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THE ROLE OF TNF-β GENE POLYMORPHISM IN OVARIAN CANCER

MASTER OF SCIENCE THESIS

SHIYA HUSSEIN IBRAHIM, BSc

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SUPERVISOR

Assoc Prof. Dr. SEDA GÜLEÇ YILMAZ

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	Title, Name-Surname (Institution)
Chair of the Jury:	Prof. Dr. S. Ayşe Özer Yeditepe University Institute of Health Sciences Department of Molecular Medicine
Supervisor:	Assoc Prof. Dr. G. Seda Güleç Yılmaz Yeditepe University Institute of Health Sciences Department of Molecular Medicine
Member/Examiner:	Assoc Dr. İrem Peker Eyüboğlu Marmara University Institute of Basic Medical Sciences Department of Medical Biology

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 the Administrative Board of the Institute with decision date 24.07.2023 and numbered 610.

Prof. Dr. Bayram YILMAZ

Director of the Institute of Health Sciences

DECLARATION

I hereby affirm that I am the solitary author of this thesis and have not previously presented it, whether in its entirety or partially, for any academic degree program. With the exception of explicitly stated cases within the text, all of the content contained herein is entirely my own. Any references or acknowledgments have been noted.

24.07.2023

Shiya Hussein Ibrahim

DEDICATION

To my beloved family

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LIST OF ABBREVIATIONS AND SYMBOLS

AJCC: American Joint Committee on Cancer

BRCA: Breast Cancer gene

BMI: Body Mass Index

CA: Cancer Antigen

CdKs: Cyclin-dependent kinases

CT: Computed Tomography

CST: Charge Switch Technology

C: Celsius

CI: Confidence Interval

DNA: Deoxyribonucleic Acid

EOC: Epithelial Ovarian Cancer

EDTA: Ethylenediaminetetracetic acid

FIGO: International Federation of Obstetrics and Gynecology

FAM: Fluorescein amidites

gDNA: Genomic DNA

HGSOC: High Grade Serous Ovarian Cancer

HOX gene: Homeobox genes

HNPCC: Hereditary Nonpolyposis Colorectal Cancer

HRT: Hormone Replacement Therapy

HR: Homologous Recombination

HE4: Human Epididymis Protein

HLA: Human Leukocyte Antigen

IV: Intravenous

IP: Intraperitoneal

IKK: Inhibitory Kappa B Kinase

kD: Kilo Dalton

LT-β, LT-α: Lymphotoxin beta , Lymphotoxin alpha

MLH1: MutL Homolog 1

MSH2: MutS Homolog 2

MR: Magnetic Resonance

MHC: Major Histocompatibility

ml: mililitter

µl: Microlitter

NFkB: Nuclearfactor Kappa B

nm: Nano meter

OC: Ovarian Cancer

OSE: Ovarian Surface Epithelium

OCP: Oral Contraceptive Pill

OD: Optical Density

OR: Odd Ratio

PMS1: Postmeiotic Segregation Increased1

PID: Pelvic Inflammatory Disorder

P53,P50: Protein 53 and Protein 50

PET: Positron Emission Tomography

RT-PCR: Realtime Polymerase Chain Reaction

RNA: Ribonucleic Acid

SNP: Single Nucleotide Polymorphism

SPSS: Statistical Package for Social Sciences

SD: Standard Deviation

TNF-β: Tumor Necrosis Factor Beta

TNFR: Tumor Necrosis Factor Receptor

UTR: Untranslated Region

UV: Ultraviolet

WHO: World Health Organization

ABSTRACT

Shiya Hussein Ibrahim, investigation of Tumor Necrosis Factor Beta (TNF-β) Gene polymorphism and Ovarian Cancer susceptibility among the Turkish population. Yeditepe University, Institute of Health Science, Department of Molecular Medicine, MSc Thesis. Istanbul,2023.

This study explored the relationship between ovarian cancer (OC) risk in Turkish women and polymorphisms in TNF-β (+252A>G), which are known to play a critical role in malignancy development. The study included 47 cases and 45 controls and used RT-PCR for genotyping analysis. Statistical analysis was conducted to compare significant differences between the groups. The outcomes showed that the control group had an AA genotype at 64.4% (29 out of 45), an AG genotype at 35.5% (16 out of 45), and a GG genotype at 0% (0 out of 40). In the ovarian cancer patient group, the AA genotype was at 53.1% (25 out of 47), the AG genotype at 29.7% (14 out of 47), and the GG genotype at 8.5% (4 out of 47). However, no significance was observed when comparing the groups, with a p-value of 0.802. more extensive studies with larger population scales are necessary to determine the exact association.

Key words: Ovarian cancer, TNF-β, polymorphism

ÖZET

Shiya Hussein Ibrahim, Türk popülasyonunda Tümör Nekroz Faktör Beta (TNF-β) Gen polimorfizmi ve Yumurtalık Kanseri duyarlılığının araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Moleküler Tıp Anabilim Dalı, Yüksek Lisans Tezi. İstanbul,2023.

Çeşitli çalışmalar, yumurtalık kanserine yatkınlıkta inflamatuar yanıtın değiştiğini göstermiştir ve inflamatuar sitokinlerdeki polimorfizmlerin, yumurtalık kanseri (YK) dahil olmak üzere malignitelerin gelişiminde önemli bir rol oynadığı kanıtlanmıştır. Bu nedenle bu çalışmada Türk kadınlarında TNF-β (+252A>G) polimorfizmleri ile OK riski arasındaki ilişkiyi araştırdık. Bu çalışma 47 kişilik bir vaka grubu ve 45 kişilik kontrol grubu ile yürütülmüştür. Genotipleme analizi için RT-PCR ve gruplar arasındaki anlamlı farklılıkları karşılaştırmak için istatistiksel analizler yaptık. Sonuçlarımıza göre kontrol grubunda AA genotipi %64,4 (45 üzerinden 29), AG genotipi %35.5 (45 üzerinden 16) ve GG genotipi %0 (40 üzerinden 0) olarak tespit edilmiştir. Over kanseri hastalarında AA genotipi %53,1 (47'den 25'i), AG genotipi %29.7'si (47'den 14'ü) ve GG genotipi %8.5'i (47'den 4'ü) dağılmıştır. p değeri 0.802 olan gruplar arasında anlamlı fark saptanmadı. Kesin ilişkiyi araştırmak için daha büyük popülasyonlarla daha ileri çalışmalar gereklidir.

Anahtar kelimeler: Yumurtalık kanseri, TNF-β, polimorfizi

1. INTRODUCTION AND PURPOSE

Among females, ovarian cancer (OC) is a primary malignant tumor responsible for 52% of deaths caused by gynecological cancers. It is challenging to detect due to its asymptomatic nature and the lack of sensitive and specific screening tests, with CA-125 serum determination and trans-vaginal ultrasound examination currently being used for diagnosis, but neither providing the required accuracy. Unfortunately, 75% of ovarian cancers are not diagnosed early hence leading to advanced stage III or IV, giving patients a five-year survival rate of just 20%. Cancer's immunogenic malignancy is due to an immunosuppressive microenvironment, which allows it to evade immune recognition and elimination. The pro-inflammatory cytokine tumor necrosis factor beta (TNF-β), which lymphocytes excrete in substantial amounts, has both pro-cancer properties and anti-tumor. Although numerous TNF-β polymorphisms have been identified, many epidemiological studies have focused on the relation between the A252G polymorphism and various diseases associated with an inflammatory response and cancer (6).

Studies suggest a connection between changes in the body's inflammatory response and the risk of developing ovarian cancer. Specifically, certain variations in inflammatory cytokines have been linked to the development of malignancies, including ovarian cancer (OC) (1). Tumor cells and the surrounding inflammatory cells can promote tumor growth, progression, and immunosuppression, rather than triggering an effective anti-tumor response in the body. However, if genetic damage is the leading cause of cancer, certain types of inflammation may provide the conditions that allow that damage to thrive (3).

TNF is a critical player in inflammation, aiding tissue recovery and destroying it (3). While it can destroy tumor blood vessels, it can also cause the release of angiogenic factors. TNF can selectively destroy tumor blood vessels in malignant diseases when used in high doses. However, if produced chronically, it can act as a promoter for tumors (3). Cytokines, including TNF-beta, significantly impact inflammation, immune responses, and cancer development (5). TNF-beta is involved in all stages of tumorigenesis, such as cellular

survival, promotion, transformation, metastasis, angiogenesis, invasion, and proliferation (4). TNF is typically detected in human ovarian cancer cells, often alongside ILs 1 and 6 (3).

This study is the first to investigate whether the SNP TNF-β+252A>G(rs909253) is linked to the likelihood of developing ovarian cancer in the Turkish population. However, conducting more extensive studies on a larger scale is crucial to accurately determine the connection between tumor suppressor genes and ovarian cancer development in Turkish women.

2. LITERATURE REVIEW

2.1. Ovarian Cancer

Ovarian cancer is categorized into three groups based on the cell type they originate from, epithelial, germ layer, and stromal. The ovary consists of three different cell types, each capable of transforming into a tumor with varying characteristics such as spreading, handling, and prognosis. The most prevalent type of ovarian cancer is high-grade serous ovarian cancer (HGSOC), often referred to when discussing the disease (7).

2.1.1. Anatomy of The Ovaries

The pelvic area has a diameter that varies from 2 to 4 cm. They house the ova and are in charge of producing and synthesizing sex hormones. The ovarian ligament stretches from the ovary to the uterus, and the broad ligament runs from the ovary to the uterus, serving as the principal supporting membrane for the uterus and uterine tubes. Eggs, often referred to as ova, which are essential for reproduction, are stored in and released by the ovaries. Only about 300 of a woman's one million to two million eggs will mature into fertile eggs afterbirth (8). The ovaries are situated above the fallopian tubes, also known as oviducts or uterine tubes (11). The distal fallopian tubes, a pair of skinny ducts of about 10 cm in length with fingerlike extensions (fimbriae) on either side of the ovary, are where the eggs migrate from the ovaries to the uterus. By transporting sperm and egg cells to the uterus, the Fallopian tube plays a critical part in the reproductive process. The peritoneum, which in females also comprises the omentum and the pelvic and abdominal viscera, lines the anterior (front) and posterior (back) sides of the uterus. Metastases typically appear in the peritoneum rather than the primary tumor (9). The essential elements of the female reproductive system are shown in Figure 2.1.1-1.

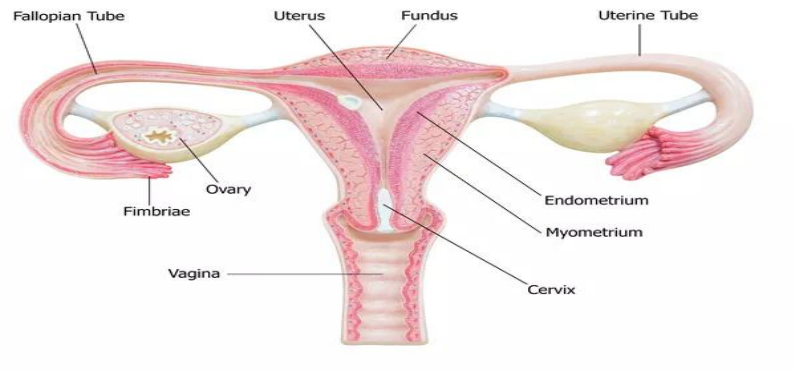

Figure 1.2.1.1: structures of the female reproductive system (11).

2.1.2. Microscopic Anatomy

According to the histological approach, the ovary contains two main parts: the cortex on the exterior and the medulla on the inside. A germinal layer of cuboidal epithelial cells covers ovarian surfaces. Follicles and oocytes in both the developing and degenerating stages may be present in the cortex. Connective tissue is tightly packed throughout the cortex. Spindle-shaped fibroblasts make up the stroma of this cortical connective tissue, which responds to hormonal cues. The medulla, which is primarily made up of stromal tissue with an amorphous consistency, houses the ovarian vascular system. The distinction between the cortex and the medulla in humans is, at best, hazy. The medulla and cerebral cortex may both contain stroma. It is the tissue that contains the arteries and follicles (8). Figures 2.1.1-2 and 2.1.1-3 show the anatomy of the ovary.

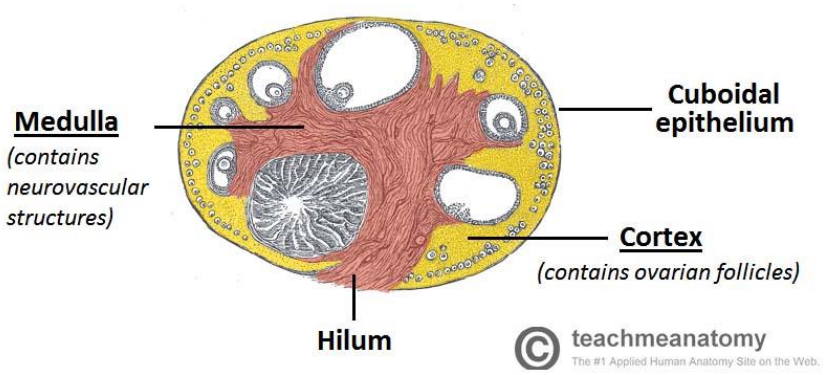

Figure 2.2.1.1: Cross section of an ovary showing the three major components (11).

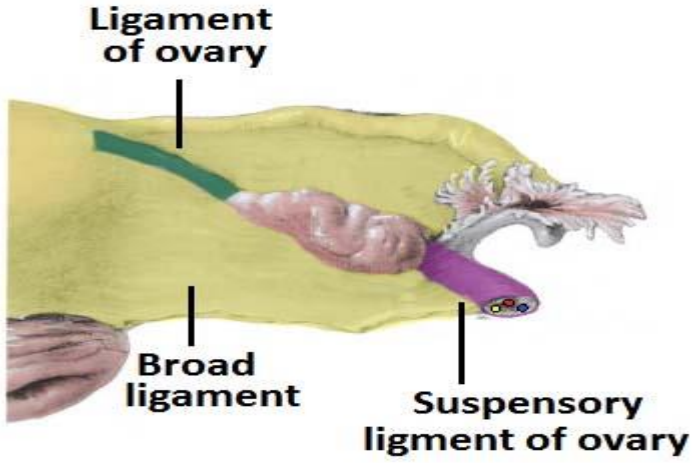

Figure 3.2.1.1: The major ligaments of the ovary (11).

The stroma of the ovarian cortex contains the ovarian follicles. Ovarian follicles are little sacs filled with fluid. Around 300,000 to 400,000 of these hormone-secreting cells, which control the menstrual cycle, exist in women at the time of adolescence. Each has the ability to release an egg for fertilization. An oocyte is contained within a follicle by granulosa cells, which are follicular cells. In order to mature and release the egg for the purpose of fertilization and reproduction, follicles go through different stages of development each month. When the follicle ceases to release the egg, degeneration follows (8). The maturation of ovarian follicles is shown in Figure 2.1.1-4.

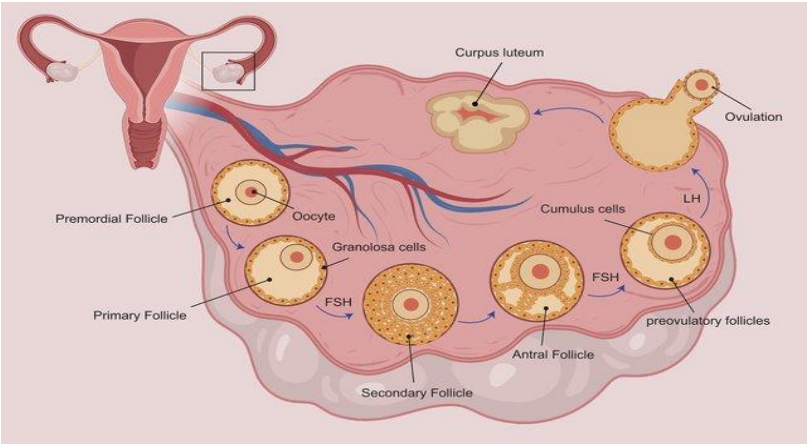

Figure 4.2.1.1: Ovarian follicle maturation illustrated (10).

2.1.3. Etiology, Risk Factors and Epidemiology

The early stages of OC are often uncertain, so clinical data is used to identify at-risk women. Family history is the most crucial risk factor. This study explores various hypotheses about the causes and pathophysiology of ovarian cancer. The epidemiology of ovarian cancer encompasses heredity, reproductive health, hormones, inflammation, diet, surgery, and location. All these factors can double the risk of ovarian cancer (13). Some hypotheses suggest that women who ovulate more frequently throughout their lives are more likely to develop ovarian cancer due to repeated trauma to the ovaries' epithelial surface during each ovulation event, predisposing it to malignant changes over time (14).

2.1.4. Etiology

Since the ovaries are positioned intra-abdominally, and most ovarian malignancies typically present with advanced disease at presentation, it has been challenging to define changes in the ovarian surface epithelium (OSE) consistent with intraepithelial neoplasia. Therefore, the early molecular and genetic pathways that cause ovarian cancer are poorly understood. As a result, the cause of ovarian cancer remains unknown, and it is still unclear exactly which cell is responsible for epithelial ovarian cancer's genesis. Researchers have recently put out different hypotheses, suggesting that the cells responsible for ovarian cancer could have their roots in fallopian tube cells. Though speculative, this hypothesis is backed

by the fact that the majority of ovarian tumors have histologies similar to those of the fallopian tube. Additionally, prophylactic oophorectomy samples taken from women who were genetically predisposed to developing OC showed an unusually high incidence of histologic and molecular markers linked to dysplasia in the fimbriated end of the fallopian tube (13). Long-term exposure to hormones or other cytokines may cause pathways (such the HOX genes) to become aberrantly activated, which may explain the Müllerian characteristics and morphologic heterogeneity of epithelial ovarian cancer (14).

2.1.5. Risk Factors

Numerous genetic, environmental, and reproductive risk factors have been studied in the context of ovarian cancer etiology and pathogenesis. A family history of breast or ovarian cancer is the most important factor. A germline mutation that raises risk from the mother or father may be present in up to 10% of individuals with ovarian cancer. A woman's lifetime chance of getting ovarian cancer is three times higher if her mother, daughter, or sister did. A portion of this greater risk is caused by the presence of known high-penetrance cancer susceptibility genes such (BRCA1, BRCA2, MLH1, and MSH2) (15). Few reliable serum signs can be used to identify women who would develop ovarian cancer early, despite substantial research into serum biomarkers. Contrary to other cancers like colon or cervical cancer, there is not enough tissue or other biomarker information to help doctors in identifying women at risk, and risk assessment is mostly relied on epidemiologic factors. The risk factors for epithelial ovarian cancer are listed in Table 1-2.1.5 (16).

Table 1-2.1.5: Risk factors for developing epithelial ovarian cancer (16).

Factors		Protective	Predisposing	Controversial
Demographic	Age		✓	
Reproductive	Menstrual-related factors		✓	
	Age of menarche and menopause			✓
	Parity	✓		
	Pregnancy characteristics			✓
	Higher age of childbirth	✓		
Gynecologic	Pelvic inflammatory disease			✓
	Endometriosis		✓	
Hormonal	Contraceptive methods	✓		
	Hormone Replacement Therapy (HRT)			✓
	Infertility treatments			✓
Genetic	Family history		✓	
	BRCA mutations		✓	
	Lynch syndrome		✓	
Lifestyle	Nutrition and Diet			✓
	Obesity and physical activity			✓
	Alcohol, caffeine and cigarettes			✓
Other	Lactation	✓		
	Lower socioeconomic status		✓	

2.1.6. Hereditary

A family record of ovarian cancer, particularly among first-degree relatives, is considered a significant and lasting risk factor for the disease. Research has found that females with first- or second-degree relatives with ovarian cancer are at a 3.6- or 2.9-fold higher risk, respectively than those without a family disease record (13). Most hereditary ovarian cancers, over 90%, are caused by germline mutations in the genes BRCA1 or BRCA2. The remaining cases are due to unknown gene mutations or DNA mismatch fix gene mutations that cause Lynch syndrome. The unraveling discovery of the genes BRCA1 and BRCA2 has supported the idea that ovarian cancer is more common in females having a genetic predisposition to the disease. It is estimated that 10-12% of ovarian cancer patients have genetic mutations in the genes BRCA1 or BRCA2, while an additional 2-3% come from families with Lynch syndrome or hereditary nonpolyposis colorectal cancer. Therefore, women with a family history that suggests a high risk of ovarian cancer should be offered genetic testing and counseling (13,15).

2.1.7. Lynch Syndrome

Lynch syndrome, a hereditary cancer risk disorder, can be caused by a genetic mutation in one of four mismatch repair genes (PMS2, MSH6, MSH2, and MHL1) (16). Clinicians should be aware of this syndrome, also known as HNPCC if a family record of early-onset endometrial or colon cancer is present before age 50. Women diagnosed with Lynch syndrome are at a higher risk for colon cancer (30-54%), endometrial cancer (42-60%), and ovarian cancer (9-12%) due to mutations in DNA mismatch-repair genes (MSH6, MSH2, or MLH1) (15).

2.1.8. Reproductive Factors

2.1.8.1. Parity

Research has demonstrated that pregnancy can reduce the risk of developing ovarian cancer. Each normal pregnancy can lower the risk by about one-third. This protective effect can continue up to 20 years after the last birth, although it may decrease over time. Furthermore, pregnancy may help the ovary eliminate damaged or premalignant cells. Women with first pregnancy after age 35 may be twice as protected against ovarian cancer compared to those who have their first child before age 25. Also, delaying the first childbirth age by five years can reduce the risk of ovarian cancer by up to 10%. It is worth noting that infertility treatments do not decrease the pregnancy protective effect, but infertility itself may increase the risk of ovarian cancer by up to two-fold (13,16).

2.1.8.2. Oral Contraceptive Pills (OCP)

Most studies indicate that oral contraceptives reduce the risk of all histological subtypes of ovarian cancer (16). In addition, OCP usage for a minimum of three years lowers the incidence of epithelial ovarian cancer by 30% to 50%. (13). However, the period of use and consumption is crucial, as early usage at ages below 25 portrays more reliable protection than when used above the age of 25. That being said, the risk reduction of OCP might last up to twenty years (16). Comparable to the epidemiologic evidence on parity, our results imply that OCP usage and parity may share a biological mechanism that protects against ovarian cancer (13).

2.1.9. Gynecological Factors

2.1.9.1. Pelvic Inflammatory Disease (PID)

PID is a multi-microbial infection of the pelvic structures, fallopian tubes, the uterus, and upper genital tract in females, caused by germs ascending from the lower genital tract, which leads to tubo-ovarian abscess, pelvic peritonitis, salpingitis, and endometritis. Around 800,000 women are treated for PID annually in the United States, and 6–20 percent of all Western women are diagnosed with PID at some time (17). Sexually active, young females are disproportionately impacted. Most females having PID may be approached effectively as outpatients with at least two weeks of antibiotics. However, if not appropriately treated, PID may cause significant consequences such as ectopic pregnancy, pelvic discomfort, abscesses, and infertility (18). Several studies have found that PID can increase the chances of developing ovarian cancer. Hence common complications of PID, low parity and infertility, are also risk factors for ovarian cancer. In a case-control study conducted by Risch and Howe in Canada, it was found that PID increased the likelihood of primary epithelial ovarian cancer. Based on an analysis of 564 randomly chosen controls and 450 confirmed primary epithelial ovarian cancer cases. This correlation is due to inflammation in the ovarian surface epithelium (9).

2.1.9.2. Endometriosis

Endometriosis, specifically ovarian endometriomas, is now universally regarded as a potentially malignant neoplastic illness, although it was formerly considered benign. Endometriosis, known as the growth of endometrial glands and stroma external to the uterine cavity, is an illness that, in addition to being relatively common, can impact patients' health and way of life on several levels. Pain (which may be life-threatening for specific individuals) and infertility are hallmarks of a disease with a pervasive presence. Common issues include dysmenorrhea, dyspareunia, chronic pelvic discomfort, irritable bowel motions, tenesmus, urinary dysfunction, and lower back pain (20). Various research has shown a link between endometriosis and ovarian cancer through various routes. Endometriosis-associated ovarian cancer is diagnosed earlier and at an initial phase than other ovarian cancer (16). According to Erzen and Kovacic, the patient's age determines the relation between ovarian cancer and

endometriosis. In less than 30 years, the likelihood of epithelial ovarian cancer in females having endometriosis has climbed from 4.99 per 10,000 people per year to 35.81 per 10,000 people per year in more than 50 years (21).

2.1.9.3. Ovarian Cysts

It is unknown if benign ovarian cysts may evolve into cancerous cysts. If this is regarded as the case for the vast majority of ovarian cancers, it might be viable to excise benign cysts as part of a screening program before turning cancerous (22). However, specific benign ovarian cysts have been linked to developing malignant ovarian tumors. Ovarian cysts have been linked to an increased risk of borderline ovarian cancers in case-control studies**,** and this risk is larger in postoperative patients. Additionally, ovarian cancer risk is higher in postmenopausal women with complicated ovarian cysts (16).

2.1.10. Hormonal Factors

Extensive evidence suggests that the ovarian epithelium is highly responsive to hormones and influenced by its local hormonal environment. The normal ovarian epithelium produces several members of the steroid hormone superfamily, such as androgens, vitamin D, retinoids, progestins, and estrogens. A recent study has revealed that reproductive hormones can significantly impact the ovarian epithelium, potentially altering ovarian cancer risk. For example, progestins have been found to induce apoptosis, a vital molecular pathway for cancer prevention, and are mediated by several known chemoprotective medications. In a similar way the ovarian epithelium may be affected by the cancer preventive properties of retinoids, vitamin D, and nonsteroidal anti-inflammatory medications. (13).

2.1.10.1. Hormone Replacement Therapy (HRT)

Studies on hormone replacement therapy and ovarian cancer risk have conflicting findings. Some suggest no increase in risk, while others link prolonged estrogen use to higher risk. However, combining progesterone with hormone therapy may lower the risk. (16)

2.1.11. Lifestyle Factors

2.1.11.1. Nutrition and Diet

Research has shown that excessive cholesterol consumption increases the risk of ovarian cancer but taking beta-carotene and B-complex vitamins as supplements may help reduce the risk. Eating fish daily positively correlates with ovarian cancer risk, while daily milk intake is negatively associated with the risk. Consuming plant-based diets and vegetables during early adolescence can decrease the incidence of hormone-related ovarian cancer. Saturated fat is linked to a higher prevalence of ovarian mucinous tumors, but a higher vitamin D level in the blood may reduce the risk. According to case-control research, calcium, and lactose consumption have also been associated with a reduced risk of ovarian cancer (16, 23).

2.1.11.2. Obesity

According to studies, obesity decreases the likelihood of surviving ovarian cancer and increases the likelihood of dying from the illness. Ovarian cancer is associated with central adiposity, suggesting that androgen is transformed in peripheral tissue. The issue of obesity and its relevance to ovarian cancer as a precursor is yet another disputed topic. Numerous studies have found a correlation between a patient's body mass index and their risk of developing ovarian cancer due to a hormonal mechanism in obese individuals that increases their risk of malignancy and mortality. Meanwhile, other studies have found that weight and BMI have no direct bearing on ovarian cancer prognosis (24,25).

2.1.11.3. Alcohol, Caffeine, and Smoking

Studies have proven that the type of alcohol consumed may affect the risk of developing ovarian cancer rather than alcohol consumption itself. Wine, beer, and liquor have not been associated with an increased risk of ovarian cancer. However, research suggests that premenopausal women who consume caffeine and coffee may be at a higher risk (26). Smoking does not notably raise the risk of ovarian cancer, although 20 years of smoking a pack a day may double the risk of certain tumors. Additionally, smoking duration and frequency may increase the likelihood of borderline tumors and death in ovarian cancer patients (27).

2.2. Pathogenesis

Repeated ovulation can cause genetic abnormalities in the ovarian epithelial cells, leading to an increased risk of ovarian cancer. Research have indicated that frequent ovulation, as observed in chickens, is linked to a higher incidence of spontaneous ovarian cancers. Moreover, p53 mutations are commonly found in epithelial ovarian cancer, and the number of ovulatory events throughout a person's lifetime corresponds to the occurrence of these mutations. These mutations can result in deficient DNA repair and increased susceptibility to ovarian cancer. However, it remains unclear how these changes might contribute to the development of fallopian tube neoplasms (28). The malfunction of the tumor p53 suppressor gene is the most prevalent genetic mutation found in human cancer. Usually, p53 helps prevent cell proliferation by binding to DNA transcriptional regulatory regions and producing genes that inhibit the function of cdks, which are necessary for cell cycle progression. However, p53 is also believed to have an important role in prevention of cancer by halting genetically damaged cells in the phase G1 of the cell cycle for DNA repair. Insufficient DNA repair may cause p53 to trigger programmed cell death. The second copy of the gene p53 is often deleted as a result of mutations in the gene p53, leaving the cancer cell with just the mutant protein p53. The mutant protein p53 may combine with the normal protein p53 and prevent it from interacting with DNA in circumstances where the cancer cell still has one normal copy of the gene p53.

Additionally, up to 82% of sporadic malignancies may have altered BRCA function due to epigenetic alterations, alternative splicing, and other genetic variables. The proteins BRCA2 and BRCA1 are necessary for the (HR) DNA system to repair DNA double-strand breaks and activate apoptosis and monitor cell cycle checkpoints. Approximately 10%-15% of sporadic ovarian cancers have mutant BRCA genes.

When the BRCA gene is not functioning properly, cells are more likely to experience aneuploidy, centrosome amplification, and chromosomal abnormalities, that makes them susceptible to further mutations. Additionally, BRCA is essential in helping several transcription factors, such as p53, function properly. People with germline BRCA1 mutations may more likely to develop tubal intraepithelial carcinoma. In contrast, patients with BRCA2 mutations (but not BRCA1 deficiency) tend to have better survival rates, respond better to

chemotherapy, and experience less genome instability (30). Molecular features unique to each histological subtypes are shown in figure 2.2-5 (31).

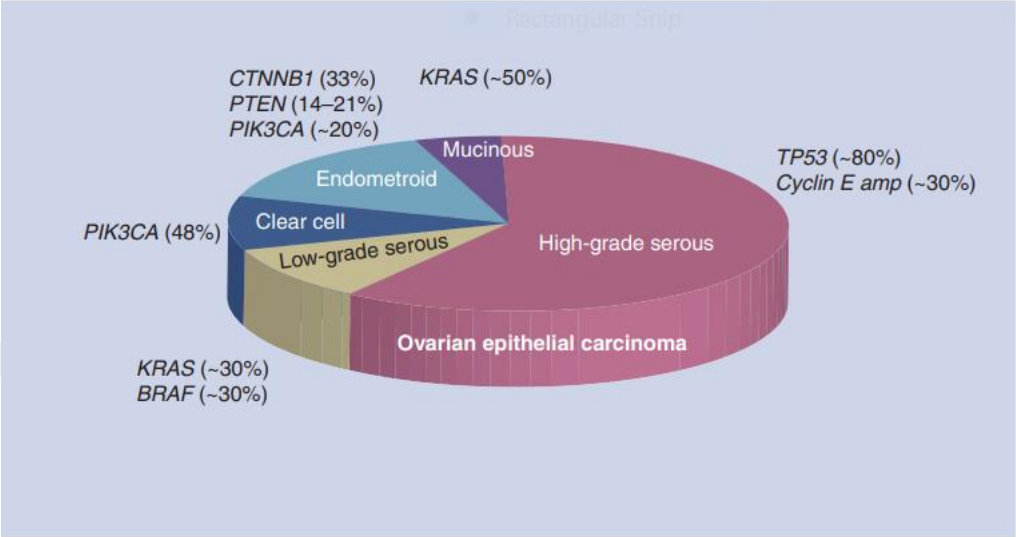

Figure 5.2.2: The main molecular genetic alterations in various subtypes of ovarian carcinoma (31).

2.2.1 Classification of Ovarian Cancers

The different types of ovarian cancer:

- Germ Cell ovarian cancers
- Small Cell ovarian cancers
- Stromal Cell ovarian cancers
- Epithelial ovarian cancers

Ovarian cancer is a complex disease with various clinicopathological and molecular characteristics. Surface epithelial tumors comprise almost two-thirds of all ovarian neoplasms, including a higher proportion of malignant neoplasms. Typically, adults are more susceptible to these tumors, especially the malignant forms that occur later in life. The tumor cells' predominant differentiation pattern is used to categorize epithelial malignancies. Traditional ovarian tumor categories include endometrioid, mucinous, serous, and clear cell

cancers corresponding to numerous kinds of epithelia in the woman reproductive system organs. Additionally, according to their clinical characteristics, tumors are classified as carcinoma (malignant), cystadenoma (benign), or borderline carcinoma (borderline) (low-malignant propensity). Recent research has identified specific molecular elements for each distinct histologic subtype of ovarian cancer (31,32).

2.2.1.1. Serous Tumors

Neoplasms that are categorized as serous are very common. These can take the form of cystadenomas, carcinomas, and borderline tumors, each with its distinct microscopic characteristics. High-grade serous ovarian carcinoma, which also includes fallopian tubes and primary peritoneal carcinoma, is the most typical kind of severe epithelial ovarian cancer. Unfortunately, this type of cancer is often diagnosed in later stages of Stage III or Stage IV around 70% of the time. The reason for that may be that many of these cancers begin in the fallopian tubes, eventually spreading to the ovaries from there, which explains why they are often discovered at later stages. Recent research has shown that high-grade serous cancers originating in the fallopian tubes take around 6.5 years on average to make their way to the ovaries before spreading elsewhere (7).

2.2.1.2. Mucinous Tumors

Mucinous cystadenoma is a common type of ovarian tumor known for its large size. While mucinous ovarian cancer is rare, it is essential to differentiate between primary and metastatic mucinous carcinoma tumors in order to provide the most effective treatment. Primary mucinous carcinoma tumors are typically limited to the ovaries and can be successfully treated when detected early, with an average diameter of 18 to 20 cm. However, it can be complicated to separate between primary mucinous carcinoma tumors and metastatic tumors from other organs. In some cases, primary mucinous carcinoma may require different treatments than other types of epithelial ovarian cancer. Nevertheless, patients with early detection of primary mucinous carcinoma have a good prognosis (7).

2.2.1.3. Endometrioid Tumors

John A. Sampson was the first to introduce the concept of endometrioid ovarian cancer in the 1920s this concept was later resurrected by FIGO in 1961 followed by WHO. The great majority of them are cancerous and invasive. They're frequently diagnosed as complex nonspecific solid-cystic masses on imaging and are linked to endometriosis. 8-15 percent of all ovarian carcinomas are endometrioid carcinomas. It's the second most prevalent malignant ovarian neoplasm. Moreover, because most endometrioid carcinomas are considered to arise from the epithelium surface rather than endometriotic tissue, the patterns and cellular characteristics of the poorly differentiated forms may blend almost unnoticeable with those of other common epithelial tumors, especially serous carcinomas, complicating differential diagnosis. The distinction between endometrioid and serous carcinoma is critical because to the differences in their biologic activity. Unlike serous carcinoma, which affects both ovaries in two-thirds of cases if all stages are taken into account, endometrioid carcinoma affects both ovaries in just one-third of instances. ' Furthermore, unlike serous carcinoma, which has frequently migrated throughout the abdomen by the time of surgical exploration, the latter tends to stay isolated to the pelvis and ovary for prolonged periods of time. The prognosis and therapy of the two types of cancer are different as a result of these variances (33).

2.2.1.4. Clear Cell Tumors

Clear cell tumors of the ovary were formerly considered to originate from the mesonephric or mesometanephric tissues or at least have some differentiation towards these tissues. However, recent studies suggest that these tumors are of Mullerian origin and nature, specifically in the genital tract. In the United States, ovarian Clear Cell carcinoma affects 4.8% of white women, 3.1% of black women, and 11.1% of Asian women, according to recent Surveillance and Epidemiology data. It is even more prevalent in Japan, accounting for 25% of epithelial ovarian malignancies. Clear Cell carcinoma makes up approximately 6% of epithelial ovarian malignancies and is more resistant to the most commonly used platinum-based chemotherapy than other types of epithelial ovarian malignancies. Most clear cell tumors are carcinomas, similar to endometrioid tumors (7,33).

2.2.2. Symptoms, Diagnosis and Treatment of Ovarian Cancer

Early detection is critical for successful ovarian cancer treatment. Unfortunately, it is challenging to detect EOC early because the disease can be asymptomatic for years, and its symptoms are vague when it is restricted to the ovary. According to statistics, only 15 percent of ovarian cancer is confined to the ovary, while 17 percent is regional, and 62 percent is metastatic, this is why ovarian cancer is defined as the "silent killer." Patients with metastatic tumors to the upper abdomen and pelvis usually experience early satiety, dyspepsia, abdominal swelling, and abdominal or pelvic pressure or pain. In addition, patients may experience weight loss, increased pain, and bowel or ureteral obstruction as the condition progresses. Many patients may recall a several-month record of mild abdominal discomfort that was usually nonspecific and was not initially considered a sign of severe underlying disease (41).

Since the early discovery and diagnosis of ovarian cancer is very unlikely, the diagnostic bases of the disease have proven no significance in a favorable prognosis for ovarian cancer patients. Therefore, a primary means of diagnosis is a physical pelvic, pulmonary, nodal, and abdominal examination looking for any visible and palpable abnormalities that could suggest ovarian cancer.

Radiographic imaging is another criterion of diagnosis usually recommended following a physical examination. For example, if ovarian cancer is suspected, imaging for the disease typically contains computed tomography (CT), transvaginal ultrasound, and magnetic resonance (MR) of the chest, abdomen, and pelvis to characterize the extent of peritoneal disease and check for distant dissemination. PET imaging is rarely employed in the early stages of a diagnosis. Instead, PET is often used in the recurrent scenario or for ambiguous lesions on CT/MR that would limit primary surgery.

Typically doctors do not use concurrent tumor marker testing for patients with average ovarian cancer risk. However, these tests can help assess the effectiveness of ovarian cancer treatment and keep track of the progress or recurrence of the disease. Some biomarkers commonly screened for ovarian cancer include CA-125, HE4, and CA19-9. For example,

CA-125 levels are high in 80 percent of patients in the last phases of the disease and 50 percent in the early stages of ovarian cancer (42).

Typically, treatment approaches are based on the type of ovarian cancer, its stage, and any other conditions. Most females with ovarian cancer will have their tumors surgically removed. However, additional effective treatments may be necessary prior to or post-surgery, depending on the kind and stage of ovarian cancer.

Most of ovarian malignancies are treated with surgery. Surgery for epithelial ovarian cancer has two main objectives: staging and debulking. Staging is to determine how far the cancer has metastasized from the ovaries this procedure usually includes a hysterectomy, bilateral salpingo-oophorectomy and omentectomy. Biopsies from all the removed tissues are further examined to assess an accurate staging of the cancer.

Debulking on the other hand is the removal of the tumor as extensively as possible, its essential to leave behind no apparent tumor larger than 1cm this is called optimal debulking patient who have their tumors optimally debulk usually have a better prognosis.

Radiation therapy is a cancer treatment approach that makes use of high-energy X-rays or particles to eradicate tumor cells. It is often administered similarly to a regular X-ray. While chemotherapy is generally more effective as the primary treatment for ovarian cancer, radiation therapy can help treat cancer that has spread to nearby tissues or distant organs like the brain or spine.

Chemotherapy is nearly always a systemic treatment, implying that the medication enters the bloodstream and reaches nearly every body part. Chemotherapy can eradicate tiny numbers of tumor cells persisting after surgery, treat malignancies that have metastasized (spread), or reduce enormous tumors to make surgery easier. Chemotherapy often involves medications administered intravenously (IV) or orally. It is also possible to inject chemotherapy directly into the abdominal cavity using a catheter, this is known as intraperitoneal chemotherapy (IP).

Chemotherapy for epithelial ovarian cancer is administrated throughout 3 to 6 cycles depending on the stage and type of ovarian cancer. Chemotherapy is highly effective on epithelial ovarian cancer, but over time, cancer cells may regenerate (43).

2.2.2.1. Stages of Ovarian Cancer

The classification of ovarian cancer staging involves three factors: the size of the tumor, whether it has spread to neighboring lymph nodes, and if it has metastasized to distant sites; this is also known as the TNM system (US Joint Cancer Committee), where T stands for tumor, N for nodes, and M for metastasis. Combined with numbers, these letters provide information on the invasive nature of the tumor and the overall stage grouping assessment (43). The AJCC staging system for peritoneal, fallopian tubes, and ovarian cancer can be found in Table 2-2.2.2.1(43).

Table 2-2.2.2.1: AJCC staging of ovarian cancer (43).

Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor invades lamina propria or submucosa
T2	Tumor invades muscularis propria
T3	Tumor invades adventitia
T4	Tumor invades adjacent structures
Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant metastasis	
MX	Distant metastasis cannot be assessed
M	No distant metastasis
M1	Distant metastasis
Tumors of the lower thoracic esophagus	
M1a	Metastasis in celiac lymph nodes
M1b	Other distant metastasis
Tumors of mid-thoracic esophagus	
M1a	Not applicable
M1b	Non regional lymph nodes and/or other distant metastasis
Tumors of the upper thoracic esophagus	
M1a	Metastasis in cervical nodes
M1b	Other distant metastasis

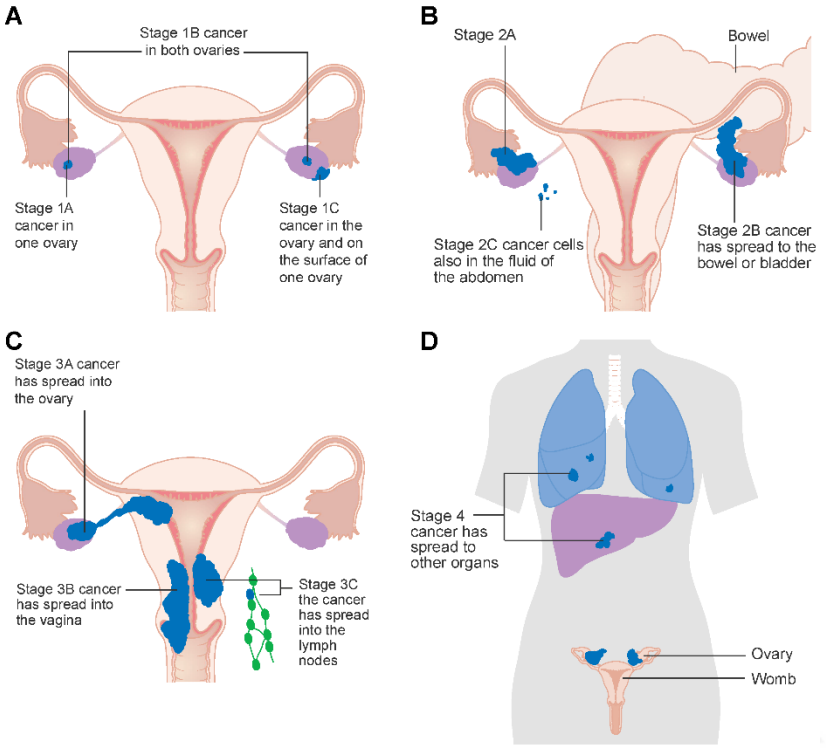

Figure 6.2.2.2.1: staging of ovarian cancer (43).

2.2.3. Tumor Necrosis Factor-Beta (TNF-β)

TNF is a substance that triggers inflammation and immune responses in the body. It interacts with cells through TNFR1 and TNFR2 when activated by a TNF family 1 ligand. TNF-beta, produced by activated T and B cells, affects various biological processes such as cell growth, differentiation, cell death, lipid metabolism, blood clotting, and neurotransmission, and can bind to lymphotoxin-alpha to fight cancer cells. TNF has been studied for its role in usual cancer-related inflammation, autoimmune diseases, inflammation, and physiology (34).

TNF has various effects ranging from physiological to pathological. For example, it promotes tumor cell necrosis and can cause apoptosis. In addition, TNF affects embryo development, sleep-wake cycle, lymph node formation, and defense against infections. It is also the main cause of fever; chronic exposure can lead to cachexia, wasting syndrome, and depression.

Research has shown that TNF plays a significant role in chronic inflammation associated with cancer and increases the risk of tumorigenesis, progression, invasion, and metastasis. Aspirin can reduce the release of TNF and lower the incidence of colorectal cancer when taken over an extended period. TNF is frequently found in biopsy specimens from breast, ovarian, and kidney cancer, as well as adjacent stromal cells. TNF is considered to be an important element in the development of malignant mesothelioma caused by exposure to asbestos, as it is not pathogenic when TNFR is absent. In addition, experimental models of mouse cancer have demonstrated that TNF polymorphisms can promote skin and early-stage liver cancer besides metastases of colon cancer to the lungs.

TNF-β is a 25kD glycoprotein produced by T cells encoded by an MHC gene. It consists of a polypeptide core and a carbohydrate and is similar to another non-glycoprotein, TNF-α, produced by macrophages. Both proteins interact with cells through high-affinity receptors, TNFR1 and TNFR2, in varying amounts in different cells. TNFR1 is found in all human tissues, while TNFR2 is primarily activated in immune cells and has a limited role in biological reactions. Both TNF-β and -α can bind to TNFR2 (35).

2.2.3.1.TNF-β Mediated Signaling Pathway

NF-β is a part of the TNF family that binds to various receptors, activating the response of innate immune for immunological regulation. To activate, TNF-β needs to form a complex with LT-β, resulting in the creation of the LT-α1-β2 complex. This complex allows TNF-β to bind to LT-β, activate signaling pathways, and anchor TNF-β to cell membrane receptors. The activation of the NF-κB signaling pathway triggers biological events like cell proliferation and death. When the LT-β receptor is activated, IKK-α, β, and γ are generated, which degrade NF-kB inhibitor I-κB and form ReIA (p60) and NF-kB1 (p50). This synthesis boosts gene transcription rates for cytokines and inflammatory-inducing molecules (36).

New studies have revealed that TNF-β signaling can contribute to cancer development. Although TNF-β signaling can strengthen inflammatory reactions, prolonged inflammation can disrupt cells and accelerate certain illnesses risk, such as cancer. As a result, mutations in regulatory components of the TNF-β pathway can disrupt cell signaling and promote the growth of cancerous cells. One such change involves the LT-α1-β2complex constantly binding to receptors LT, leading to the alternative pathway's continuous activation. A NF-κB pathway of constitutively active is present in numerous types of cancers, among them is multiple myeloma. Studies have shown that removing LT-β receptors can inhibit angiogenesis and tumor growth. Therefore, lymphotoxin and its downstream signaling through the NF-κB pathway indicate that the cytokine is involved in the spread and growth of tumors (37).

2.2.3.2. Chromosome Localization of TNF-β

The gene responsible for encoding TNF-β is found on the short arm of the sixth chromosome, specifically on 6p21.3, which is part of the major histocompatibility complex (MHC) locus referred as HLA. This gene is made up of four exons and spans approximately three kilobases. It includes both the 5′- and 3′-untranslated regions, and the 3'-UTR of TNF contains an Adenylate-Uridylate-rich element that codes for proto-oncogenes, nuclear transcription factors, and cytokines, and the last exon of the gene codes for over 80 percent of mature TNF-β (38). Chromosomal location of TNF-β is shown in figure 2.2.3.2-7.

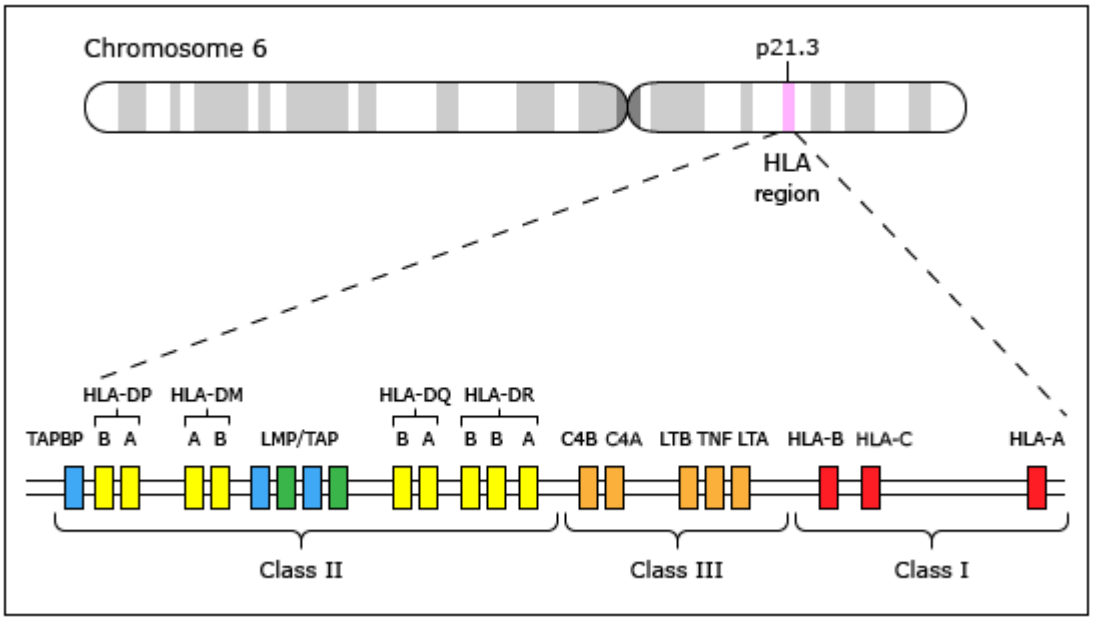

Figure 7.2.2.3.2: chromosome localization of TNF-β (39).

3. MATERIALS AND METHODS

3.1. Study Population

This research was conducted in a hospital setting using a case-control method. Participants were selected from the Obstetrics and Gynecology Departments of Yeditepe University Hospital in Istanbul, Turkey. The study included 47 diagnosed ovarian cancer patients between the ages of 18 and 83 who were confirmed through histological analysis and 45 non-cancer controls who met the eligibility criteria. The ethics committee at Yeditepe University approved the research (ethics committee decision no: (1508).

3.2. Laboratory Materials

3.2.1. Materials Used In DNA Isolation

Ethylenediaminetetraacetic acid (EDTA) tubes were used to collect the peripheral blood samples of 5ml from case and control groups and stored at +4C. To stop the collected blood from clotting, EDTA tubes were utilized. DNA extraction was performed by using an iprep Purification instrument (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA) and an iprep pure link gDNA blood isolation kit (Invitrogen, Life Technologies; USA).

3.2.2. Equipment Used In This Study

Types of equipment used in this study are; (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA), nanodrop 2000 (Thermo Fisher Scientific Inc.), Real-time PCR (Light cycle 480 II instruments, Roche Diagnostics, fast Real-time 7500, Applied Biosystems), 7500 fast Real-time PCR instrument, +4C and -20 refrigerator (Haier), Plate Centrifuge (Hettich), ultra-pure water (PURELAB option Quest), Vortex (V.I Plus Biosan) and pipette kit (Thermo Fishe Scientific Inc.) pure water (PURELAB option Quest), Vortex (V.I Plus Biosan) and pipette kit (Thermo Fishe Scientific Inc.).

3.3. Laboratory Methods

3.3.1. DNA Isolation from Blood

The blood samples for both controls and case groups were collected into EDTA tubes with 5ml volume and stored at +4°C. An iPrep Purification Instrument (Invitrogen) was used to perform the DNA extraction within 30 minutes, and iPrep™ PureLink® gDNA Blood Kit allows the isolation of genomic DNA from fresh blood samples. This automatic system can extract DNA from 350 µl of peripheral blood. These robots use magnetic bead technology to process 13 blood samples per run. Prepackaged reagents in cartridges were used to set up variation and allow for simple process validation. The prepackaged reagents were agitated for a few seconds to mix the magnetic beads in the reagents thoroughly.

IPrep robotic isolation equipment takes advantage of ChargeSwitch® (CST®), a robust and automated system in DNA purification. ChargeSwitch® allows rapid and efficient purification of DNA from small volumes of blood samples. This technology uses magnetic bead-based technology to isolate amounts of genomic DNA from samples. This method is based on a distinct principle compared to the silica-based purification methods. Magnetic bead charges switch according to the pH of the surrounding buffer to facilitate nucleic acid purification. The negatively charged DNA backbone binds to the positively charged beads at low pH. As a result, proteins and other particles do not bind and are washed away in an aqueous wash buffer. DNA is eluted by neutralizing the beads' charge using a low salt and high pH 8.5 buffer. Purified DNA passes instantly into the elution buffer and is ready for use in the next step.

3.3.2 Measurement of DNA Purity (Nanodrop)

In this study, purified DNA was measured by Nanodrop 2000 (Thermo Fisher Scientific Inc.) for purity and nucleic acid concentrations. Nucleic acid absorbs UV light around a wavelength of 260nm. Unlike the other classical spectrophotometer method, nanodrop measurement does not require special cuvettes or capillaries. Both proportions of this instrument measured OD260/OD280 and OD260/OD230 of DNA concentration. The amount of DNA used in this study was 1.5µl. The DNA was pipetted directly onto an optical measurement surface and closed to complete the automated measurement. Once the

measurement was done, the surface was wiped with clean paper tissue for the accuracy of the measurements. For every ten measurements, the surface was cleaned with distilled water for better accuracy. The computer detected the results, and we were able to detect DNA purity as well as DNA concentration. The optimal ratio of OD260/OD280 is between 1.7-1.9ng/µl for Genotyping, values higher or lower than the optimal ratio is determined to be contaminated with RNA or protein respectively.

3.3.3. Genotyping Analysis

Genotyping analysis was performed by Real-Time PCR (light cycle 480 II instrument, Roche Diagnostics, fast real-time 7500, Applied Biosystems), a powerful technology in molecular biology. Real-time PCR uses fluorescence dyes of probes to detect single nucleotide polymorphism (SNP). This method allows Genotyping by reading the fluorescence radiation. This study used two TaqMan probes: FAM dye and VIC dye. TaqMan probes are covalently joined to the amplified region of the DNA template. Taq polymerase hydrolyzes the probes detaching the reporter from the quencher and increases fluorescent signals at the end of each cycle. Therefore, real-time PCR instruments can monitor and record this increase in fluorescence, and with its two allele-specific wavelengths, wildtype and mutant alleles can be detected, Vic binding to the wild allele while Fam binding to the mutant allele.

Real-Time RT-PCR is used to detect polymorphism in the TNF-β gene by amplifying the target sequences in the TNFβ gene. DNA amplification of the polymorphism-containing region was performed using

reverse 5′- AGA GGG GTG GTA GCT TGG GTT C-3′ and forward 5′-CCG TGC TTC GTG CTT TGG ACT A −3′ primers. This primer was determined according to the sequence of the TNF-β gene in human cells(40). First, genotyping of the target gene region, TNF-β rs909253, was analyzed. Then allele discrimination is illustrated in the 7500 fast Real-time PCR.

The preparation of the reaction mixture is the initial step in PCR amplification. A concentrated pre-mixed solution known as master mix is utilized in this stage. This solution consists of buffer, , nucleotide, primers (reverse and forward), Taqman probe, then DNAse,

RNAse free water, Taqman genotyping assay, and DNA template is added to the reaction mixture. The components and quantity of the reaction mixture are illustrated in Table 3-3.3.3.

Table 3-3.3.3: The contents of the reaction mixture

Used Products	Quantity(µl)
Master Mix	5
Taqman Genotyping Assay	0,2
DNAse,RNAse Free Water	3.75
Template DNA	1

The reaction mixture is loaded into the RT-PCR plate consisting of 96 wells, allowing the analysis of several samples at once. Then the plate is sealed and centrifuged for 2 min at 3000 rpm to make the solution more homogeneous and to precipitate all liquids to the bottom, next the plate is placed in the RT-PCR machine, which is essentially a thermal cycler. In a real-time PCR reaction, three significant steps make up each cycle, and the reactions (Enzyme activation,denaturation and elongation) tend to run for 40 cycles.

Real-time PCR conditions are programmed as a 10-minute waiting time at 95°C, then denaturation at 92°C for 15 seconds, followed by elongation at 60°C for one minute at every cycle. PCR conditions are shown in Table 4-3.3.3.

Table 4-3.3.3: PCR Conditions.

Steps	Temperature (°C)	Time	Cycle
Enzyme activation	95	10 minutes	Hold
Denaturation	92	15 seconds	40
Annealing	60	1 minutes	

3.3.4. Statistical Analysis

We analyzed and studied the data using IBM SPSS Statistics version 23 (IB Corp, Armonk, NY, USA). After completing the PCR experiment and receiving allelic discrimination data, we entered all the data into the SPSS 23.0 program. We had previously acquired and documented the clinical parameters in the SPSS document. To analyze and determine the significance of differences between groups, we used the independent sample t-test, and for analyzing demographic information, we used the chi-square and Fisher's exact tests. We designated statistical significance as $p<0.05$ to identify and evaluate potential ovarian cancer risk variables.

4. RESULTS

4.1. Demographic Data Analysis

This study is composed of a control group of 45 individuals and a case group of 47 patients of the same gender (female), we compared the two groups against our documented clinical and demographic variables to observe if there is any significant difference. These variables included age, BMI, smoking, alcohol consumption, family history, pregnancy, and menopause status, using the independent t-test and chi-square test. Demographic characteristic analysis of the study group is shown in Table 5-4.1.

Table 5-4.1: Demographic characteristic analysis of the case and control groups.

Parameters	Control group n=45	Case group n=47	P value
Age X± SD	51.71±12.23	54.62±12.24	0.258 NS
BMI X± SD	23.2±3.78	29.1±5.14	0.001* S
Smoking status Non-smoker	n=22 (48.8%)	n=37 (78.7%)	0.004* S
Smoker	n=18 (40%)	n=7 (14.9%)	
Alcohol Consumps alcohol	n=15 (33.3%)	n=0	0.001* S
No consuption	n=25 (55.5%)	n=47 (100%)	
Family history Has family history	n=23 (51.1%)	n=15 (31.9%)	0.048* S
No family history	n=17 (37.7%)	n=29 (61.7%)	
Pregnancystatus Pregnancy	n=27 (60%)	n=37 (78.1%)	0.063 NS
No pregnancy	n=13 (28.8%)	n=7 (14.8%)	
Menopause Pre-menopause	n=26 (57.7%)	n=16 (34%)	0.036* S
Post menopause	n=19 (42.2%)	n=31 (65.9%)	

*S= significant difference ($p<0.05$), **NS= non significant ($p>0.05$).

n= number of sample. X± SD = Mean ±Standard Deviation,

The demographics characteristics of the groups were analyzed by fishers exact test test and the independant t-test

Table 5-4.1 shows statistically significant differences in BMI, smoking status, alcohol consumption, family history, and menopause status, with p-values of (.001, .004, .001, .048, and .036) respectively. In comparison, no significant difference was found in age with p=0.258 and pregnancy with p=0.063 in our study population, including cases and controls.

4.2. Case Group Clinical Data

This study consists of 47 cases of ovarian cancer, table 6-4.2 explains the clinical data of the case group.

Table 6-4.2: Clinical data of ovarian cancer case group.

Cell spectrum	Number (n) and percentage value (%)
Mixed epithelial tumors	n=5 10.6%
Mucinous tumors	n=3 6.3%
Serous tumors	n=24 51%
Endometrioid tumors	n=2 4.2%
Clear cell tumors	n=2 4.2%
Sex cord stromal tumors	n=1 2.1%
Germ cell tumors	n=2 4.2%
Total	n=47

n= number of sample. %= percentage values based on sample group in total.

Table 6-4.2 showed that the most prevalent type of ovarian cancer in our case study group was of serous origin, followed by mixed epithelial tumors.

4.3. Real-Time PCR Results

The 7500 Fast-Real Time PCR instrument evaluated allele types in both the patient and control groups. The allelic discrimination plot derived from this device is depicted below in Figure 4.3-8. The allelic discrimination was detected automatically by the software of this device. The fluorescence irradiation analysis and readings used the contribution of dyes contained in the probes. Despite this, several samples could not be distinguished or analyzed. This experiment used two different dyes that corresponded to the colors FAM blue and VIC green.

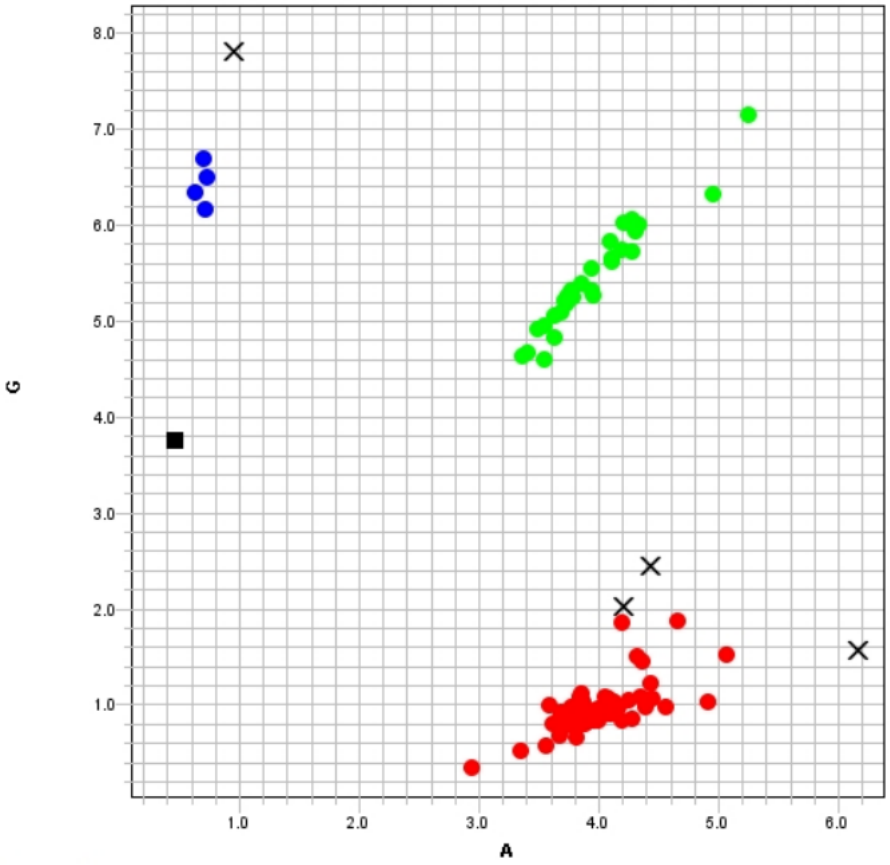

Figure 8.4.3: Allelic discrimination plot (SNP Assay: TNF-β).

(AA) is the homozygous wild type, (GG) is homozygous mutant type, and (AG) is the heterozygous type.

The figure 4.3-9 below illustrates the amplification plot of allele A. The yellow line corresponds to Threshold value (0.030145).

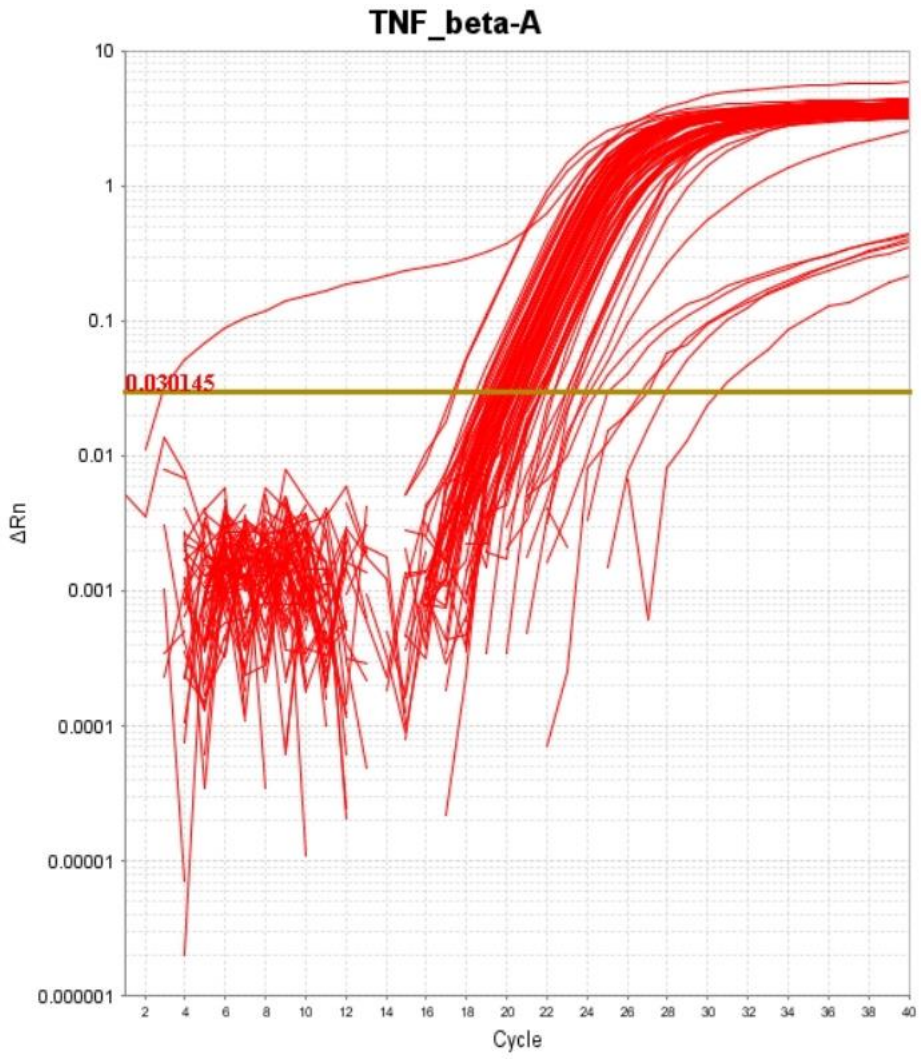

Figure 9.4.3: Amplification plot of allele A.

Figure 4.3-10 illustrates the amplification plot of allele G. The yellow line corresponds to threshold value (0.028655).

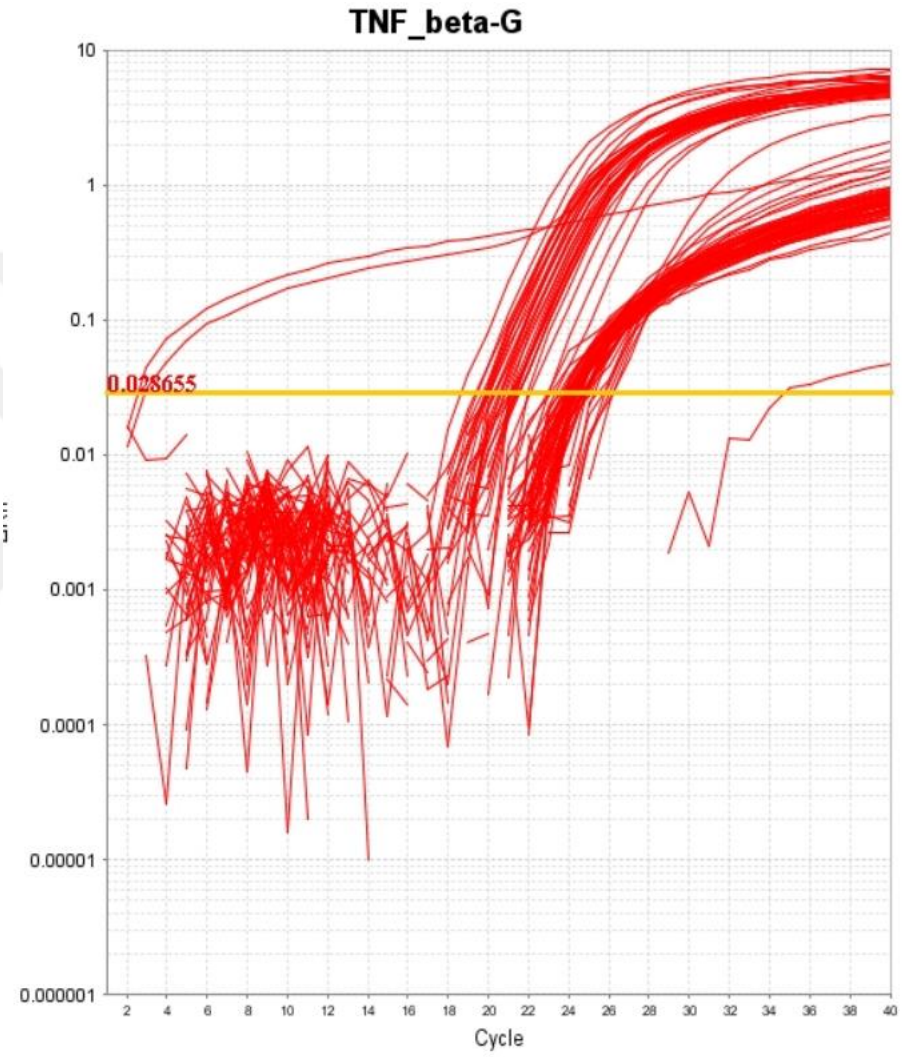

Figure 10.4.3: Amplification plot of allele G.

4.4. Genotyping Analysis of Study Groups

In this study, we examined the TNF-β polymorphism in both case and control groups. Table 7-4.4. shows the genotypes (AA, AG, GG) and alleles (A, G) distribution of TNF-β in case and control groups.

The Fisher s Exact Test was used to analyze the statistically significant differences between the ovarian cancer case and healthy control groups.

Table 7-4.4: genotype and allelic distribution of case and control groups.

Genotypes	Control n=45	Case n=47	P value	Odd Ratio (OR)	95% CI
AA	n= 29 64.4%	n=24 51.06%	0.545 NS	1.305	0.552 - 3.084
AG	n= 16 35.5%	n=14 29.7%	0.767 NS	1.143	0.473 - 2.764
GG	n= 0 0%	n=4 8.51%	0.053 NS	0.464	0.369 - 0.584
Allelic distribution					
A	n =45 100%	n=39 82.9%	0.053 NS	2.154	1.712 – 2.710
G	n =17 37.7%	n=14 38.3%	0.696 NS	0.843	0.359 – 1.982

*S= significant different (p<0.05), **NS= non significant (p>0.05), n= number of sample, OR:Odd Ratio, CI: Confidence Interval. The differences between case and control groups were analyzed by Fishers Exact Test.

The homozygous mutant genotype (GG) has a p-value of 0.053, the heterozygous genotype (AG) has a value of 0.767, and the homozygous wild type (AA) genotype has a value of 0.544, as seen in Table 8-4.4.

According to our findings, 51.06 percent of patients are homozygous wild type (AA), 29.7 percent are heterozygous (AG), and 8.51 percent are homozygous mutant type (GG).

In the comparable group, however, homozygousus wild type (AA), heterozygote (AG), and homozygous mutant type (GG) percentages are 64.4 percent, 35.5 percent, and 0 percent, respectively. As demonstrated in (Table 8-4.4), the percentage of homozygote mutant type (GG) was higher in the case group than in controls, but the p-value of 0.053 is not significant.

With a p-value of 0.544, the homozygote wild type (AA) percentage was more significant in controls than in cases, but the difference was not statistically significant.

The heterozygous genotype (AG) was more abundant in the control group in comparison to the case group; that being said, the p-value of 0.767 is not statistically significant. Among the individuals of the case group, 39 had Allele A and 18 had allele G. While in the control group, 45 had Allele A and 17 had allele G. According to these results, the TNF-β alleles distribution is relatively similar, and no significant difference was detected between the two groups in which the p values of Allele A is 0.053, and Allele G is 0.696.

TNF-β genotypes and alleles were analyzed in this study, and no significant correlation between the individuals of both case and control groups was reported ($p>0.05$). According to our findings, there is no significant relation between TNF-β rs909253 and ovarian cancer susceptibility in five genetic models: homozygous mutant GG p=0.053 (OR:0.464, 95% Cl 0.369 - 0.584), homozygous wild AA p= 0.544 (OR:1.503, 95% Cl 0.552 - 3.084), and heterozygous AG p= 0.767 (OR:1.143, 95% Cl 0.473 - 2.764), Alleles A p= 0.053(OR: 2.154, 95% Cl 1.712 – 2.710) and G p=0.696 (OR: 0.843, 95%Cl 0.359 – 1.982).

5. DISCUSION AND CONCLUSION

Understanding the pathophysiology of early cancer development is essential for discovering biomarkers for early diagnosis and researching cost-effective cancer preventive measures. This endeavor indicates an unmet need for malignancies for which regular cancer screening and primary preventions are inefficient. Such is the case with ovarian cancer, which is buried deep inside the pelvis; it is difficult to diagnose clinically and is particularly fatal. There are currently few viable methods for preventing the advancement of the disease from a benign precursor state to an advanced, terminal state.

Ovarian cancer is third among the most prevalent gynecologic malignancies, after uterine and cervical cancer. Unfortunately, it has the greatest death rate and the poorest outlook. Ovarian cancer is less common than breast cancer, but it is three times more deadly, and by 2040, the death rate is anticipated to rise dramatically. Ovarian cancer is often diagnosed late due to the tumor's silent and gradual growth, delayed symptoms, and lack of reliable screening methods. Moreover, ovarian cancer is not a single disease but a combination of various malignancies, making it challenging to study its early course. As a result, ovarian cancer is often called the "silent killer."(43).

Studies have shown numerous predisposing factors for the onset and pathogenesis of ovarian cancer, such as genetics and hereditary, reproductive, environmental, and lifestyle. Although an exact precursor for the disease has not been pinpointed, family history remains one of the most accurate approaches for preliminary detection and prevention, as individuals with a family history of ovarian cancer, specifically in first-degree relatives, have a much higher chance of developing OC compared to individuals with no family history hence regular screening can used for suspected individuals.

Extensive research has been done on the reproductive risk factors for epithelial ovarian cancer (EOC). A 2016 meta-analysis of 32 studies found that pregnancy and nursing can reduce the likelihood of spontaneous genetic mutations. This risk reduction is due to the incessant ovulation theory and the hyperproliferation of inclusion cysts on the gonadotropin hypothesis. Furthermore, increased progesterone levels may eliminate abnormal cells from

the ovarian epithelium through apoptosis during pregnancy. In addition, nursing can prevent ovulation by blocking the release of gonadotropin-releasing and luteinizing hormones (45).

Other factors contributing to ovarian cancer are underlying diseases such as (PID and Endometriosis) since PID contributes to poor parity, which is a mentioned risk factor, and environmental factors such as (dietary habits and physical activity) that have been said to increase body mass and practicing unhealthy habits are precursors for all cancers and other pathogenesis and usually lead to poor prognosis.

Research has revealed that ovarian tumors are affected by many genetic and epigenetic changes. Gene deactivation can occur due to the loss of large chromosome regions or localized loss of heterozygosity at specific loci, as well as promoter methylation. Meanwhile, activation can occur due to promoter hypomethylation, mutation, and amplification. Additionally, 10-15% of ovarian tumors have a connection to germline mutations in BRCA1, BRCA2, or heritable non-polyposis colorectal cancer, DNA mismatch repair genes. These mutations can lead to transformation and genetic instability (43).

The tumor suppressor gene TP53 undergoes mutations in many types of human cancers. When the DNA is damaged or under oncogenic stress, the p53 protein it encodes manages various molecular activities that lead to cell cycle arrest or death. Conversely, without proper p53 activity, the regulation of cell cycle checkpoints is lost, leading to abnormal cell cycle growth and cancer (44).

In 2003, a study was conducted in New York on 73 cases of ovarian cancer at stage I and stage II with different histologies. The study found that 37% of cases had a mutation in the Tp53 gene, and the prevalence was higher in stage I serous carcinomas. These results suggest that early stage ovarian carcinomas with a serous histology tend to have TP53 mutations, which are also likely to develop early in the course of the most common histologic variety of ovarian carcinoma. (46).

TNF-β gene polymorphism-related cancers can diversly be noticed in previously done research and studies that have been said its correlation with ovarian cancer probability is extremely faint and very diminutively studied.

TNF is a protein that activates different pathways to produce biological effects. These include activating nuclear factor (NF-κB) and c-Jun N-terminal kinase (stress-activated protein kinase). NF-κB is essential in promoting cell survival, while persistent JNK activation can lead to cell death. TNF has a dual role in cancer. It can promote tumor growth, proliferation, invasion, metastasis, and angiogenesis in cells resistant to TNF apoptotic activity, thus making it an endogenous tumor promoter. However, TNF can also function as a cancer killer.

In 2010, a case-control study was conducted in India with 900 individuals from two different ethnic groups - Northern Indians and Southern Indians. The study investigated the correlation between TNF-α (rs361525, −238G>A and rs1800629, −308G>A) and TNF-β intron 1 (rs909253, +252A>G) polymorphisms and the breast cancer development. The study found that none of the polymorphisms significantly correlated with breast cancer development in Northern Indians. However, all polymorphisms significantly correlated with breast cancer in Southern Indians, particularly in pre-menopausal breast cancer. A resembling study was done in Korea in March 2005, which also reported that polymorphism of gene TNF-β posed an increased risk of developing breast cancer in Korean women. But, it was more prevalent in post-menopausal individuals compared to Indian women (46,6).

Therapeutic techniques for TNF have been extensively researched due to its tumorigenic effects. In 1998, the European Agency for the Evaluation of Medicinal Products granted a license for treating limb threatening Soft Tissue Sarcoma in isolated limb perfusion. TNF-α, when combined with melphalan, has proven effective in targeting tumor vasculature and controlling melanoma and high-grade soft tissue sarcomas.

TNF-α anti-angiogenic activity in isolation limb perfusion settings targets and destroys tumor vasculature and eventually necrotizes the tumor while the normal vasculature and endothelial cells remain intact. In several studies, the anti-tumor and antivascular effects

of TNF-α therapy were observed after only five weeks of treatment by magnetic image resonance and angiography. Revealing destroyed tumor vasculature and a significant reduction in tumor size (47).

Our study examined the correlation between the polymorphism of TNF-β+252A>G(rs909253) and the risk of ovarian cancer. Specifically, we analyzed the single nucleotide polymorphism of the TNF-β gene in cases of ovarian cancer within the Turkish population.

For this study, a total sample size of 92 was established; of this number, 47 were cases, and 45 were controls. The genotype and allelic distribution of TNF-β were analyzed employing ChiSquare and Fisher's exact test, as shown in Table 8-4.4.

Our research found no significant difference in TNF-β genotypes and alleles distribution between cases and controls. The p values for homozygote wild (AA), heterozygote (AG), and homozygote mutant (GG) genotypes were p=0.544, p=0.767, and p=0.053 respectively. Furthermore, the p values for the two alleles were allele A p=0.053 and allele G p=0.696, further confirming the lack of significance.

According to our clinical data in Table 6-4.2, the most prevalent type of ovarian cancer among the case group is serous tumors, making up 51% of cases. Mixed epithelial tumors make up 10% of cases. Overall, serous tumors are the most prevalent type of ovarian cancer worldwide.

In evaluating the demographic characteristics and ovarian risk parameters, we observed significant differences ($p<0.05$) in (BMI, smoking status, alcohol consumption, family history, and menopause status) with p values of (0.001, 0.004, 0.001, 0.048, and 0.036) and no significant difference ($p>0.05$) in age and pregnancy status among the case and control study groups.

This case-control study is the first to analyze the link between ovarian cancer risk and TNF-β polymorphism in the Turkish population. We found similar results to previous studies, which showed no relation between TNF-β SNP and ovarian cancer development risk. In other words, none of the genotypes (AA, AG, GG) increase the risk of ovarian cancer. However, our research had a relatively small sample size, which could significantly impact the

statistical outcomes. Therefore, further research on a larger scale and population is necessary to better understand the relationship between the TNF-β rs909253 gene and ovarian cancer risk.

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7. APENDICES

7.1. Ethics Committee Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2225 **14/10/2021**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Shiya Hussein Ibrahim)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, Kadın Hastalıkları ve Doğum AD. Prof. Dr. Rukset Attar'ın sorumlu araştırmacı olduğu **"TNF-ß Gen Polimorfizminin (+252 A/G) Over Kanser Hastalığında Rolü"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2279)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **13.10.2021** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir **(KAEK Karar No: 1508)**

Prof. Dr. Hasan AYDIN

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkan Yrd.

7.2. Case Report Form

ÇALIŞMANI ADI: TNF-β gen polimorfizminin *(+252 A/G)* Over kanse Hastalığında Rolü

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Grupları için;

- Gönüllü Olmak
- Over kanseri hastası olmak
- 18 yaşından büyük olmak
- Yukarıda belirtilenler haricinde bir hastalığa sahip olmama

Kontrol Grupları için,

- Gönüllü olma
- Sağlıklı olma (yukarıda belirtilenlerde dahil olmak üzere hiçbir hastalığa sahip olmama)
- 18 yaşından büyük olmak

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü olmama
- 18 yaşından küçük olmak
- Yukarıda belirtilenler dışında bir hastalığa sahip olma

7.3. Raw Materials

T-Test

Notes

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Group Statistics

	patient control group	N	Mean	Std. Deviation	Std. Error Mean
age range	case	47	.7234	.45215	.06595
	control	45	.6000	.49543	.07385

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
age range	Equal variances assumed	5.708	.019	1.249	90	.107	.215	.12340	.09882	-.07292	.31972
	Equal variances not assumed			1.246	88.391	.108	.216	.12340	.09902	-.07336	.32017

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
age range	Cohen's d	.47381	.260	-.151	.670
	Hedges' correction	.47780	.258	-.150	.665
	Glass's delta	.49543	.249	-.164	.660

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.

T-Test

Notes

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	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=casecontrol(1 2) /MISSING=ANALYSIS /VARIABLES=BMIrange /ES DISPLAY(TRUE) /CRITERIA=CI(.95).
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Group Statistics

	patient control group	N	Mean	Std. Deviation	Std. Error Mean
BMI range	case	44	1.4545	.50369	.07593
	control	40	1.0500	.22072	.03490

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
BMI range	Equal variances assumed	169.467	<.001	4.685	82	<.001	<.001	.40455	.08634	.23278	.57631
	Equal variances not assumed			4.841	60.127	<.001	<.001	.40455	.08357	.23739	.57170

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
BMI range	Cohen's d	.39523	1.024	.565	1.477
	Hedges' correction	.39889	1.014	.560	1.463
	Glass's delta	.22072	1.833	1.237	2.416

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

Notes

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	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Smoker BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.03
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Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
smoking status * patient control group	84	91.3%	8	8.7%	92	100.0%

smoking status * patient control group Crosstabulation

			patient control group		
			case	control	Total
smoking status	smoker	Count	7	18	25
		% within smoking status	28.0%	72.0%	100.0%
	non-smoker	Count	37	22	59
		% within smoking status	62.7%	37.3%	100.0%
Total		Count	44	40	84
		% within smoking status	52.4%	47.6%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	8.482[a]	1	.004	.004	.004	
Continuity Correction[b]	7.148	1	.008			
Likelihood Ratio	8.675	1	.003	.004	.004	
Fisher's Exact Test				.004	.004	
Linear-by-Linear Association	8.381[c]	1	.004	.004	.004	.003
N of Valid Cases	84					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.90.

b. Computed only for a 2x2 table

c. The standardized statistic is -2.895.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-.318	.004	.004
	Cramer's V	.318	.004	.004
N of Valid Cases		84		

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for smoking status (smoker / non-smoker)	.231	.083	.641
For cohort patient control group = case	.446	.231	.863
For cohort patient control group = control	1.931	1.280	2.914
N of Valid Cases	84		

Crosstabs

Notes

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	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Alcohol BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
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	Cells Available	524245
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Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Alcohol Intake * patient control group	84	91.3%	8	8.7%	92	100.0%

Alcohol Intake * patient control group Crosstabulation

			patient control group		
			case	control	Total
Alcohol Intake	user	Count	0	15	15
		% within Alcohol Intake	0.0%	100.0%	100.0%
	non-user	Count	44	25	69
		% within Alcohol Intake	63.8%	36.2%	100.0%
Total		Count	44	40	84
		% within Alcohol Intake	52.4%	47.6%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	20.087[a]	1	<.001	<.001	<.001	
Continuity Correction[b]	17.612	1	<.001			
Likelihood Ratio	25.904	1	<.001	<.001	<.001	
Fisher's Exact Test				<.001	<.001	
Linear-by-Linear Association	19.848[c]	1	<.001	<.001	<.001	.000
N of Valid Cases	84					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.14.

b. Computed only for a 2x2 table

c. The standardized statistic is -4.455.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-.489	<.001	<.001
	Cramer's V	.489	<.001	<.001
N of Valid Cases		84		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
For cohort patient control group = control	2.760	2.018	3.774
N of Valid Cases	84		

Crosstabs

Notes

Output Created		13-JUN-2022 18:34:46
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Familyhistory BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.05
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.02

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Family History * patient control group	84	91.3%	8	8.7%	92	100.0%

Family History * patient control group Crosstabulation

			patient control group		
			case	control	Total
Family History	has family history	Count	15	23	38
		% within Family History	39.5%	60.5%	100.0%
	no family history	Count	29	17	46
		% within Family History	63.0%	37.0%	100.0%
Total		Count	44	40	84
		% within Family History	52.4%	47.6%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	4.635[a]	1	.031	.048	.026	
Continuity Correction[b]	3.738	1	.053			
Likelihood Ratio	4.673	1	.031	.048	.026	
Fisher's Exact Test				.048	.026	
Linear-by-Linear Association	4.580[c]	1	.032	.048	.026	.018
N of Valid Cases	84					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 18.10.

b. Computed only for a 2x2 table

c. The standardized statistic is -2.140.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-.235	.031	.048
	Cramer's V	.235	.031	.048
N of Valid Cases		84		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for Family History (has family history / no family history)	.382	.158	.925
For cohort patient control group = case	.626	.399	.984
For cohort patient control group = control	1.638	1.038	2.585
N of Valid Cases	84		

Crosstabs

Notes

Output Created		13-JUN-2022 18:38:25
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=gravida BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.08
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.03

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
gravida * patient control group	84	91.3%	8	8.7%	92	100.0%

gravida * patient control group Crosstabulation

			patient control group		
			case	control	Total
gravida	been pregnent	Count	37	27	64
		% within gravida	57.8%	42.2%	100.0%
	no pregnancy	Count	7	13	20
		% within gravida	35.0%	65.0%	100.0%
Total		Count	44	40	84
		% within gravida	52.4%	47.6%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	3.179[a]	1	.075	.123	.063	
Continuity Correction[b]	2.330	1	.127			
Likelihood Ratio	3.206	1	.073	.123	.063	
Fisher's Exact Test				.123	.063	
Linear-by-Linear Association	3.141[c]	1	.076	.123	.063	.043
N of Valid Cases	84					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 9.52.

b. Computed only for a 2x2 table

c. The standardized statistic is 1.772.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	.195	.075	.123
	Cramer's V	.195	.075	.123
N of Valid Cases		84		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for gravida (been pregnent / no pregnancy)	2.545	.896	7.231
For cohort patient control group = case	1.652	.877	3.110
For cohort patient control group = control	.649	.422	.999
N of Valid Cases	84		

Crosstabs

Notes

Output Created		13-JUN-2022 18:41:20
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=menopause BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.06
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.04

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
menopause status * patient control group	92	100.0%	0	0.0%	92	100.0%

menopause status * patient control group Crosstabulation

			patient control group		
			case	control	Total
menopause status	pre-menopause	Count	16	26	42
		% within menopause status	38.1%	61.9%	100.0%
	post-menopause	Count	31	19	50
		% within menopause status	62.0%	38.0%	100.0%
Total		Count	47	45	92
		% within menopause status	51.1%	48.9%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	5.220[a]	1	.022	.036	.019	
Continuity Correction[b]	4.307	1	.038			
Likelihood Ratio	5.269	1	.022	.036	.019	
Fisher's Exact Test				.036	.019	
Linear-by-Linear Association	5.163[c]	1	.023	.036	.019	.013
N of Valid Cases	92					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 20.54.

b. Computed only for a 2x2 table

c. The standardized statistic is -2.272.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-.238	.022	.036
	Cramer's V	.238	.022	.036
N of Valid Cases		92		

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for menopause status (pre-menopause / post-menopause)	.377	.162	.878
For cohort patient control group = case	.614	.395	.956
For cohort patient control group = control	1.629	1.064	2.495
N of Valid Cases	92		

Crosstabs

Notes

Output Created		12-JUN-2022 19:09:00
Comments		
Input	Data	C:\Users\shiya\Desktop\SPSS shıya_1.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=TNFBAA BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.06
	Elapsed Time	00:00:00.05
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.01

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
TNFB AA genotype * patient control group	88	95.7%	4	4.3%	92	100.0%

TNFB AA genotype * patient control group Crosstabulation

			patient control group		
			case	control	Total
TNFB AA genotype	NO	Count	18	16	34
		% within TNFB AA genotype	52.9%	47.1%	100.0%
	YES	Count	25	29	54
		% within TNFB AA genotype	46.3%	53.7%	100.0%
Total		Count	43	45	88
		% within TNFB AA genotype	48.9%	51.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	.369[a]	1	.544	.662	.349	
Continuity Correction[b]	.151	1	.698			
Likelihood Ratio	.369	1	.544	.662	.349	
Fisher's Exact Test				.662	.349	
Linear-by-Linear Association	.364[c]	1	.546	.662	.349	.145
N of Valid Cases	88					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.61.

b. Computed only for a 2x2 table

c. The standardized statistic is .604.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	.065	.544	.662
	Cramer's V	.065	.544	.662
N of Valid Cases		88		

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for TNFB AA genotype (NO / YES)	1.305	.552	3.084
For cohort patient control group = case	1.144	.746	1.754
For cohort patient control group = control	.876	.568	1.353
N of Valid Cases	88		

Crosstabs

Notes

Output Created		12-JUN-2022 19:10:31
Comments		
Input	Data	C:\Users\shiya\Desktop\SPSS shıya_1.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=TNFBAG BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.06
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.02

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
TNFB AGgenotype * patient control group	88	95.7%	4	4.3%	92	100.0%

TNFB AGgenotype * patient control group Crosstabulation

			patient control group		
			case	control	Total
TNFB AGgenotype	NO	Count	29	29	58
		% within TNFB AGgenotype	50.0%	50.0%	100.0%
	YES	Count	14	16	30
		% within TNFB AGgenotype	46.7%	53.3%	100.0%
Total		Count	43	45	88
		% within TNFB AGgenotype	48.9%	51.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	.088[a]	1	.767	.824	.472	
Continuity Correction[b]	.005	1	.943			
Likelihood Ratio	.088	1	.767	.824	.472	
Fisher's Exact Test				.824	.472	
Linear-by-Linear Association	.087[c]	1	.768	.824	.472	.170
N of Valid Cases	88					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.66.

b. Computed only for a 2x2 table

c. The standardized statistic is .295.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	.032	.767	.824
	Cramer's V	.032	.767	.824
N of Valid Cases		88		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for TNFB AGgenotype (NO / YES)	1.143	.473	2.763
For cohort patient control group = case	1.071	.676	1.699
For cohort patient control group = control	.938	.615	1.430
N of Valid Cases	88		

Crosstabs

Notes

Output Created		13-JUN-2022 02:51:34
Comments		
Input	Data	C:\Users\shiya\Desktop\SPSS shıya_1.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=TNFBalleleA BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.05
	Elapsed Time	00:00:00.05
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.01

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
TNFB allele A * patient control group	88	95.7%	4	4.3%	92	100.0%

TNFB allele A * patient control group Crosstabulation

			patient control group		
			case	control	Total
TNFB allele A	NO	Count	4	0	4
		% within TNFB allele A	100.0%	0.0%	100.0%
	YES	Count	39	45	84
		% within TNFB allele A	46.4%	53.6%	100.0%
Total		Count	43	45	88
		% within TNFB allele A	48.9%	51.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	4.385[a]	1	.036	.053	.053	
Continuity Correction[b]	2.503	1	.114			
Likelihood Ratio	5.929	1	.015	.053	.053	
Fisher's Exact Test				.053	.053	
Linear-by-Linear Association	4.336[c]	1	.037	.053	.053	.053
N of Valid Cases	88					

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.95.

b. Computed only for a 2x2 table

c. The standardized statistic is 2.082.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	.223	.036	.053
	Cramer's V	.223	.036	.053
N of Valid Cases		88		

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
For cohort patient control group = case	2.154	1.712	2.710
N of Valid Cases	88		

Crosstabs

Notes

Output Created		13-JUN-2022 02:50:36
Comments		
Input	Data	C:\Users\shiya\Desktop\SPSS shıya_1.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	92
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=TNFBalleIG BY casecontrol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW /COUNT ROUND CELL /METHOD=EXACT TIMER (5).
Resources	Processor Time	00:00:00.14
	Elapsed Time	00:00:00.13
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00.06

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
TNFB allele G * patient control group	88	95.7%	4	4.3%	92	100.0%

TNFB allele G * patient control group Crosstabulation

			patient control group		
			case	control	Total
TNFB allele G	NO	Count	25	28	53
		% within TNFB allele G	47.2%	52.8%	100.0%
	YES	Count	18	17	35
		% within TNFB allele G	51.4%	48.6%	100.0%
Total		Count	43	45	88
		% within TNFB allele G	48.9%	51.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	.153[a]	1	.696	.828	.431	
Continuity Correction[b]	.030	1	.862			
Likelihood Ratio	.153	1	.696	.828	.431	
Fisher's Exact Test				.828	.431	
Linear-by-Linear Association	.151[c]	1	.697	.828	.431	.160
N of Valid Cases	88					

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 17.10.

b. Computed only for a 2x2 table

c. The standardized statistic is -.389.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-.042	.696	.828
	Cramer's V	.042	.696	.828
N of Valid Cases		88		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for TNFB allele G (NO / YES)	.843	.359	1.982
For cohort patient control group = case	.917	.597	1.410
For cohort patient control group = control	1.088	.711	1.664
N of Valid Cases	88		

8. Curriculum Vitae

Personal Information

Name	Shiya	**Surname**	Hussein Ibrahim

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate			
Master	Molecular medicine	Yeditepe University	2023
University	Medical microbiology	Hawler Medical University	2016
High school		Nilufer Girls Highh School	2011

Languages	level
English	Fluency in reading, writing and speaking
Arabic, Kurdish (Mother language)	Fluency in reading, writing and speaking
Turkish	Fluency in reading, writing and speaking

Work Experience

Position	Institute	Duration (Year - Year)
Laboratory supervisor	Life Support Team (Erbil)	2018-2019
Field officer	Heartland Alliances NGO	2017-2018
Laboratory technician	PAR private Hospital	2016-2017

Computer Skills

Program	Level
Microsoft word,powepoint,excell	Good
SPSS	Good

Publications

Shiya, H., Nabaa, Q., Fatma, A. Isolation and Identification of Multidrug Resistant Klebsiella spp. Causing Urinary Tract Infection, *International Journal of Research Studies in Science, Engineering and Technology*,
Volume 4, Issue 11, 2017, PP 1-6.

Projects / Certificates / Rewards

CRISPR: Revolutionising Genome Editing Advanced Certificate Program CPD
“Hastalıkların Tanısında Real-Time PCR”. (Katılım Belgesi). Cellestetix