptable ratio of samples purity of the OD260/OD280 is between 1.7 and 1.9

The formula of Calculating the concentration of DNA:

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

Which 50 µg/mL of double stranded DNA at a wavelength of 260 nm is equal to one optical density OD unit.

3.3. Determination of CYP24A1 Gene (rs2248137) Single Nucleotide Polymorphism

To Analyse and indicate the single nucleotide polymorphism within CYP24A1 gene, we applied genotyping with real-time polymerase chain reaction technique using (Applied Biosystems)7500 Fast-Real Time PCR.

In PCR, DNA polymerase enzyme takes place during amplification of DNA which requires high degrees and the enzyme is temperature resistant beside it amplifies DNA amount in-vitro. Allelic determination assay of TaqMan (Applied biosystems) Foster city, CA, USA was used for the analysis. (Applied Biosystems)the primer Express software used to design the probes of TaqMan.

Two different dyes used for analysis of allelic discrimination on rt-PCR, for wild-type allele (C) FAM (fluorescein amidite) and for mutant allele (G) VIC fluorescent probes were applied.

Table 3.1. The sequence of TaqMan assay used

Polymorphism	C/G, Transversion Substitution
Sequence	TCCTCCTAGGGGACCGGGGACCCTC**[C/G]**CTGCCCAGACGCCG AAGCGCACCAT

Table 3.2. Contents of Real-Time PCR.

Material	Quantity (For Each Sample)
Master Mix	5 µl
Template DNA	1 µl
DNAse, RNAse	3.75 µl
TaqMan Genotyping Assay	0.25 µl

Table 3.3 Real-Time PCR conditions

Step	Temperature (°C)	Time	Cycle
Enzyme Activation	95	10 min	Hold
Denaturation	92	10 sec	40
Annealing	60	1 min	40

3.4. Statistical Analyses

SPSS version 25.0 software used for statistical analysis. Student t-test was used to evalute the different significane between the 2 groups of the collected date after the gynotyping. Chi-squared test (X^2) and fishers extract tests were used to evaluate the frequency of genotypes and alleles among groups. p-values less than 0.05 indicates statistical significance. Risk factor for parameters was evaluated using logistic regression Analysis.

4. RESULTS

4.1. Assessment of Real Time PCR Findings

The software of the 7500 Fast-Real time PCR instrument automatically evaluate the Allelic discrimination.

In Figure 4.1 below the allelic discrimination plot is shown. The readings and interpretations of the fluorescence irradiation are performed by dyes of the probes. FAM dye shows blue color (Allele G) while VIC dye shows green color (Allele C).

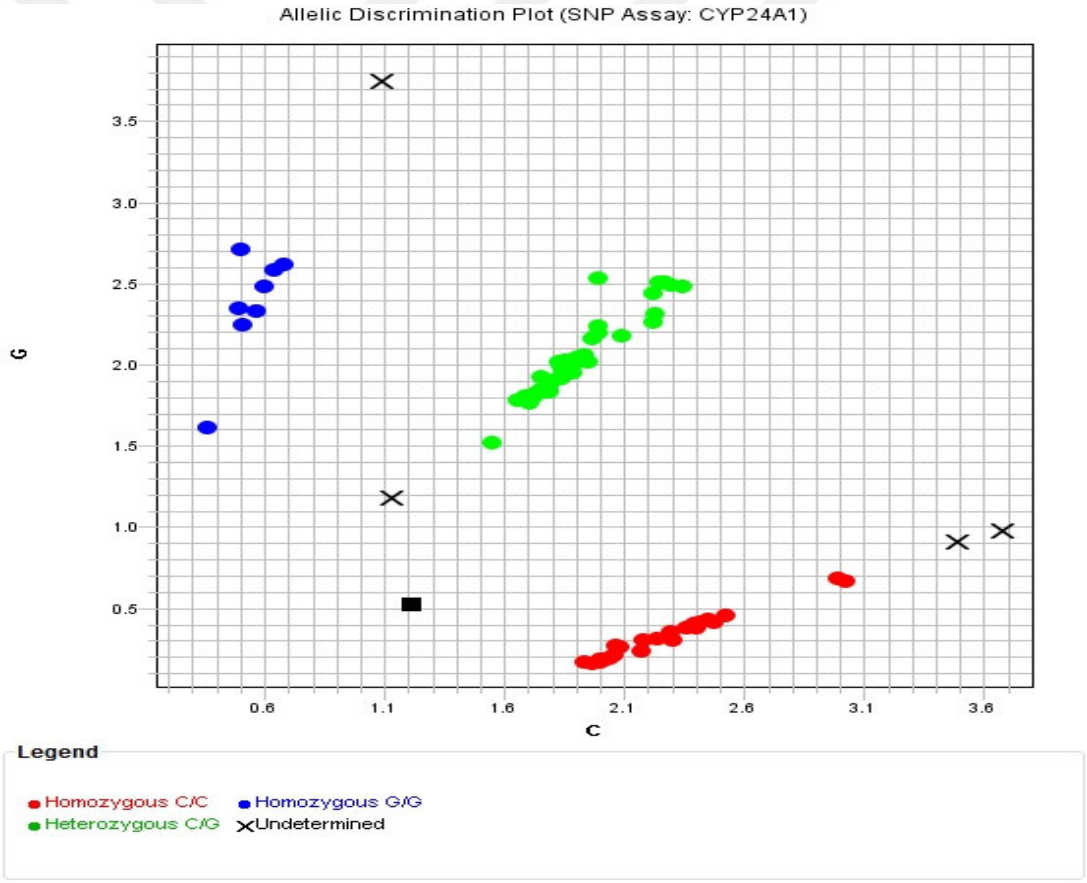

Figure 4.1. Allele discrimination display of CYP24A1 genotype

CC: Homozygote Wild Type, C/G: Heterozygote, GG: Homozygote Mutant

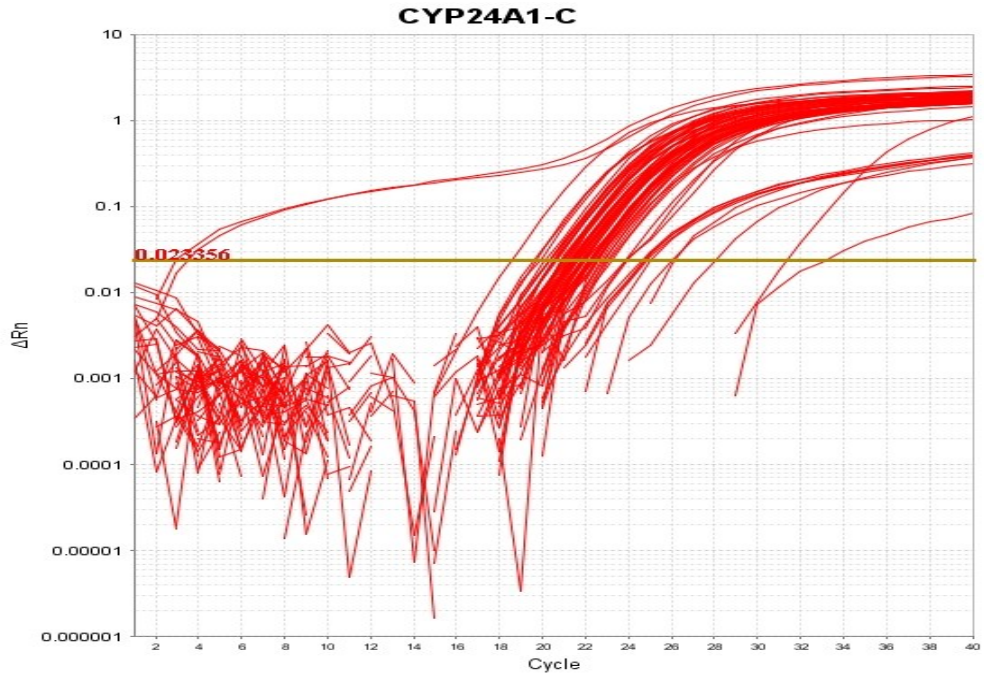

Figure 4.2. Amplification plot (ΔRn vs. Cycle) display of Allele C.

Figure 4.2 shows the amplification plot of Allele C, threshold value (0.023) is shown as a gold line.

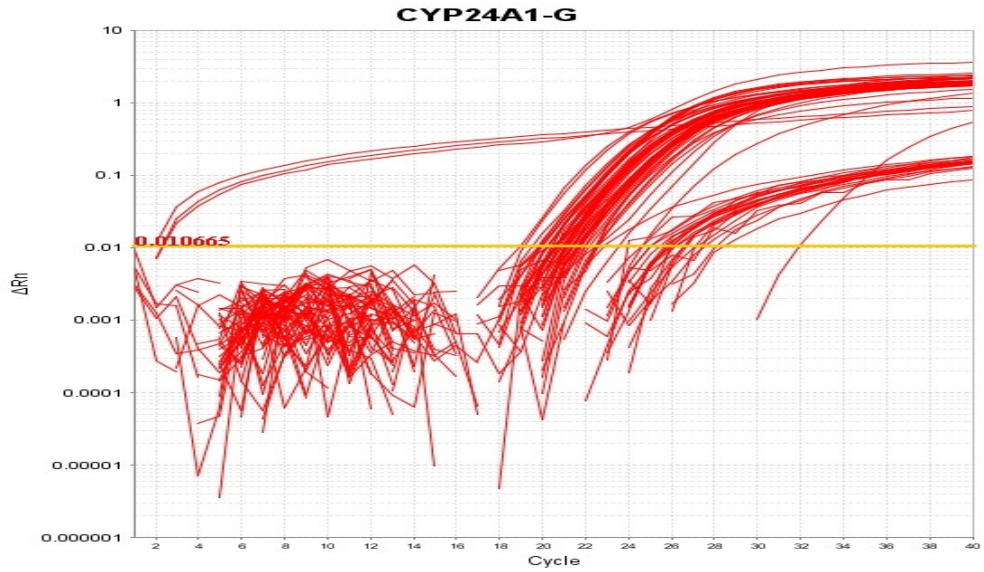

Figure 4.3. Amplification Plot (ΔRn vs. Cycle) display of Allele G.

Figure 4.3 shows the amplification plot of Allele G, threshold value (0.010) is shown as a gold line.

4.2. Statistical Analysis of Real Time PCR Findings

This study worked with 50 patients with ovarian cancer, and 50 healthy controls in this study, CYP24A1 gene variations and Ovarian Cancer data distribution were given in Table 4.1.

4.2.1. Analysis of Genotype and Allele Distributions for the CYP24A1 Gene in Patients and Controls

Rs2248137 polymorphism is a C>G transition, as this case (C) allele is the wildtype and (G) allele is the mutant. In our study, no statistically significant findings (p=0.051) for CYP24A1 gene polymorphism genotype between patients and the controls have been found.

As in table 4.1, our results shown that in Heterozygote Genotype (CG) (p=0.027) and in Homozygote Mutant Genotype (GG) (p= 0.065) the frequency was found a low sigificancy in patients comparing it with controls. Same with (CC) Homozygote Wild Type Genotype (p=0.288) no significant difference among the two groups.

According to our result, homozygote wild type genotype (CC), Homozygote Mutant Genotype (GG) and Heterozygote Genotype (CG) rates are calculated respectively: 57.6%, 75% and 40% among the control group. While for the patients' group, rates of homozygote wild type genotype (CC), Homozygote Mutant Genotype (GG) and Heterozygote Genotype (CG) were respectively: 42.4%, 25% and 60%.

For the allelic distribution, our findings indicate that there is no statistically significant difference for the wild allele (C) (p=0.065) between patients and the controls and for the mutant allele (G) (p=.288).

In the controls group, the wild allele (C) rate was 53.4%, while the mutant allele (G) rate was 53.7%. For the patients' group, the wild allele (C) rate was 46.6%, and the mutant allele (G) rate was 46.3%.

Table 4.2.1. Genotype and allele distribution.

Genotype	Case	Control	P-value
CC	n=19 57.6%	n=14 42.4%	0.288 NS
CG	n=22 40%	n=33 60%	0.027 S*
GG	n=9 75%	n=3 25%	0.065 NS
Allelic discrimination			
C	n=41 46.6%	n=47 53.4%	0.065 NS
G	n=31 46.3%	n=36 53.7%	0,288 NS

*S= significant different ($p<0.05$), **NS= non significant ($p>0.05$). n= number of samples.
The difference between groups was analyzed by the exact fisher test.

Table 4.2.2. Demographic data and explanation

Parameters	Case	Control	P-value
Age	41.60±8.35	43.69±15.721	0.038 S*
Smoking Smoker Non-smoker	 14 (87.5%) 32 (69.9%)	 2 (12.5%) 14 (30,4%)	0,200 NS
Oral contraceptive User Non-user	 11 22% 33 66%	 35 (70%) 15 (30%)	0.139 NS

*S= significant difference ($p<0.05$), **SN= non significant ($p>0.05$).
X± SD = Mean ±Standard Deviation, n= number of sample.
The parameters of the groups were analyzed by chi-square test and the independant t-test.

5. DISCUSSION AND CONCLUSION

A somewhat prevalent disease among women is ovarian cancer. Ovarian cancer kills more people each year than any other gynecologic malignancy because it can't be detected at an earlier stage. As a result, ovarian cancer is a serious public health issue. Numerous risk variables can be changed, despite the fact that many of them cannot be changed due to genetics and unavoidable exposures. Ovarian cancer risk is decreased by increasing parity and using OC.

From breastfeeding and unfinished pregnancies, the same is likely true, but to a lesser extent. While transient reproductive issues in parous women do not higher the risk of cancer in ovaries, infertility does in nulliparous women. It is critical to clarify that the known risk variables do not provide significant increases in risk and do not explain for all of the variation in the frequency of this disease. As a result, some triggers of ovarian cancer remain unknown. More study is required to better understand the origin of this lethal illness.

It’s indicated that progestins PR-dependently block CAL-induced CYP24A1, thus extending CAL activity. Progestins and vitamin D together decrease viability of the cell and cause apoptosis in the cells of the ovaries (28).

In 1980, The Garland brothers who were first to declare that the relation between vitamin levels and survival of cancer through a conducted study on latitude area population finding that increased level of vitamin D elevate the chance of developing cancer (17).

According to a previous study on epithelial ovarian cancer cases, it is indicated that excessive 25(OH)D concentrations have been linked to prolonged survival rates. Thus, high pre-diagnostic 25(OH)D levels in colorectal cancer people lead to decrease the risk of cancer and mortality. Therefore, vitamin D supplements show an effective early-stage cancer therapy (17).

A study results collected from participants on their own from participants concluded that Obesity and the risk of ovarian cancer are linked, however the evidence is few and contradictory (18). Excess body weight has been reported to increase the risk of ovarian cancer, with extreme obesity having a greater risk impact (19).

After adjusting for age and family history of breast or ovarian cancer on Val/Met COMT gene, Women over the age of 50 were higher at risk, as were those with invasive serous cancers (23). On The other hand, CYP17A2 mutation seemed to increase the risk of ovarian cancer, but the Val/Met COMT variant appears to lessen mucinous carcinoma risk (24).

A study included females with HDP found in 105. After controlling for relevant factors, it was discovered that 25(OH)D level changes between T1 and T2 and vitamin D deficiency at T2 were inversely related to DBP at T2 and T3, but not HDP. Polymorphisms in the CYP24A1 gene was associated to blood pressure and Hypertensive Disorder in Pregnancy. Vitamin D also functioned on blood pressure polymorphisms in the CYP24A1 gene. In addition, individuals with CYP24A1-rs2248137 polymorphisms and deficiency of Vitamin D at T2 revealed to higher risk of HDP (29).

Additionally, it has been found that the pathogenesis of ccRCC may be influenced by an increase in the genes that catabolize vitamin D, such as CYP24A1, as well as those that synthesize it, such as CYP2R1 and CYP27B1(30).

Cancer patients and Caucasian controls significantly different genotypic frequencies for the rs4809960, while Asian ancestry controls had significantly different genotypic frequencies for the rs6022999 SNP (31).

To genotype CYP24A1 gene polymorphisms, the Sanger sequencing test was utilized. Single-locus research found the SNP rs6022999 strongly related to lung and liver cancer risk, whereas stomach cancer was linked with rs6068816 (32). Other findings revealed that the CYP24A1 polymorphism rs6068816 is linked to lung cancer susceptibility in Chinese female nonsmokers (33).

In Caucasian population a large-scale study included five polymorphisms in the CYP24A1 gene found that the rs2585428 and rs4809960 were shown to be highly associated to overall cancer risk while the SNPs rs2585428 and rs4809960 lowered the cancer risk.

Meanwhile, the rs2585428 considerably decreased the risk of pancreatic cancer and rs4809960 significantly lowered breast cancer risk. There was no correlation discovered between rs6068816, rs6022999, or rs2181874 and cancer risk (34). On the Other hand, research discovered that the CYP24B1 gene polymorphism rs4588 was strongly related with cancer risk. A study. Relying on ethnicity revealed that the rs4588 polymorphism raises the risk in Asians and Caucasians (35).

A study reveals that CYP24A1 has a causative role in various cases of IIH by significantly increasing calcium absorption in the intestines, implying that genetic identification might be useful for IIH patients (36). However, Some CYP24A1 gene variations have been identified, with rs2248137 regarding autoimmune illnesses being related to vitamin D deficiency, like asthma and diabetes 1. (37)

Despite the fact that the capacity to do BRCA1 and 2 genetic testing is a huge therapeutic achievement, ovarian cancer related genes' mutation are still uncommon at many areas (38). It's been proven that frequent low penetrance genetic variations may impact ovarian cancer susceptibility, as has been proven for other prevalent illnesses such as diabetes and breast cancer (39). Recent study on invasive ovarian cancer among African women detected that a strong relation with Apa1 (40).

However, a study included postmenopausal females using daily dose of Calcium and vitamin D had not shown a dramatic decline in cancer incidence (41). Tumor metastasis is the leading cause of therapy failure in ovarian cancer patients. The omentum is the frequently affected tissue type by ovarian cancer metastasis because it has a high concentration of microvascular cells, adipocytes, fibroblasts, and immune cells which produce a favorable microenvironment for proliferation for a cancer cell (42).

In vitro and in vivo research demonstrated that 1,25(OH)2D3 may prevent cancer cell spread by atatching to Vitamin D Receptor in cancer cells (43). Furthermore, 1,25(OH)2D3 discovered to express VDR E-cadherin in high levels in ovarian cancer, opposing the expression level of catenin (44).

According to some findings, the differentiated thyroid carcinoma (DTC) risk is getting higher in case of conversion reduction to 1,25(OH)2D3 and low 25(OH)D3 circulation in CYP24A1 gene (45).

HNPCC syndrome results in an exceptionally elevated risk of colon cancer, uterine and ovarian cancer. Many genes are responsible for the disease including MLH1, MSH2, MSH6, PMS2, and EPCAM. Females with genetic nonpolyposis colon cancer have a 10% chance of acquiring ovarian cancer in their lifetime. HNPCC syndrome is only being seen in approximately 1% of all ovarian epithelial malignancies. HNPCC is also known as Lynch syndrome. Aside from the genes described above, other genes have been associated to ovarian cancer. Examples include RAD51D, PALB2, BRIP1 and ATM. Cancers of the breast and pancreas have also been associated to some of these genes (46).

A trial found no benefit of vitamin D supply on cancer rate or death. However, randomized controlled trials on postmenopausal women utilizing vitamin D plus calcium demonstrated a stronger positive impact in lowering cancer risk than vitamin D doses alone(47). Another study found that women who used vitamin D and calcium daily doses for long time lower chance of having breast colorectal cancer (48). There is a chance that combining calcium and vitamin D treatment that might be beneficial for avoiding cancer. More study is required to determine the best therapeutic dosage for preventing the development of ovarian cancer (49). Furthermore, its claimed that larger dosages of Ca plus vitamin D had no effect on cancer rates comparing it to lesser doses of vitamin D with Ca (50).

Our experiment clarifies the role of CYP24A1(rs2248137) C>G gene polymorphism and its relationship with ovarian cancer. We investigated the SNP (single nucleotide polymorphism) of CYP24A1 gene among patients with ovarian cancer within the Turkish population.

Samples of ovarian cancer patients were conducted with healthy control people with an n=50 for each group, we statistically evaluated and analyzed our genotype distributions outcomes of for both groups and as in table 4.1 results were wild type (p=0.288),

heterozygous (p=0.027) and homozygous mutant type (p= 0.065). The statistical analysis made use of the Chi-squared test (X2).

Genotypic of CYP24A1(rs2248137) frequencies between ovarian cancer patients and controls were statistically significant (p=0.051), our findings suggest that there were no significant differences among patient and control groups in (CC) Homozygote Wild Type Genotype (p=0.288). While the frequency in Heterozygote Genotype (CG) (p=0.027) was significantly higher in controls compared to patients. For the Homozygote Mutant Genotype (GG) (p= 0.065), we found the frequency was significantly lower in patients than in the controls, meaning that having (GG) genotype is not a risk elevating factor for developing ovarian cancer.

For the allelic distributions, our study found no significant difference in neither allele (G) mutant allele nor (C) wild allele, between patient and control groups. (P=0.065) which mean having the mutant (G) or the wild allele (C) is not considered to be an ovarian cancer increasing risk factor.

We also evaluated the major parameter that determine the risk factors for ovarian cancer, such as age, oral contraceptive use and smoking. But only age of these parameter has shown a significant difference between case and control group (p>0.05) while other parameters shown no significant difference.

To conclude, the outcome of our study declares that no statistically significant differences of carrying any of the Genotypes (CC), (CG), or (GG) of CYP24A1 gene and ovarian cancer disease. Thus, further studies are needed to clarify more of the ovarian cancer incidence.

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7. APPENDICES

7.1 Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15-2307
Konu: Klinik Araştırmalar Etik Kurul Başvurusu hk.

03/03/2022

İlgili Makama (Riman Nabil Shamallakh)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, Tıp Fakültesi Kadın Hastalıkları ve Doğum AD. Prof. Dr. Rukset Attar'ın sorumlu araştırmacı olduğu, Tıp Fakültesi Tıbbi Biyoloji AD. Doç. Dr. Seda Güleç Yılmaz'ın yardımcı araştırmacı olduğu **"CYP24A1 Geni Polimorfizimin Over Kanserdeki Rolünün Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2364)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **02.03.2022** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No:1574)**

Prof. Dr. Turgay ÇELİK

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7.2 Raw Data

T-test

Group Statistics					
	grup	N	Mean	Std. Deviation	Std. Error Mean
yas	over ca	48	41.60	8.315	1.200
	kontrol	35	43.69	15.721	2.657

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
yas	Equal variances assumed	16.008	<.001	-.781	81	.219	.437	-2.082	2.666	-7.386	3.223
	Equal variances not assumed			-.714	47.844	.239	.479	-2.082	2.916	-7.945	3.782

Independent Samples Effect Sizes					
		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
yas	Cohen's d	11.994	-.174	-.609	.263
	Hedges' correction	12.107	-.172	-.604	.261
	Glass's delta	15.721	-.132	-.568	.305

a. The denominator used in estimating the effect sizes.

Cohen's d uses the pooled standard deviation.

Hedges' correction uses the pooled standard deviation, plus a correction factor.

Glass's delta uses the sample standard deviation of the control group.

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
sigara * grup	62	62.0%	38	38.0%	100	100.0%

sigara * grup Crosstabulation					
			grup		
			over ca	kontrol	Total
sigara	içmiyor	Count	32	14	46
		% within sigara	69.6%	30.4%	100.0%
	içiyor	Count	14	2	16
		% within sigara	87.5%	12.5%	100.0%
Total		Count	46	16	62
		% within sigara	74.2%	25.8%	100.0%

Chi-Square Tests						
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.994[a]	1	.158	.200	.139	
Continuity Correction[b]	1.168	1	.280			
Likelihood Ratio	2.216	1	.137	.200	.139	
Fisher's Exact Test				.200	.139	
Linear-by-Linear Association	1.962[c]	1	.161	.200	.139	.105
N of Valid Cases	62					

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.13.

b. Computed only for a 2x2 table

c. The standardized statistic is -1.401.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for sigara (içmiyor / içiyor)	.327	.065	1.632
For cohort grup = over ca	.795	.609	1.037
For cohort grup = kontrol	2.435	.620	9.563
N of Valid Cases	62		

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ok * grup	44	44.0%	56	56.0%	100	100.0%

ok * grup Crosstabulation				
			grup	
			over ca	Total
ok	kullanmıyor	Count	33	33
		% within ok	100.0 %	100.0%
	kullanıyor	Count	11	11
		% within ok	100.0 %	100.0%
Total		Count	44	44
		% within ok	100.0 %	100.0%

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CYP24A1 rs2248137 * grup	100	100.0%	0	0.0%	100	100.0%

CYP24A1_rs2248137 * grup Crosstabulation					
			grup		Total
			over ca	kontrol	
CYP24A1 rs2248137	C/C	Count	19	14	33
		% within CYP24A1_rs2248137	57.6%	42.4%	100.0%
	C/G	Count	22	33	55
		% within CYP24A1_rs2248137	40.0%	60.0%	100.0%
	G/G	Count	9	3	12
		% within CYP24A1_rs2248137	75.0%	25.0%	100.0%
Total		Count	50	50	100
		% within CYP24A1_rs2248137	50.0%	50.0%	100.0%

Chi-Square Tests				
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	5.958[a]	2	.051	.054
Likelihood Ratio	6.115	2	.047	.054
Fisher-Freeman-Halton Exact Test	5.843			.059
N of Valid Cases	100			

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.00.

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotype CC * grup	100	100.0%	0	0.0%	100	100.0%

genotype CC * grup Crosstabulation					
			grup		
			over ca	kontrol	Total
genotype CC	yes	Count	19	14	33
		% within genotype CC	57.6%	42.4%	100.0%
	no	Count	31	36	67
		% within genotype CC	46.3%	53.7%	100.0%
Total		Count	50	50	100
		% within genotype CC	50.0%	50.0%	100.0%

Chi-Square Tests						
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.131[a]	1	.288	.395	.198	
Continuity Correction[b]	.724	1	.395			
Likelihood Ratio	1.134	1	.287	.395	.198	
Fisher's Exact Test				.395	.198	
Linear-by-Linear Association	1.119[c]	1	.290	.395	.198	.097
N of Valid Cases	100					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 16.50.
b. Computed only for a 2x2 table
c. The standardized statistic is 1.058.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for genotype CC (yes / no)	1.576	.680	3.654
For cohort grup = over ca	1.244	.842	1.839
For cohort grup = kontrol	.790	.501	1.245
N of Valid Cases	100		

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotype CG * grup	100	100.0%	0	0.0%	100	100.0%
genotype GG * grup	100	100.0%	0	0.0%	100	100.0%

genotype CG * grup

Crosstabs

			grup		
			over ca	kontrol	Total
genotype CG	yes	Count	22	33	55
		% within genotype CG	40.0%	60.0%	100.0%
	no	Count	28	17	45
		% within genotype CG	62.2%	37.8%	100.0%
Total		Count	50	50	100
		% within genotype CG	50.0%	50.0%	100.0%

Chi-Square Tests						
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	4.889[a]	1	.027	.044	.022	
Continuity Correction[b]	4.040	1	.044			
Likelihood Ratio	4.931	1	.026	.044	.022	
Fisher's Exact Test				.044	.022	
Linear-by-Linear Association	4.840[c]	1	.028	.044	.022	.014
N of Valid Cases	100					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 22.50.
b. Computed only for a 2x2 table
c. The standardized statistic is -2.200.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for genotype CG (yes / no)	.405	.180	.909
For cohort grup = over ca	.643	.433	.955
For cohort grup = kontrol	1.588	1.030	2.448
N of Valid Cases	100		

genotype GG * grup

Crosstab

			grup		
			over ca	kontrol	Total
genotype GG	yes	Count	9	3	12
		% within genotype GG	75.0%	25.0%	100.0%
	no	Count	41	47	88
		% within genotype GG	46.6%	53.4%	100.0%
Total		Count	50	50	100
		% within genotype GG	50.0%	50.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	3.409[a]	1	.065	.121	.061	
Continuity Correction[b]	2.367	1	.124			
Likelihood Ratio	3.549	1	.060	.121	.061	
Fisher's Exact Test				.121	.061	
Linear-by-Linear Association	3.375[c]	1	.066	.121	.061	.047
N of Valid Cases	100					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.00.

b. Computed only for a 2x2 table

c. The standardized statistic is 1.837.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for genotype GG (yes / no)	3.439	.872	13.563
For cohort grup = over ca	1.610	1.083	2.392
For cohort grup = kontrol	.468	.172	1.271
N of Valid Cases	100		

Crosstabs

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ALLELE C * grup	100	100.0%	0	0.0%	100	100.0%
ALLELE G * grup	100	100.0%	0	0.0%	100	100.0%

ALLELE C * grup

Crosstab

			grup		Total
			over ca	kontrol	
ALLELE C	yes	Count	41	47	88
		% within ALLELE C	46.6%	53.4%	100.0%
	no	Count	9	3	12
		% within ALLELE C	75.0%	25.0%	100.0%
Total		Count	50	50	100
		% within ALLELE C	50.0%	50.0%	100.0%

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ALLELE C (yes / no)	.291	.074	1.147
For cohort grup = over ca	.621	.418	.923
For cohort grup = kontrol	2.136	.787	5.803
N of Valid Cases	100		

ALLELE G * grup

Crosstab

			grup		Total
			over ca	kontrol	
ALLELE G	yes	Count	31	36	67
		% within ALLELE G	46.3%	53.7%	100.0%
	no	Count	19	14	33
		% within ALLELE G	57.6%	42.4%	100.0%
Total		Count	50	50	100
		% within ALLELE G	50.0%	50.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.131[a]	1	.288	.395	.198	
Continuity Correction[b]	.724	1	.395			
Likelihood Ratio	1.134	1	.287	.395	.198	
Fisher's Exact Test				.395	.198	
Linear-by-Linear Association	1.119[c]	1	.290	.395	.198	.097
N of Valid Cases	100					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 16.50.

b. Computed only for a 2x2 table

c. The standardized statistic is -1.058.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ALLELE G (yes / no)	.635	.274	1.471
For cohort grup = over ca	.804	.544	1.187
For cohort grup = kontrol	1.267	.803	1.997
N of Valid Cases	100		

8. CURRICULUM VITAE

PERSONAL INFORMATION

Name	Riman Nabil	**Surname**	Shamallakh

EDUCATION INFORMATION

Degree	**Department**	**The name of the Institution Graduated From**	**Graduation year**
Master	Molecular Medicine	Yeditepe University-Health Sciences institute	2022
University	Molecular Biology and Genetics	Bahcesehir University	2019
High school	Scientific	Ibad Ur-Rahman Private School	2013

Language experiance

Language	**Level**
Arabic	Native
English	Excellent
Turkish	B2