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YEDITEPE UNIVERSITY

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THE RELATION BETWEEN GPX1 GENE POLYMORPHISM AND OVARIAN CANCER

DOCTOR OF PHILOSOPHY THESIS

BAKER ALSHEIKH

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PhD THESIS

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ………………and numbered ....

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Director of the Institute of Health Sciences

DECLARATION

I hereby declare that this thesis, titled "The relation between GPX1 polymorphism and ovarian cancer" represents my original work, conducted under the guidance of my academic advisors, and based on my own investigation and analysis. I have correctly cited all sources of material and ideas used in this thesis, and I confirm that all data contained herein are accurate and have not been changed. This work has yet to be previously submitted for any academic degree or examination at any institution. I understand this thesis submission's ethical and academic standards and express my commitment to respecting these standards.

Baker Alsheikh

DEDICATION

I would like to dedicate this thesis to the everlasting support, prayers, and affection of my mother, whose encouragement and believe in me have been constant sources of inspiration throughout my academic path.

To my beloved wife, your patience, understanding, and sacrifices during these long hours of writing and researching have been the cornerstone of my success; this achievement is as much yours as it is mine.

To my friends, your friendship, and the innumerable moments of respite you provided have made this endeavor more joyful and manageable. This work is a memorial to the collective power of my family and friends, and I am really grateful to each of you.

And finally, I wish to dedicate my sincere commitment of this thesis to those individuals whose wisdom and profound knowledge have not only been a source of inspiration but have also acted as guiding lights for the researchers of tomorrow. It is my honest goal that my study can become a part of their enduring legacy and bright a spark in others to continue forward their significant contributions to the profession.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

OC:	Ovarian Cancer
GPX1:	Glutathione Peroxidase 1
PCR:	Polymerase Chain Reaction
OR:	Odd Ratio
CI:	Confidence Interval
EOC:	Epithelial Ovarian Cancer
HBOC:	Family history of breast and/or ovarian cancer
BRC1:	Breast Cancer Gene 1
BRC2:	Breast Cancer Gene 2
SNPs:	Single Nucleotide Polymorphisms
MLH1:	MutL homolog 1
MSH2:	MutS homolog 2
HGSC:	High grade serous ovarian cancer
LGSC:	Low grade serous ovarian cancer
PTEN:	Phosphatase and TENsin homolog deleted on chromosome 10
K RAS:	Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
STIC:	Serous tubal intraepithelial carcinoma
IBS:	Irritable Bowel Syndrome
FIGO:	International Federation of Gynecological Oncologists
GLOBOCAN:	Global Cancer Incidence, Mortality and Prevalence
CST:	Charge Switch Technology
PH:	potential of hydrogen

OD: Optical Density

UV: Ultraviolet

dsDNA: Double Strand DNA

WHO: World Health Organization

Tm: Melting Temperature

PARP: Poly ADP Ribose Polymerase

ROS: Reactive oxygen species

Sec: Selenocysteine

C: Cytosine

T: Thymine

EDTA: Ethylenediamine tetra acetic acid

PMS2: PMS1 Homolog 2, Mismatch Repair System Component

HNPCC: Hereditary Non-Polyposis Colorectal Cancer

DNA: Deoxyribonucleic Acid

MSH6: MutS homolog 6

TUV: Transvaginal Ultrasound

CA125: The Cancer Antigen 125

HE4: The Human Epididymis Protein 4

ACOG: American College of Gynecologist

NCCN: National Comprehensive Cancer Network

CEA: the carcinoembryonic antigen

CA19-9:	cancer antigen 19-9
CA15-3:	cancer antigen 15-3
HRP:	Hormone Replacement Therapy
BMI:	Body Mass Index

ABSTRACT

Alsheikh, B. (2024) The Relation Between GXP1 Gene Polymorphism and Ovarian Cancer in the Turkish Population. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. PhD thesis, Istanbul.

Ovarian cancer (OC) is defined as a common type of cancer among women and has the highest mortality rate. Difficulty in diagnosing the disease in its early stages can lead to a poor prognosis. Genetic or environmental factors can increase the risk of ovarian cancer. Glutathione peroxidase 1 (GPX1) is an important antioxidant enzyme that regulates the levels of reactive oxygen species in cells. While high levels of GPX1 are found in some cancer types, some studies suggest that low levels of circulating GPX1 may increase the risk of cancer. Consequently, the role of the GPX1 enzyme and gene variants in cancer risk is debated, being described as both a tumor suppressor and promoter. GPX1 gene polymorphism (rs1050450) has been identified as the most common functional variant of the human GPX1 gene and has been associated with various cancers, including breast, prostate, bladder, lung, leukemia, and colorectal cancers. The aim of this thesis study is to examine the relationship between GPX1 (rs1050450) polymorphism and the risk of ovarian cancer. Ninety patients diagnosed with OC and 90 healthy control subjects were included in our study. GPX1 (rs1050450) polymorphism genetic analyses were performed using real-time PCR (RT-PCR), and statistical analyses of the results were carried out using the SPSS program. According to the findings, individuals with the homozygous CC genotype had a statistically significantly lower risk of OC ($p=0.002$, Odds Ratio (OR): 0.304, 95% CI: 0.161 - 0.577), while individuals with the TT polymorphism had a threefold higher risk of OC ($p=0.036$, OR: 3.308, 95% CI 1.024 - 10.682). Additionally, individuals carrying the T allele were observed to be approximately three times more sensitive to this type of cancer ($p=0.0002$). In light of the obtained data, it was concluded that GPX1 (rs1050450) polymorphism may be associated with ovarian cancer.

Keywords: Ovarian Cancer, Glutathione Peroxidase 1 (GPX1), rs1050450, Gene Polymorphism

ÖZET

Alsheikh, B. (2024) Türk Toplumunda GXP1 Gen Polimorfizmi ve Yumurtalık Kanseri Arasındaki İlişkinin Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Bölümü. Doktora çalışması, İstanbul.

Yumurta kanseri (YK), kadınlarda sık görülen ve en yüksek mortaliteye sahip kanser türü olarak tanımlanmaktadır. Hastalığın erken aşamalarındaki teşhis zorluğu nedeniyle kötü bir prognoze yol açabilmektedir. Genetik veya çevresel faktörler yumurtalık kanseri riskini artırabilmektedir. Glutathione peroksidaz 1 (GPX1) hücrelerde reaktif oksijen türlerinin seviyelerini düzenleyen önemli bir antioksidan enzimdir. GPX1 seviyesi bazı kanser türlerinde yüksek bulunurken, bazı çalışmalarda ise dolaşımdaki düşük GPX1 seviyesinin kanser riskini artırabileceğini öne sürmektedir. Sonuç olarak hem bir tümör baskılayıcısı hem de teşvikçisi olarak tanımlanabilen GPX1 enziminin ve gen varyantlarının kanser riskinde rolü tartışmalıdır. GPX1 gen polimorfizmi (rs1050450) insan GPX 1 geninin en yaygın fonksiyonel varyantı olarak tanımlanmış ve meme, prostat, mesane, akciğer, leukemi ve kolorektal kanserler de dahil olmak üzere çeşitli kanserlerle ilişkisi gösterilmiştir. Bu tez çalışmasının amacı, GPX1 (rs1050450) polimorfizminin yumurtalık kanseri riskini arasındaki ilişkiyi incelemektir. Çalışmamıza YK tanısı almış olan 90 hasta ve 90 sağlıklı kontrol grubuna dahil edilmiştir. GPX1 (rs1050450) polimorfizmi genetik analizleri gerçek zamanlı PCR (RT PCR) kullanılarak yapılmış ve elde edilen sonuçların istatistik analizleri SPSS programı kullanılarak yapılmıştır. Elde edilen bulgulara göre, homozigoz CC genotipine sahip olan bireylerde YK riskinin istatiksel olarak anlamlı seviyede düşük olduğu belirlenirken (p=0.002, Odds Ratio (OR): 0.304, 95% CI: 0.161 - 0.577), TT polimorfizmli kişilerde YK riskinin 3 kat fazla olduğu belirlenmiştir (p=0.036, OR: 3.308, 95% CI 1.024 - 10.682). Bunun yanında, T alleli taşıyan bireylerin, bu kanser türüne yaklaşık üç kat daha duyarlı oldukları gözlenmiştir (p=0,0002). Elde edilen veriler ışığında GPX1 (rs1050450) polimorfizminin, yumurtalık kanseri ile ilişkili olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Yumurtalık kanseri, Glutathione Peroksidaz 1 (GPX1), rs1050450, gen polimorfizmi

1. INTRODUCTION

Ovarian cancer stood as the third most often occurring form of gynecological malignancy on a global scale, as per the latest figures available for the year 2020[1]. It had the most elevated mortality rate among any type of female malignancies, mostly owing to late diagnosis [2]. Compared to other types of malignancies that affect females, the prognosis for ovarian cancer is substantially less favorable, demonstrated by an overall survival rate after five years of less than 50% among women diagnosed with this cancer [3]. However, once cancer has metastasized to some more sites or has advanced to stage IV, the chance of survival drops to 17%. [5]. Breast cancer, on the other hand, has a five-year survival rate of around 90% [4]. It is believed that in industrialized countries, the frequency of ovarian cancer is expected to ascend with age, with a forecasted increase of 9.4 incidences per 100,000 individuals. In less developed regions, the rate is 5 cases per 100,000 persons [6].

There are several types of ovarian malignancies, including malignant tumors that arise within the epithelial cells and nonepithelial forms such as embryonic or sex cord-stromal cancer. However, the vast majority of ovarian malignancies (approximately 90% of all cases) consist of epithelial ovarian cancer (EOC) and its subtypes.[7][8].

Ancestral history of breast or ovarian cancer (HBOC) stands as one of the most significant risk factors for EOC." An abundance of mutations in the germline of breast cancer vulnerability genes (BRCA 1 / 2) enhances the risk severalfold. Recently, single-nucleotide polymorphisms (SNPs) in genes other than (BRCA 1 / 2) were revealed to possibly impact ovarian cancer risk in females with congenital mutations in these genes. [9]. Other high-risk cancer susceptibility genes, often including MLH1 or MSH2, that are related to Lynch's syndrome (colorectal cancer nonpolyposis) tend to enhance the lifetime risk of ovarian cancer by 8% [10]. Several other potential non-inherited variables may boost the chance of getting ovarian cancer, including early onset of menstruation, increasing age, infertility, late menopause, nulliparity, and obesity [11].

Genetic polymorphism refers to heterozygous DNA sequence variations in more than 1% of the general population. It can encompass single nucleotide polymorphism (SNP) or length polymorphism involving copies of DNA sequences such as minisatellites or microsatellites, which is a prevalent phenomenon in humans. However, the incidence of genetic polymorphism might differ among various racial groupings. While a majority of polymorphisms do not have a noticeable effect, others may be able to change protein structure and expression, making functional gene polymorphisms potentially critical molecular factors in developing diseases, including tumors and malignancies [12].

The GPX1 gene lies on the short arm (p-arm) of chromosome 3." Its role is to produce a necessary antioxidant enzyme called glutathione peroxidase 1. This enzyme regulates and maintains the cells' reactive oxygen species (ROS) levels, protecting the oxidation-reduction balance and minimizing oxidative damage. [13].

GPX1 gene has multiple single nucleotide polymorphisms (SNPs). However, the main form that has been extensively investigated over the past 20 years was rs1050450 (Pro198Leu) polymorphism; prior analyses have displayed the connection between lower levels of circulating GPX1 and raised risk of cancer, which was seen in different types of cancers, such as prostate cancer, lung cancer, breast cancer, and colorectal cancer [14]. Therefore, this investigation intended to explore the potential influence of the GPX1 (rs1050450) polymorphism on the susceptibility to ovarian cancer within the Turkish female population.

2. LITERATURE REVIEW

2.1. Cancer at A Glance

Cancer refers to a grouping of abnormal conditions distinguished by the unregulated growth and spread of mutant cells. When it's not appropriately controlled, it can be lethal by many distinct means [15]. Cancer stands as the second leading cause of global mortality, yielding roughly 10 million fatalities annually [16]. Malignant tumors can develop from numerous tissues and organs, resulting in a vast spectrum of cancer forms. In each situation, the normal control of cell division and differentiation is disrupted, enabling a single cell to proliferate abnormally, generating a primary malignant tumor. As a consequence, these cancerous cells may spread throughout the body, resulting in potentially deadly metastases [17].

Hanahan and Weinberg have suggested a cancer progression model defining six "hallmarks" of cancer that enable it to originate, progress, and metastasize. According to this hypothesis, cancer cells must acquire all six of these characteristics in order to mature and spread across the body [18]. The six hallmarks are:

1. The ability to develop and proliferate without requiring external stimuli like growth signals, Cancer cells possess the capability to trigger signaling pathways that drive division and self-cell creation, even in the absence of external growth stimuli. This causes them to continue to grow and divide unchecked [19].

2. Resistance to growth-inhibitory signals: Cancer cells also possess the ability to avoid signals that would typically impede cell growth and division. This allows them to continue growing and dividing even in the presence of signals limiting their growth [18].

3. Avoiding apoptotic cell death: Cancer cells exhibit the power to elude planned cell death (usually called apoptosis). Apoptosis is a normal process that occurs when cells become damaged or aberrant and is a mechanism for the body to get rid of those cells. Cancer cells can evade this mechanism, allowing them to survive and continue to grow and divide [20].

4. Infinite replicative potential: Cancer cells may divide and replicate endlessly, unlike normal cells with a fixed number of divisions they can undergo. This permits them to accrue mutations and continue to develop and divide over time [17].

5. Sustained angiogenesis: Cancer cells need a steady supply of nutrients and oxygen to survive. They might stimulate the forming of novel blood vessels, a mechanism called angiogenesis, to fulfill their requirements. This permits them to develop and propagate. [20].

6. The spread of metastatic and Tissue invasion: Through this process, Cancer cells travel to various regions of the body. Metastasis occurs when cancer cells break out from the primary tumor and migrate to other bodily regions through the blood circulation or lymphatic circulatory system. Cancer cells could begin to develop and generate new tumors when they reach a new location. And it is the most harmful element of cancer as it permits cancer to spread to other organs and is a main cause of death from cancer [17].

2.2. Ovarian Cancer (OC)

Ovarian cancer is a chain of events that end in a malignant tumor in the ovaries (Figure 2.2.1.), the female reproductive organs generating eggs and hormones [21]. As revealed by the latest GLOBOCAN numbers for the year 2020, 314,000 women were identified with ovarian cancer on a global scale, with an age-standardized incidence rate (ASR) of 6.6 per 100,000. Furthermore, there were 207,252 newly recorded fatalities associated with ovarian cancer on a worldwide level, accompanied by an age-standardized mortality rate (ASR) of 4.2 per 100,000. [22]. The start of ovarian cancer is generally asymptomatic, making it a silent killer usually discovered at an advanced stage. On average, ovarian cancer patients are 52 years old. Usually, one female out of 70 has the risk of experiencing ovarian cancer in her entire existence.[3][8].

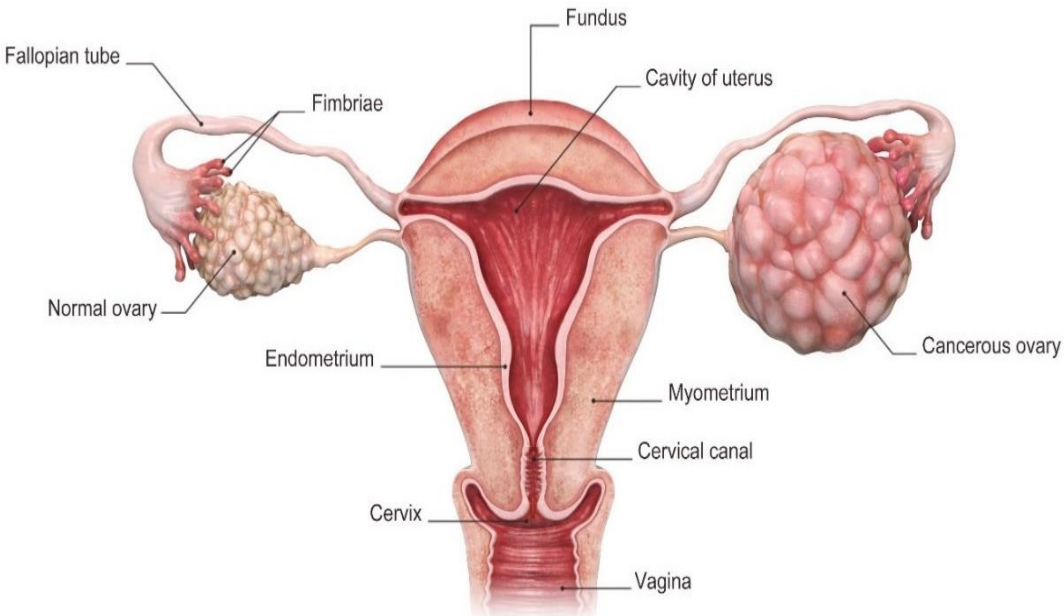

Figure 1. Ovarian cancer versus normal ovary.

2.2.1. Ovaries Anatomy and Physiology.

The ovaries are reproductive organs in women that develop in the upper abdomen of the fetus. They typically descend into the true pelvis by birth and increase in size, taking on an almond shape with the onset of puberty, and descend further into the pelvis, measuring 2.5-5cm in length, 1.5-3cm in width, and 0.6-1.5cm in thickness (figure 2) [23].

In the structure of the ovary, there are two distinct portions: the inner medulla and the outer cortex. The medulla encompasses connective tissue, blood vessels, lymphatic vessels, and nerves. Conversely, the cortex serves as the site of ovarian follicles, tiny sacs filled with fluid that contain the primary oocyte, the precursor of the egg. At birth, the ovary's outer cortex initially holds millions of primordial follicles. However, as puberty approaches, the count of oocytes diminishes significantly, ultimately resting at around 300,000. [24].

Functionally, besides their role in ovum production, ovaries also have the function of producing and delivering hormones. They constitute an integral part of the female reproductive system's hypothalamic-pituitary-ovarian system. Through this intricate interplay, the ovaries synthesize and release progesterone and estrogen during the luteal and follicular phases of the period cycle, respectively [24]. These hormones substantially impact the development of feminine characteristics such as breasts and the uterus. Furthermore, estrogen helps females' cardiovascular, musculoskeletal, and nervous systems work correctly. If a woman's menstrual cycle began at the age of 13 and lasted uninterrupted until menopause at about the age of 51, she would have released 456 eggs in her lifetime (figure 3) [23][24]."

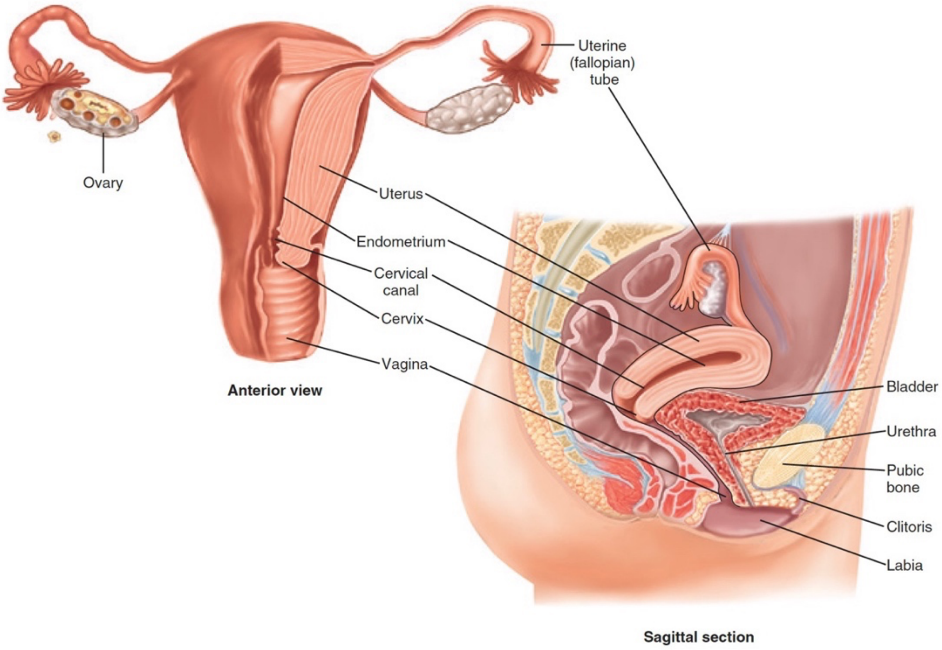

Figure 2. The female reproductive organs including ovaries.

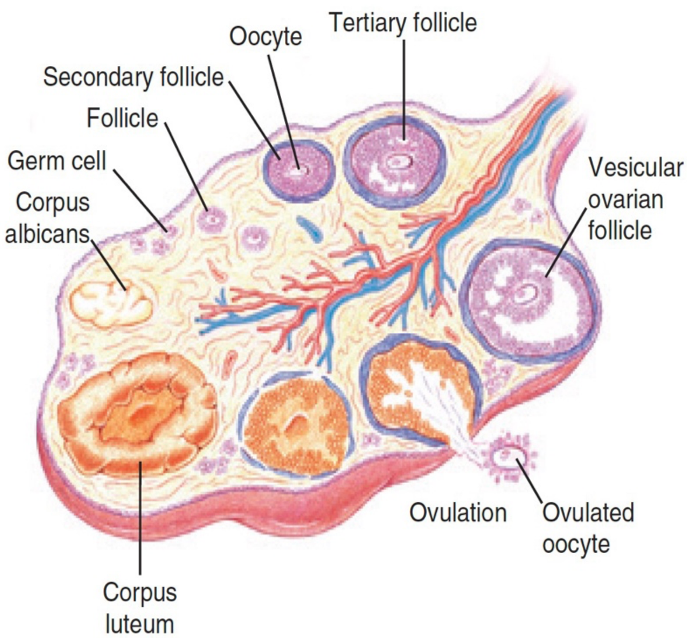

Figure 3. Ovarian follicle development.

2.2.2. Epidemiological of Ovarian Cancer

Ovary cancer contributes roughly 4% of the overall occurrences of cancer among females [8]; globally, during 2020, there were a projected 314,000 newly diagnosed cases of ovarian cancer, and 207,000 fatalities from this form of cancer. [16]. EOC comprises 90% of total incidences of this cancer, and the prevailing and hazardous subtype is high-grade serous carcinoma (HGSC) [4][11]. Ovarian cancer incidence and death rates increase with age. While most ovarian cancer instances surface in females aged 50 and beyond, the disease may appear at any age, even in infancy situations, although this is exceptionally uncommon [4].

2.2.3. Histopathology and Classification

From a histological perspective, ovarian cancer is divided into three fundamental groups: epithelial tumors, sex-cord-stromal tumors involving gonadal stromal cells, and germ-cell tumors [28]. Epithelial tumors are the most prevalent, accounting for almost 95% of all ovarian cancer cases. Within the category of epithelial ovarian cancer, there are four primary types: serous, endometrioid, mucinous, and clear cell. Among these, serous tumors are usually classified into low-grade and high-grade serous carcinomas (HGSC). The high-grade serous carcinomas (HGSCs) are the most frequent subtype, accounting for 70-80% of all epithelial ovarian malignancies, while the low-grade serous carcinomas (LGSCs) compose fewer than 5%. The remaining subtypes—endometrioid, mucinous, and clear cell represent 10%, 3%, and 10% of epithelial ovarian malignancies, respectively [25].

A novel classification system for epithelial ovarian cancer was proposed ten years ago, breaking ovarian cancer into two main types: type I and type II tumors (Figure 4). The type one group covers low-grade serous endometrioid, clear-cell, and mucinous cancers and contains the less common Brenner and seromucous tumors [26]. Type 1 tumors commonly result from atypical proliferative (borderline) malignancies that exhibit alterations in PTEN, BRAF, KRAS, and with microsatellite instability.
Conversely, tumor type 2 is made up of high-grade malignancies such as serous carcinoma, carcinosarcoma, and undifferentiated carcinoma. They typically originate from tubal intra-epithelial carcinoma of serous type and are commonly diagnosed later

than Type 1 tumors. Type 2 tumors have a much faster and more aggressive rate of proliferation than Type 1 tumors and are categorized as high-grade. They showed a high degree of chromosomal instability and had an increased prevalence of p53 mutations. While Type 1 tumors are usually low-grade and have a relatively slow growth rate, Type 2 tumors are considered more severe and often have a poor prognosis [25][27].

- Type I *v* type II tumor types
  - Type I
    - Less lethal than type II
    - Causes are continued ovulation cycles, inflammation, and endometriosis
    - Typically present as low-stage disease
    - Ovary origin
  - Type II
    - Associated with fatal outcomes
    - Diagnosed later
    - Linked to genetic mutations
    - Fallopian tube origin
- High-grade *v* low-grade serous tumors
  - High-grade serous tumors
    - 90% of all tumor types
    - More fatal prognosis
    - 10-year mortality rate of 70%
  - Low-grade serous tumors
    - 10% of all tumor types
    - Diagnosed at younger age
    - Better prognosis than high-grade serous tumors
- Endometrioid carcinomas
  - Originate from endometriosis
  - Good prognosis
- Clear cell carcinomas
  - 10% of epithelial ovarian cancers
  - Often diagnosed in early stages
  - If diagnosed late has poor prognosis
- Mucinous carcinoma
  - Least common type of epithelial ovarian cancer
  - Associated with metastasis from gastrointestinal tract
- Germ cell ovarian cancer
  - Rare
  - Make up only 3% of all ovarian cancers
  - Frequently diagnosed in younger women
  - Histologic type similar to that of germ cell tumors in the testes of men
- Sex cord-stromal ovarian cancer
  - Least common ovarian cancer
  - Less than 2% of all primary ovarian cancers
  - Rarely malignant
  - Usually diagnosed early
  - Smoking can decrease risk

Figure 4.types of epithelial ovarian cancer.

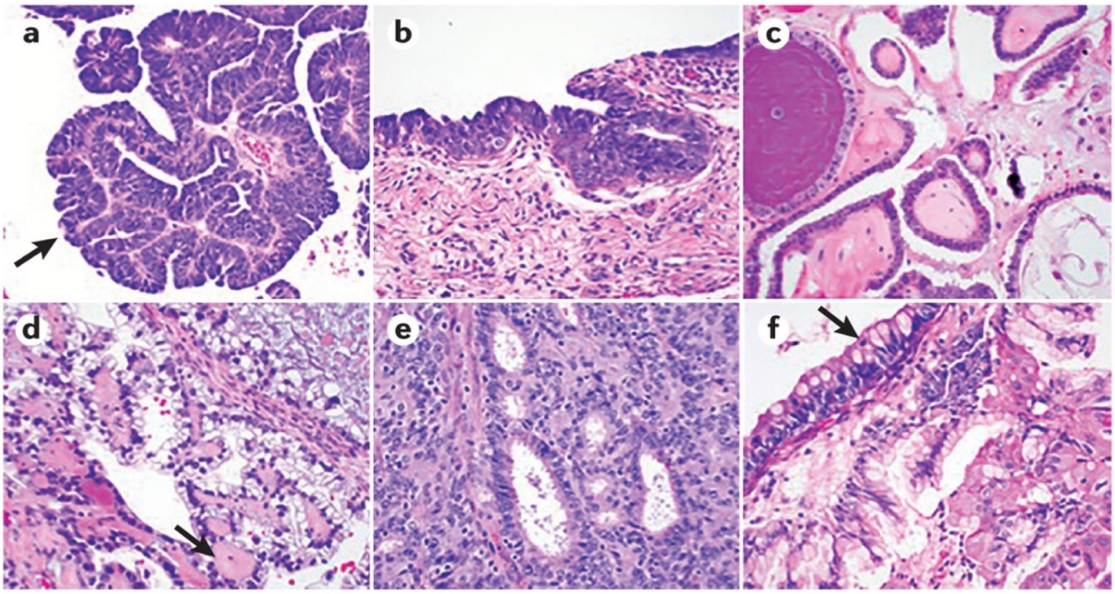

Figure 5. Types of ovarian cancer based on their cell of origin (Histological subtypes).

(a) High-grade serous carcinoma (HGSC) displays an elevated nuclear-to-cytoplasmic ratio, visible nuclear atypia, systematic mitoses, (b) Serous tubal intraepithelial carcinoma (STIC) shares morphological features with HGSC, including strong atypia, lack of polarity, and abundant mitoses. It is considered a forerunner of HGSC. (c) Low-grade serous carcinoma (LGSC) exhibits a papillary organization, modest nuclear atypia, and a reduced nuclear-to-cytoplasmic ratio. (d) Clear-cell carcinoma is defined by larger, atypical tumor cells with substantial cytoplasmic clearance and stromal hyalinization. (e) Endometrioid adenocarcinoma is diagnosed by gland secretion similar to endometrial glands and is graded based on cellular architecture and nuclear atypia. (f) Mucinous adenocarcinoma has mucin-filled tumor cells and typically incorporates goblet cell types.

2.2.4. Symptoms, Manifestations, and Signs.

The symptoms of OC are not unique, and lack specificity, however most women diagnosed with this disease often remark sensations of swelling, tenderness in the belly or pelvic area, eating difficulty, or urinary concerns. These symptoms can sometimes lead to misdiagnosis and might be attributed to irritable bowel syndrome (IBS) [29]. Notably, these symptoms sometimes go undiscovered until a diagnosis has been verified.

Research investigations suggest that over 90% of females encountered at least one symptom of ovarian cancer in their lives, including those in the early and advanced stage of the disease. Remarkably, the symptoms expressed by females in both early and late phases of the disease demonstrate no substantial variations. This similarity in characteristics provides a barrier for patients and healthcare practitioners as well, as it makes it difficult to correlate these symptoms with gynecological conditions, consequently preventing early detection efforts [28].

Genealogical background or (a family history) of ovary cancer can enhance a female's chance of having the disease. Furthermore, if a female or a family member has a past record of breast cancer, there is a probability of an enhanced chance of having ovarian cancer for her. The existence either (BRCA1 /2) gene alterations or mutations within a family can indicate a hereditary inclination towards breast or ovarian cancer, which can appear through the following indications [28]:

- A significant number of family members were diagnosed with cancer.
- An increased prevalence of cancer among relatives compared to those who are not.
- Significant genetic connections among individuals who have been identified with cancer, such as parent-child or sibling-sibling.
- Cancer diagnosis identified at an earlier age, generally spanning from 45 to 50 years old.
- A person who has been diagnosed with both malignancies of the breast and ovary.
- Individual with breast cancer that metastasized to other parts or organs.
- The co-occurrence of ovarian, prostate, colon, or pancreatic malignancies within the same family.
- Male members of the family who were previously verified to have breast cancer.

2.2.5. Ovarian Cancer Metastasis and Staging.

In general, any metastasis should pass through five steps:
local invasion, intravasation, systemic transport, extravasation, and colonization [33]. Meanwhile, Ovarian cancer metastasis spreads via 4 primary ways [34]:

- Spread to neighboring organs through direct extension.
- Infiltration of the ovarian membrane and subsequent invasion of the peritoneum(invasion)
- The lymphatic system extent to the pelvic and abdominal lymph nodes (lymphatic dissemination)
- Dissemination via the bloodstream (systemic dissemination)

In 2014 (FIGO), the International Federation of Gynecology and Obstetrics designed a new staging system for ovarian cancer that incorporates ovarian, fallopian tube, and peritoneal cancer groups (Table 1)[26].

Table 1. FIGO staging system for ovarian, fallopian tube, and peritoneal cancers [26].

Stage I: Tumor confined to ovaries or fallopian tube(s)	T1-N0-M0
IA: Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	T1a-N0-M0
IB: Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	T1b-N0-M0
IC: Tumor limited to 1 or both ovaries or fallopian tubes, with any of the following:	
IC1: Surgical spill	T1c1-N0-M0
IC2: Capsule ruptured before surgery or tumor on ovarian or fallopian tube surface	T1c2-N0-M0
IC3: Malignant cells in the ascites or peritoneal washings	T1c3-N0-M0
Stage II: Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or peritoneal cancer	T2-N0-M0
IIA: Extension and/or implants on uterus and/or fallopian tubes and/or ovaries	T2a-N0-M0
IIB: Extension to other pelvic intraperitoneal tissues	T2b-N0-M0
Stage III: Tumor involves 1 or both ovaries or fallopian tubes, or peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes	T1/T2-N1-M0
IIIA1: Positive retroperitoneal lymph nodes only (cytologically or histologically proven):	
IIIA1(i) Metastasis up to 10 mm in greatest dimension	
IIIA1(ii) Metastasis more than 10 mm in greatest dimension	
IIIA2: Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes	T3a2-N0/N1-M0
IIIB: Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes	T3b-N0/N1-M0
IIIC: Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)	T3c-N0/N1-M0
Stage IV: Distant metastasis excluding peritoneal metastases	
Stage IVA: Pleural effusion with positive cytology	
Stage IVB: Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)	Any T, any N, M1

(NX: Regional lymph nodes cannot be assessed, N0: No regional lymph node metastasis, N1: Regional lymph node metastasis, MX: Distant metastases cannot be assessed, M0: No distant metastasis, M1: Distant metastasis) [26].

2.2.6. Risk Factors of Ovarian Cancer.

Many variables and factors may enhance a female's likelihood of acquiring ovarian cancer, such as inheritance, advanced age, postmenopausal hormone medication, and infertility. Epithelial ovarian cancer primarily affects women post-menopause due to its age-related feature (Table 2) [36]. The higher incidence of this malignancy is more established in females over 65; moreover, advanced stage and shorter survival rate in this type of cancer are associated with older age [37].

Studies have indicated that postmenopausal women utilizing hormone replacement medication may have a higher likelihood of having ovarian cancer. Furthermore, the administration of estrogen-only therapy enhanced the risk by 22%, whereas both estrogen and progesterone, as a combination, led to a 10% rise. [38]. On the other hand, for women diagnosed with ovarian cancer experiencing intense menopausal symptoms, implementing hormone replacement therapy appears to be secure and does not affect overall survival chances. Thus, individuals who are battling with substantial menopausal symptoms may investigate the use of hormone replacement therapy [39]. Numerous studies have suggested that using oral contraceptives decreases the incidence of all types of OC. This shows that the longer the period of contraceptive use, the more effective it is in reducing OC risk. The protective effect of oral contraceptives has been hypothesized to be connected with inhibiting ovulation. [32]. Studies have discovered a relationship between endometriosis and some forms of epithelial ovarian malignancies. These malignancies, which result from endometriosis, often occur in younger females with a more favorable outlook. However, the evidence of a solid causative association between the elimination of endometriosis growth and a subsequent reduction in the susceptibility to ovarian cancer in women remained absent in the existing research [32]. In parallel, distinct investigations have validated the correlation between overweight conditions and an elevated chance for both the prevalence and death rates attributed to ovarian cancer. [40].

Table 2. Factors related to ovarian cancer globally.

Factors		Protective	Predisposing	Controversial
Demographic	Age		✓	
Reproductive	Menstrual pattern		✓	
	The onset of menstruation			✓
	Parity	✓		
	Pregnancy features			✓
Gynecological	Higher age of childbirth Pelvic inflammatory disease	✓		✓
	Endometriosis		✓	
Hormonal	Contraceptive medication	✓		
	Hormone Replacement Therapy (HRT)			✓
	Infertility treatments			✓
Genetic	Lynch syndrome		✓	
	BRCA mutations		✓	
	Family history		✓	
Lifestyle	Dietary habits			✓
	Body weight and physical activity			✓
	Consumption of alcohol, caffeine, and smoking			✓
Other	Breastfeeding	✓		
	Lower socioeconomic status		✓	

Numerous inherited variables have been linked to an enhanced tendency to the development of ovarian cancer. Among the most prominent contributors are hereditary abnormalities within the BRCA1 and BRCA2 genes. These mutations have tremendous influence over ovarian cancer progression, with estimates indicating their presence in as much as 17% of ovarian cancer cases. Beyond the spectrum of BRCA1 and BRCA2, other hereditary disorders exist, mainly inherited nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. The disease encompasses a range of malignancies, including ovarian, colorectal, and endometrial cancers. The fundamental reason for this condition is germline mutations among genes necessary for the DNA mismatch repair pathway, including PMS2, MLH1, MSH2, and MSH6. In cases where ovarian cancer is linked to Lynch syndrome, it tends to appear at an earlier age, commonly involving endometrioid and clear-cell carcinomas. Finally, the risk of ovarian cancer is influenced by an array of additional inherited and germline abnormalities inside genes related to DNA repair pathways, as indicated in the table 3 [9][10][41].

Table 3. Common mutated genes linked to higher Ovarian Cancer susceptibility.

Gene	Protein	Protein function
BRCA1	Breast cancer type 1 susceptibility protein	• Crucially involved in the repair of double-strand breaks by homologous recombination • Serves as a scaffold for other proteins involved in double-strand DNA repair, mostly through defective homologous recombination • Stabilizes RAD51-ssDNA complexes
BRCA2	Breast cancer type 2 susceptibility protein	
BARD1	BRCA1-associated RING domain protein 1	• Forms a heterodimer with BRCA1 • The BRCA1-BARD1 complex is essential for mutual stability
BRIP1	BRCA1-interacting protein 1 (also known as Fanconi anaemia group J protein)	• Binds to BRCA1 • The BRCA1-BRIP1 complex is required for S phase checkpoint activation
PALB2	Partner and localizer of BRCA2	• A bridging protein that connects BRCA1 and BRCA2 at sites of DNA damage • Helps load RAD51 onto ssDNA
RAD51C	DNA repair protein RAD51 homologue 3	• Strand exchange proteins that bind to ssDNA breaks to form nucleoprotein filaments and initiate DNA repair
RAD51D	DNA repair protein RAD51 homologue 4	
MSH2	MutS protein homologue 2	• Mismatch repair proteins that recognize and repair base-pairing errors occurring during DNA replication • Mutations in mismatch repair genes are associated with Lynch syndrome
MLH1	MutL protein homologue 1	
MSH6	MutS protein homologue 6	
PMS2	Mismatch repair endonuclease PMS2	

2.2.7. Diagnostic Approaches for Ovarian Cancer:

Diagnosing ovarian cancer requires a comprehensive approach, including medical history, physical examinations, and various tests. Suspicion of ovarian cancer prompts a thorough medical history assessment (83), followed by pelvic-focused physical examinations to detect signs like enlarged ovaries or abdominal fluid (84). Certain ovarian cancers, especially germ cell tumors (GCT), may be detectable through blood tests (85). Imaging techniques like transvaginal ultrasound (TVU), CT scans, and MRI scans can be employed, although they cannot definitively confirm cancer (86, 87). The widely utilized cancer antigen 125 (CA125) marker, previously considered for OC screening, has limitations due to its lack of specificity, leading to a shift away from its use (86).

Detecting ovarian cancer poses challenges due to its non-specific symptoms. Although a combination of techniques, such as TVU, CA125, and the human epididymis 4 (HE4) protein markers [31], may offer partial reliability, no single tool provides a reliable screening option [86]. Abdominal fluid detection may lead to paracentesis for cancer cell examination [88]. For precise diagnosis, tissue biopsy remains the gold standard, while TVU-guided biopsies have minimized the risk of metastasis [32,86]. Genetic screening also offers insights into the risk of ovarian cancer [86]. Guidelines for referring patients with suspected ovarian cancer have been published by the American College of Obstetricians and Gynecologists (ACOG) and the National Comprehensive Cancer Network (NCCN), summarized in the (figure 6) [30].

Ovarian Cancer Assessment Guidelines Summary

Clinical presentation

1. Detection of pelvic mass on exam
2. Symptoms: e.g., bloating, pelvic or abdominal pain, early satiety, urinary symptoms
3. Concern raised on screening assays (e.g., CA-125)
4. Incidental findings on previous surgery or tissue biopsy

Workup

- Family history of breast or ovarian cancer
- Abdominal/pelvic exam
- Imaging: chest, abdominal, and pelvic CT/MRI as indicated
- Blood tests: CBC, chemistry profile, liver function tests; CA-125 and other serum biomarkers (e.g., inhibin, AFP, beta-HCG) as indicated
- Needle aspiration should be avoided for diagnosis in patients with presumed early-stage disease to prevent tumor rupture and spread

Figure 6.Ovarian cancer assessment guidelines summery.

2.2.8. Treatment Approaches for Ovarian Cancer

A comprehensive spectrum of treatment methods is available for ovarian malignance. Conventionally, an assortment of chemotherapy and surgical interventions is used to treat ovarian cancer. This often comprises enduring surgical staging to identify the level of tissue involvement, followed by tumor reduction surgery to minimize tumor size, and subsequently delivering chemotherapy to target leftover cancer cells (89).

In advanced-stage scenarios, debulking or (removing) surgery is a recommended procedure. This procedure is highly effective when the thickness of any residual tumor formations less than 1 cm, according to standards of National Comprehensive Cancer Network (90). In some circumstances, after debulking procedures, intraperitoneal treatment may be explored. Intraperitoneal treatment has proven potential in enhancing patient survival rates in recent years. In this technique, a tiny incision is made in the wall of the abdomen, after that, the catheter is carefully set across the subcutaneous tissue to access the cavity of the peritoneum. The tube of the catheter acts as an infusion port., allowing the introduction of chemotherapy medicines to remain within the peritoneal cavity (91).

A new cancer treatment method is targeting treatments include poly (ADP-ribose) polymerase inhibitors (PARP). These inhibitors block the self-regeneration process of damaged cells, including cancer cells. By preventing this regeneration, they inhibit cancer cell growth and tumor development. Promising medications like olaparib and rucaparib are garnering recognized for their effectiveness in treating ovarian cancer alongside chemotherapy (92,93).

Ultimately, another potential development in ovarian cancer treatment involves integrating an active form of vitamin D3 (1,25(OH)2D3), as low vitamin D levels are connected to increased ovarian cancer risk. Administering 1,25(OH)2D3 together with chemotherapy has revealed substantial potential in increasing anticancer effects, especially inside omental cancer cells (94).

2.3. Glutathione peroxidase 1(GPX1) and Ovarian Cancer

2.3.1 GPX1 Molecular Structure, and Enzymes Family

Glutathione Peroxidase 1 is an essential enzyme that regulates and maintains cellular oxidative stress homeostasis. The glutathione peroxidase one enzyme is encoded by the GPX1 gene, which lies on the short arm of chromosome 3 (3p21.31). GPX1 gene has 1178 base pairs and possesses two exons. Additionally, there are five transcript variants connected with this gene. [42]. GPX1 enzyme belongs to the glutathione peroxidase family and is a powerful antioxidant in the human body. Its principal role is to protect cells from harm caused by reactive oxygen species (ROS), and therefore preventing potential cellular damage. GPX1 is primarily found in the liver and other tissues, including the kidney, pancreas, and small intestine [43]. ROSs have a dual function in the organism. In small amounts, they can function as crucial signaling molecules, but in excess, reactive molecules have the ability to prompt cellular harm by interacting with critical cellular constituents, including DNA, lipids, and proteins [44].

To maintain cells against harm caused by ROS, the body has numerous antioxidant defense systems in place, the human body possesses three separate strategies to protect itself against oxidative stress and damage. Miniscule molecular antioxidants are inside these three layers, neutralizing free radicals and minimizing oxidative stress. Furthermore, the body employs antioxidant enzymes to break down and neutralize harmful oxidizing chemicals [45]. Finally, damage-removing and repairing enzymes perform a key function in fixing any harm that may have happened due to oxidative stress. The human body can efficiently combat oxidative stress and avoid cellular damage by deploying these three defense systems. [45]

The glutathione peroxidases (GPXs) are one of the essential enzyme families in the enzymatic antioxidant layer, utilizing peroxidase capability to counteract the deleterious effects of hydrogen peroxide (H2O2) as well as lipid peroxides. This is performed by converting glutathione to its oxidized form. This procedure proficiently defends the organism from the detrimental effects of oxidative stress.[42].

Eight distinct isoenzymes make up the human GPXs. These isoenzymes are designated GPX1 through GPX8. Within this collection, five isoenzymes (GPX1-4, 6) incorporate the amino acid selenocysteine (Sec), obtaining the title SecGPXs. Conversely, the other three isoenzymes (GPX5, 7, 8) have cysteine instead of the active site Sec, categorizing them as CysGPXs. Each isoenzyme possesses a unique molecular architecture, subcellular distribution, substrate preference, and biological activity, permitting its differentiation and specialization within the human body, since they are encoded by distinct genes. [46]. For instance, GPX4 is a well investigated enzyme that can operate as a hydro peroxidase for phospholipids. Its initial task is shielding cells against peroxidation lipid in cellular membranes and ferroptosis by targeting lipid hydroperoxides [42].

There is evidence that various members of the GPX family contribute to initiating numerous diseases, including cardiovascular disease, diabetes, neurological difficulties, and cancers [47].

2.3.2 GPX1 and Cancer

The GPX1 polymorphism exhibits distinctive effects associated with specific cancer types and is considered a potential sign of the likelihood of developing cancer. However, the debate regarding the link between GPX1 variations and cancer vulnerability persists across research studies. it is essential to highlight that higher levels of GPX1 have been consistently reported in different cancer types. [48]

Conversely, independent research has revealed a relationship between decreasing circulating GPX1 levels and an elevated susceptibility to cancer. [14], Thus, its function can be contradictory, operating as both a promoter and a suppressor of tumors, dependent on the cancer type. GPX1 can regulate cell proliferation, cell apoptosis, cell migration, and the immunological response to cancer; Additionally, it is involved in the response to chemotherapy and radiotherapy.[49].

In the human GPX1 gene, many single-nucleotide polymorphisms (SNPs) have been discovered. The rs1050450 polymorphism is actively explored because of its functional relevance. This unique SNP comprises a cytosine (C) to thymine (T) substitution at exon 2. This mutation leads to an amino acid change from proline (CCC) to leucine (CTC), leading to a reduction of roughly 5% in GPX1 enzymatic activity [50].

Several studies recently established a bond between the existence of GPX1 (rs1050450) gene polymorphisms and the development of malignancies, such as breast [51], prostate [52], bladder [50], lungs [53] leukemia [54], and colorectal cancers [55].
However, no data yet indicate the influence of the GPX1 gene polymorphisms on the development and progression of ovarian cancer. Therefore, our work intends to establish a relationship between the genetic variation in the GPX1 gene (particularly rs1050450) polymorphism and Turkish women's susceptibility to ovarian cancer.

3. METHODS AND MATERIALS

3.1. Sample Selection and Definition

The patient and control groups were made up of people of Turkish heritage (Caucasians) from the same place in Turkey. The patient group included 90 women diagnosed with ovarian cancer by Obstetrics and Gynecology doctors, whereas the control group included 90 healthy women. All participants were at least 18 years old. Each participant was interviewed and asked to complete a standardized questionnaire on their demographics, cancer history, menopausal state, and data about their pregnancies and childbirths. However, some individuals chose not to answer all of the questions. Our study was authorized by the Ethics Committee of Yeditepe University. Blood samples were obtained from patient participants in tubes containing EDTA.

3.2. Materials and methods applied in the study.

3.2.1. Experimental Equipment

Instruments and apparatus employed for this experimental study encompassed the iPrep PureLink DNA Isolation Robot (manufactured by Invitrogen in partnership with Thermo Fisher Scientific Inc), the Nanodrop 2000 spectrophotometer (developed by Thermo Fisher Scientific Inc), Real-Time PCR devices including the LIGHT CYCLE 480 II Instrument (manufactured by Roche Diagnostics) and the Fast Real-Time 7500 system (offered by Applied Biosystems), a Plate Centrifuge sourced from Hettich, a Centrifuge provided by Beckman Coulter, refrigeration units by Haier operating at temperatures of +4°C, -80°C, and -20°C, Ultra-Pure Water obtained through the Pure Lab Option Q system by Elga, a Vortex mixer from V.I. Plus Biosan, and a Pipette Kit offered by Thermo Fisher Scientific Inc

3.2.2. DNA isolation from blood samples.

Peripheral blood samples have been collected using tubes containing 5 mL of EDTA. for both the patient and control group participants, samples have been refrigerated at +4 °C till genomic DNA isolation have been performed. EDTA in the tubes is vital to prevent clot formation in the collected blood samples.

Extraction of genomic DNA was performed employing the iPrep Purification Instrument (Invitrogen, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA) in conjunction with the iPrep Purelink gDNA blood isolation kit (Invitrogen, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA).Waltham, MA, USA) with peripheral blood samples of 350µl of volume each, the isolation capacity of this system was 13 samples at each run, This implies that 13 samples might be processed simultaneously. Each sample is placed into its own cartridge after the cartridges have been spun for a while to effectively attach the magnetic beads to the DNA.

According to ChargeSwitch® technology (CST®), the iPrep robot operates as an automated extraction procedure. With this technique, a significant amount of DNA may be extracted from materials. This technique uses just paramagnetic particles to prepare appropriate quantities of genomic DNA from samples. A DNA-binding surface surrounds these particles. Comparing the CST® (Charge Switch® Technology) extraction method to the silica-based DNA extraction method reveals a distinct concept. The pH of the buffer around the beads can alter their charge. Because the DNA's backbone is negatively charged under low pH situations, it attaches to the positively charged beads. In order to facilitate the elution of DNA, these charged beads are neutralized via the use of a low salt buffer with higher ph. DNA samples are ready for use after entering the wash buffer with purified nucleic acids. Aqueous DNA samples were collected at the conclusion of the experiment and maintained in the refrigerator at +4 ° C.

3.2.3. DNA purity measurement

NanoDrop is a brand of UV spectrophotometer that is utilized for detecting the absorption of UV light by nucleic acids at a wavelength of 260 nm. To measure the purity of DNA, the optical density (OD) OD260/OD280 and OD260/OD230 ratios are determined using NanoDrop, whereas the concentration of DNA may also be determined with this apparatus. For genotyping purposes, the ideal OD260/OD280 ratio occurs within the range of 1.7 to 1.9.

Unlike other UV spectrophotometers, Nanodrop does not require the use of cuvettes or capillaries for sample measurement. This direct assessment approach minimizes external factors' impact and results in more precise and realistic outcomes.

The present study utilized NanoDrop 2000 (Thermo Fisher Scientific Inc.) to evaluate the purity of DNA. 1µl of DNA sample from each participant in both the OC patient and control groups was measured using this device. In addition, distilled water was utilized as a blank sample, and after each sample measurement, the equipment was cleaned to offer a safe and clean zone for further studies.

The dilution factor is utilized if the DNA sample has been diluted before measurement to ensure that the OD260 reading falls within the linear range of the spectrophotometer. The conversion factor is the factor that correlates OD260 readings to the concentration of double-stranded DNA (dsDNA) in µg/mL per OD unit. The conversion factor generally employed is 50 µg/mL per OD unit for dsDNA.

3.3. DNA genotyping by real-time PCR

A high-throughput genotyping technology, the Applied Biosystems 7500 Fast-Real-Time Polymerase Chain Reaction (PCR), was utilized for the genotyping analysis.

Fluorescence dyes of probes are used in Real-Time PCR to identify single nucleotide polymorphisms (SNPs). This method makes genotyping possible by reading fluorescence radiations. The first TaqMan probe, tagged with a VIC dye, detects the first allele sequence, and the second probe, labeled with a FAM dye and detecting the second allele sequence. Additionally, they have a quencher molecule near to the reporter dye, which prevents fluorescence emission until the Taq polymerase in the PCR process cleaves the probe. The reporter dye is released from the quencher during cleavage, allowing it to produce fluorescent light that is picked up by a fluorescence detector. A fluorescence detector detects the amount of fluorescent light emitted concerning how much-amplified target DNA is present.

A real-time PCR was done for the GPX1 (rs 1050450) polymorphism genotyping, utilizing a Taqman assay. The amplification of the GPX1 gene region spanning the polymorphism was conducted using the AGGAGGGGCGCCCTAGGCACAGCTG forward primer and GCCCTTGAGACAGCAGGGCTTCGAT reverse primer.

3.4. Real-Time PCR Procedure

For the Real-Time PCR method, the following components were mixed: 5 µl of Master Mix, 0.25 µl of Taqman genotyping assay, 3.75 µl of DNase, RNase-free water, and 1 µl of template DNA. The complete analysis of reagents and reaction mixture quantities are given in (Table 3.3.4.1).

The experimental technique comprised producing a combination consisted of Master Mix, DNase, RNase-free water, and TaqMan genotyping assay. This mixture was then added to the individual wells of a 96-well plate. Subsequently, the template DNA samples were added progressively to the mixture. To assure consistency and settling of liquids, The well-enclosed plate underwent centrifugation at 3000 rpm for 2 minutes.

Subsequently, the plate was situated inside the PCR instrument, initiating the commencement of the Real-Time PCR reaction."

The PCR technique consists of three primary phases: denaturation, annealing, and extension. In the denaturation phase, the reaction mixture is heated to a higher temperature to promote the separation of DNA strands, triggering the breaking of hydrogen bonds between the matching base pairs. This denaturation temperature typically remains around 95°C, with a standard time of 15 to 30 seconds.

Table 4. Real-Time PCR Conditions.

	Denaturation	Annealing	Extension
Temperature ° C	95°C	60-70°C	72°C
Duration/ Time	15-30 seconds	15-30 seconds	1 minute
Number of cycles	40 cycles	40 cycles	40 cycles

During the annealing phase, the reaction mixture is cooled to a temperature that facilitates primers' attachment (annealing) to the corresponding DNA strands. The specific temperature for annealing is contingent upon the melting temperature (Tm) of the primers, generally falling within the range of 60 to 70°C. This phase usually lasts for 15 to 30 seconds. The extension phase encompasses elevating the temperature of the reaction mixture to a level that facilitates the elongation of primers by the Taq polymerase, leading to the generation of fresh DNA strands. Typically, the temperature for extension hovers around 72°C, and the length of time is contingent on the extent of the targeted DNA sequence amplification. Commonly, the extension duration is about 1 minute for every kilobase of DNA.

Table 5. A Composition of Real-Time PCR Reaction Mixtures.

Component	Description	Amount
TaqMan Fast Advanced Master Mix	A ready-to-use master mix for real-time PCR	5 µl
TaqMan Genotyping Assay	A specific primer and probe set for detecting a particular DNA sequence	0.5 µl
Free DNase and RNase Water	A nuclease- and RNase-free water	3.5 µl
Template DNA	DNA containing the sequence of interest	1.0µl

1.1. Statistical Analysis

Statistical Analysis of this thesis utilizes the t-test to acquire numerical data. The information supplied from the DNA genotyping was then examined in SPSS 26.0 utilizing Fisher's Exact and Chi-square testing. This investigation analyzes the distribution of genotypes and alleles among the various groups. The significance value was determined to be $p<0.05$.

The study also explored the correlation between the presence of GPX1 genotypes and the risk of ovarian cancer. This method involved the computation of odds ratios (ORs) and their corresponding confidence intervals of 95% (CIs).

4. RESULTS

4.1. Demographic features

The variations in how patients with ovarian cancer are assigned certain features compared to the control categories are thoroughly summarized in (Table 6). It's important to note that only some participants could respond to all the questions posed. Some participants exhibited signs of discomfort when confronted with specific inquiries, leading to a collection of responses that were either incomplete or lacked the necessary clarity.

There was a significant variation in the distribution of alcohol consumption between women with ovarian cancer (OC) and those without OC. The p-value for this difference was less than 0.0005, indicating that the differences were highly statistically meaningful. This implies that consuming alcohol is a considerable risk factor for OC. However, regarding the mean age of the participants and their history of cancer, there were no statistically significant differences between OC patients and controls, as demonstrated by p-values of 0.582 and 0.412, respectively.

A number of factors have been identified as having a statistically significant impact on the distribution of OC. These included the menopausal status of the participants, categorized as either premenopausal or postmenopausal; the number of pregnancies a participant had experienced, divided into two groups - those with one or fewer pregnancies and those with more than one pregnancy, and finally, the parity of the participants, classified as those with one or fewer births and those with more than one birth. A closer inspection of the data revealed that the proportion of postmenopausal women in the OC group was considerably more significant, at 79.5%, compared to 17.4% in the control group. This implies a potential connection between postmenopausal status and the incidence of OC. In addition, the control group exhibited a considerably more significant percentage of participants with one or fewer births, at 56.5%, compared to the OC patient group, at just 27.3%. This could indicate a protective effect of having more than one birth against the development of OC.

Furthermore, the control group also had a higher proportion of participants with one or fewer pregnancies, at 56.5%, compared to the OC patient group, which was only 22.7%. This observation further strengthens the hypothesis that having more than one pregnancy could potentially reduce the risk of developing OC.

Table 6. Clinical features, and histopathological parameters for Ovarian Cancer patients and control group.

Characteristics		Ovarian Cancer Patients (90 samples)	Control Collection (90 samples)	P- value
Age, x̅ ± SD (years)		53.31 ± 12.53	45.51 ± 12.01	0.707 (NS)
Body Mass Index (BMI), x̅ ± SD		30.11 ± 5.89	23.761 ± 4.30	0.043 (S)
Fasting Blood Sugar, x̅ ± SD (mg/dl)		109.12 ± 33.47	86.85 ± 7.809	< 0.0002 (S)
Alcohol consumption	Yes%	(7.8%)	(21.1%)	0.000002 (S)
	No%	(64.4%)	(26.7%)	
	I prefer not to say	(27.8%)	(65.3%)	
History of Cancer	Yes %	(35.8%)	(43.5%)	0.412 (NS)
	No %	(64.2%)	(56.5%)	
Menopause	Postmenopausal %	(79.5%)	(17.4%)	0.0001 (S)
	Premenopausal %	(20.5%)	(82.6%)	
Parity (number of birth)	≤ 1	(27.3%)	(56.5%)	0.005 (S)
	>1	(72.7%)	(43.5%)	
Pregnant	≤ 1	(22.7%)	(56.5%)	0.001 (S)
	>1	(77.3%)	(43.5%)	

(The number of samples is denoted by n. Both the mean and the standard deviation are represented by x̅ ± SD. A p-value of less than 0.05 indicates a result that is statistically significant (*S), whereas a p-value of bigger than 0.05 indicates a non-significant difference (NS))

Table 7 recapitulates the clinical and histopathological traits of 90 patients diagnosed with Ovarian Cancer. The data sheds light on critical factors that significantly manage the condition effectively and make informed medical decisions.

The menopause status of the patients is an essential factor to consider, with 79.5% being postmenopausal and 20.5% being premenopausal. Menopause could impact a female's likelihood to acquire ovarian cancer and may also influence the choice of treatment options for women with ovarian cancer.

Metastasis, an indication of cancer spreading to other body parts, was observed in a significant percentage of patients (76.1%). This finding underscores the importance of early detection and intervention to prevent the disease from advancing to advanced stages, affecting the prognosis.

Relapse is another significant aspect to consider, with 45.7% of patients experiencing a cancer recurrence after initial treatment. Monitoring for relapse and implementing appropriate management strategies are critical components of long-term care for ovarian cancer patients.

Pregnancy status and parity, the number of children a woman has borne, also provide relevant insights into the disease. About 77.3% of patients were not in a pregnant state (greater than one pregnancy), while 22.7% had either been pregnant once or were currently in a pregnant state (less than or equal to one pregnancy). Understanding these factors can be important in assessing ovarian cancer risk and considering fertility preservation options during treatment.

Adjuvant chemotherapy, administered after primary therapy, was administered to 72.1% of patients.

Staging surgery was performed in 25% of cases, while 42.5% underwent debulking surgery, and 32.5% had a combination of both staging and debulking procedures.

Lastly, the table highlights the presence of different tumor marker proteins. CA125, the most prevalent marker, was observed in 84.1% of patients, followed by CA15-3 (64.5%), CA19-9 (25.7%), and CEA (14.3%). Monitoring these tumor markers is vital in diagnosing ovarian cancer, evaluating treatment response, and monitoring disease progression.

Table 7. Comprehensive Overview: Clinical Features and Histopathological Parameters of Ovarian Cancer Patients.

Clinical and histopathological characteristics		Percentage (%)
Menopause status	Premenopausal	20.5%
	Postmenopausal	79.5%
Metastasis	Yes	76.1%
	No	23.9%
Relapse	Yes	45.7%
	No	54.3%
Pregnant state	≤ 1	22.7%
	>1	77.3%
Parity	≤ 1	27.3%
	>1	72.7%
Adjuvant chemotherapy	Yes	72.1%
	No	27.9%
Surgery	Staging	25%
	Debulking	42.5%
	Staging & Debulking	32.5%
Tumor Marker Proteins	CA125	84.1%
	CEA	14.3%
	CA19-9	25.7%
	CA15-3	64.5%

4.2. A Percentage Analysis of Stages and Cell Types involved in the Ovarian Cancer patients.

Based on the statistical analysis in Table 8, Ovarian cancer exhibits varying stages and cell types, each with distinct characteristics. While the most prevalent Stage was Stage III, constituting 40.5% of cases. Stage I, the least common Stage, accounts for 23.8% of cases, followed by Stage II, which represents 21.4% of cases. Finally, Stage IV Cancer is advanced, spreading extensively to distant organs beyond the pelvic region, accounting for 14.3% of cases. Epithelial tumors are the most frequent, accounting for 90% of all occurrences. The most frequent type of epithelial tumor is a serious carcinoma, which is responsible for 55% of all patients**.** Followed by mixed epithelial tumors (15%), mucinous tumors (10%), and endometriosis tumors (7.5%). Clear cell tumors are the least prevalent form, accounting for 2.5% of cases. Sex-cord stromal and germ cell tumors account for 5% of occurrences.

Table 8. The proportional breakdowns of stages and cell types within the ovarian cancer's patients.

Clinical features	**Proportion (%)**
Ovarian cancer Stage:	
I (One)	23.8%
II (Two)	21.4%
III (Three)	40.5%
IV (Four)	14.3%
Types of Cells involved in OC:	
Epithelial ovarian cancer	90.0%
Serous tumor	55%
Mucinous carcinoma	10%
Endometrioid tumor	7.5%
Clear cell carcinoma	2.5%
Mixed epithelial tumors	15%
Germ cell cancer	5%
Sex-cord stromal tumors	5%

4.3. Genotype and Allele Analysis of Patient and Control.

The GPX1 gene (rs1050450) has three genotypes: TT, CT, and CC. The overall P-value for the GPX1 gene (0.001) reveals a highly significant link between the GPX1 gene and ovarian cancer. The results given in Table 9 reveal that the TT and CT genotypes have a greater frequency abundant in patients than in the healthy category (75%) and (58.2%) with p-values of 0.036 and 0.017, respectively, indicating a potential link with a higher risk of ovarian cancer. Conversely, the CC genotype is considerably more prevalent in the control group than in the Ovarian Cancer patients (68.2%) with a P-value of 0.0002, suggesting a possible preventive effect against ovarian cancer. T allele is more abundant in persons with ovarian cancer (37.7%) than in controls (25.5%), with a P-value of 0.001; thus, it implies that there may be a probable relationship between the T allele and ovarian cancer. On the contrary, the C allele is more prevalent in controls (47.7%) than in ovarian cancer patients (43.3%), with a P-value of 0.036; this shows that the C allele might give a preventive influence against ovarian cancer.

Table 9. The genotypes frequencies of GPX1 gene in all study groups.

Genotype	(Ovarian Cancer patients =90)		(Control group =90)		P-Value	Odd Ratio (OR)	Confidence Interval 95%(CI)
	Account	Frequency	Account	Frequency			
CC%	21	31.8%	45	68.2%	0.0002	0.304	0.161- 0.577
CT%	57	58.2%	41	41.8%	0.017	2.064	1.137-3.749
TT%	12	75%	4	25%	0.036	3.308	1.024-10.682
Alleles Distributions							
C allele	78	43.3%	86	47.7%	0.036	0.302	0,094-0.976
T allele	68	37.7%	46	25.5%	0.0002	3.286	1.73-6.230
GPX1 gene`s P-Value 0.001							

4.4. Genotype Distribution and Its Association with Demographic Data in Patients.

The table 10, illustrates the distribution of tumor marker proteins (CA125, CEA, CA19-9, and CA15-3) in patients with different GPX1. The results indicate no statistically significant associations between genotypes and tumor marker proteins. The p-values for all the associations were found to be greater than the conventional threshold of 0.05. However, Patients with the CC genotype have high CA125 levels. (83.3%) besides CA15-3 (62.5%). Similarly, patients with the TT genotype were likelier to have elevated levels of CA15-3 (100%) and CA125 (82.5%), and this is consistent with our study's findings that there are an association between the presence of the TT genotype and the occurrence of ovarian cancer. The CT genotype also commonly displayed heightened concentrations of CA125 (83.3%) and CA15-3 (61.9%).

Table 10. The distribution of GPX1 Genotypes among different tumor marker proteins in patients.

			GPX1 Genotypes		
			CC	**CT**	**TT**
Tumor marker proteins	**CA125**	≤35 (U/mL)	16.7%	11.1%	17.5%
		>35 (U/mL)	83.3%	88.9%	82.5%
		P-Value	0.841	0.469	0.362
	CEA	≤5 (ng/ml)	91.7%	78.9%	100%
		>5 (ng/ml)	8.3%	21.1%	0 %
		P-Value	0.657	0.452	0.686
	CA19-9	≤37 (U/mL)	62.5%	73.9%	100%
		>37 (U/mL)	37.5%	26.1%	0%
		P-Value	0.385	0.944	0.211
	CA15-3	≤30 (U/mL)	37.5%	38.1%	0%
		>30 (U/mL)	62.5%	61.9%	100%
		P-Value	**0.891**	**0.660**	**0.278**

Table 11 presents the data on the occurrence of metastasis and relapse in patients with different GPX1 genotypes.

The overall analysis based on the giving data reveals not a significant connection between GPX1 genotypes and metastasis or relapse in ovarian cancer patients. The P-Values of metastasis and relapse for GPX1 genotypes were 0.339 and 0.874, respectively. The table reveals that patients with the TT genotype have a higher probability of metastasis (100%) compared to those with the CC genotype (81.3%) or the CT genotype (69.2%). Similarly, Patients with the TT and CC genotypes have a 50% chance of relapse, whereas the CT genotype shows a slightly lower likelihood of relapse at 42.3%.

Table 11. Distribution of Metastasis and Relapse among Patients with GPX1 Genotypes.

Genotype	Metastasis		Relapse	
	Yes	No	Yes	No
CC	81.3%	18.8%	50%	50%
CT	69.2%	30.8%	42.3%	57.7%
TT	100%	0 %	50%	50%
P-Value	0.339		0.874	

Table 12 shows the distribution of GPX1 genotypes in patients with different stages of ovarian cancer.

Based on the presented analysis and associated p-value (0.27), GPX1 genotypes do not appear to be significantly related to different stages of ovarian cancer. However, The TT genotype is most common in Stage II patients (50.0%), while the CC genotype is most common in Stage III patients (53.3%).

Table 12. Analysis of GPX1 Genotype Distributions with Ovarian Cancer Stages.

	GPX1 Genotypes			P-Value
Stage	CC	CT	TT	
I	33.3%	17.4%	25.0 %	0.27
II	6.7%	26.1%	50.0%	
III	53.3%	39.1%	0.0%	
IV	6.7%	17.4%	25%	

Table 13 illustrates the percentage distributions of GPX1 genotypes (CC, CA, TT) with diverse subtypes of ovarian cancer in the patient group. Overall, the p-value for this table is 0.167, indicating no statistically significant relationship between GPX1 genotypes and various types of ovarian cancer in the patient individuals.

The genotype TT was the most common genotype for serous tumors, with a prevalence of 66.7%, followed by the CT genotype at 54.5%, with the CC genotype accounting for 53.3% of cases. Furthermore, the TT genotype demonstrated a notable presence in clear cell tumors, constituting 33.3% of cases. However, it is noteworthy that the TT genotype is not observed in other tumor types, including mucinous tumors, endometriosis, mixed epithelial masses, germ cell tumors, and sex-cord stromal tumors. Furthermore, CC genotype was absent in clear cell tumors.

These intriguing findings increase the possibility of potential correlations between GPX1 genotypes and specific ovarian cancer types, warranting further comprehensive investigations to discern their clinical implications.

Table 13.GPX1 gene variations among different types of ovarian cancer within the patient's group.

Types of Ovarian Cancer	GPX1 Genotypes			P-Value
	CC	CT	TT	
Serous tumors	53.3	54.5%	66.7%	0.167
Mucinous tumors	13.3%	9.1%	0.0%	
Endometriosis tumors	13.3%	4.5%	0.0%	
Clear cell tumors	0.0%	0.0%	33.3%	
Mixed epithelial tumors	13.3%	18.2%	0.0%	
Germ cell carcinoma	0.00%	9.1%	0.00%	
Sex-cord stromal carcinoma	6.7%	4.5%	0.0%	

Table 14 displays the distributions of adjuvant chemotherapy within the patients' group based on their GPX1 genotypes. GPX1 genotypes do not appear to be associated with the administration of adjuvant chemotherapy in this patient population based on a p-value of 0.847. For the patients with the CC genotype, 66.7% received adjuvant chemotherapy, while 33.3% did not. Among patients with the CT genotype, 75.0% underwent adjuvant chemotherapy, with 25.0% not receiving it. Similarly, patients with the TT genotype showed a distribution of 75.0% receiving adjuvant chemotherapy and 25.0% not receiving it.

Table 14. The distributions of adjuvant chemotherapy among the GPX1 Genotypes of the patients' group.

Adjuvants chemotherapy		CC	CT	TT	P-Value
	Yes	66.7%	75.0%	75.0%	**0.847**
	No	33.3%	25.0%	25.0%	

4.5. Evaluation, Assessment, and Interpretation of Real-Time PCR Findings.

Our study used the 7500 Fast-Real Time PCR Instrument to assess allelic discrimination automatically. The instrument's software translated the fluorescence irradiation readings using the dyes found in the probes. The FAM dye displayed a blue coloring, whereas the VIC dye had a green tint. The FAM and VIC dyes were compared to the ROX dye as a reference color. However, it is essential to note that discrimination between alleles could not appear for particular samples. In such cases, the Thermo Fisher Cloud platform was utilized to determine the undetermined samples. Our experiment was conducted multiple times for patients and control samples. Figures 7, showing some of the analyses of allelic discrimination through the examination and interpretation of radiance curves.

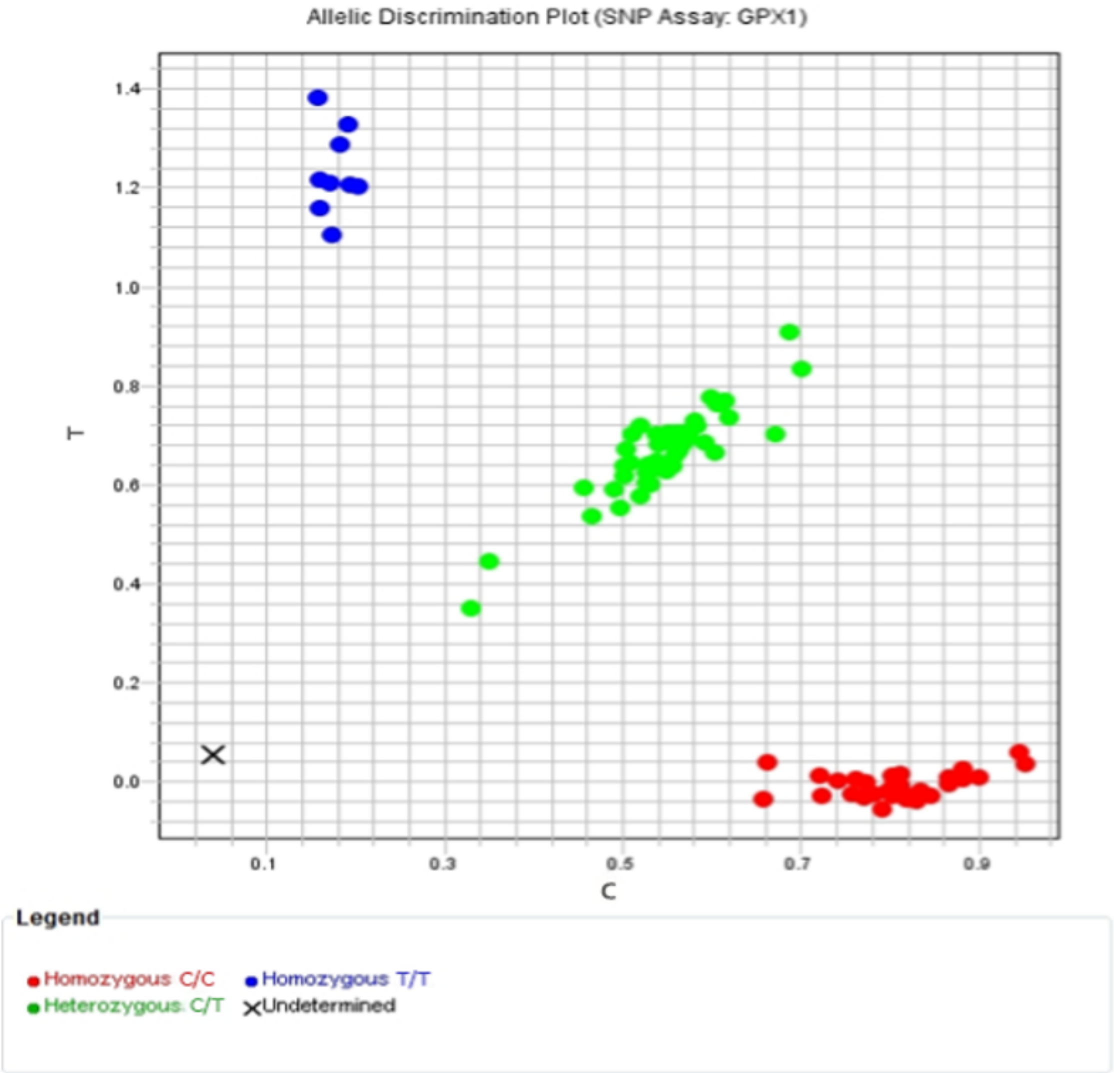

Figure 7. Allelic differentiation analysis of GPX1 genotypes was performed. The genotypes were classified as homozygous wild type (CC), heterozygous (CT), and homozygous mutant type (TT).

On the y-axis, the amplification plot displays the fluorescence intensity or signal resulting from a fluorescent dye and on the x-axis, the number of PCR cycles. In the initial cycles, the fluorescence signal is typically minimal. However, the fluorescence signal gradually increases as the PCR progresses and amplifies the target DNA.

Figure 8 displays amplification plots for the T allele of the GPX1 gene, with the threshold value set at 0.029, represented by a yellow line. Contrariwise, Figure 9, showcases amplification plots for the C allele of the same gene, with the threshold value (0.0298) indicated by light green.

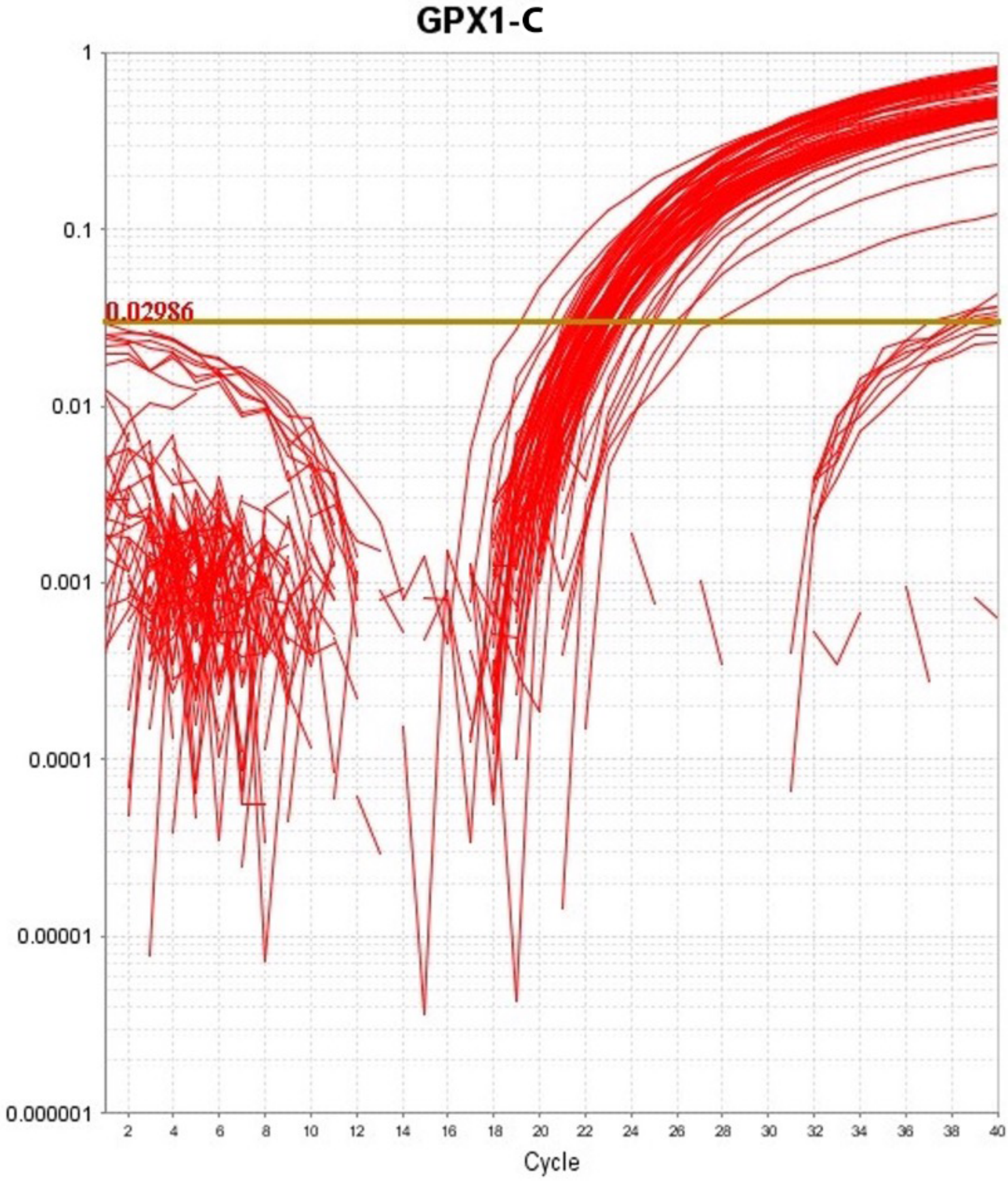

Figure 8. Plot of C allele amplification.

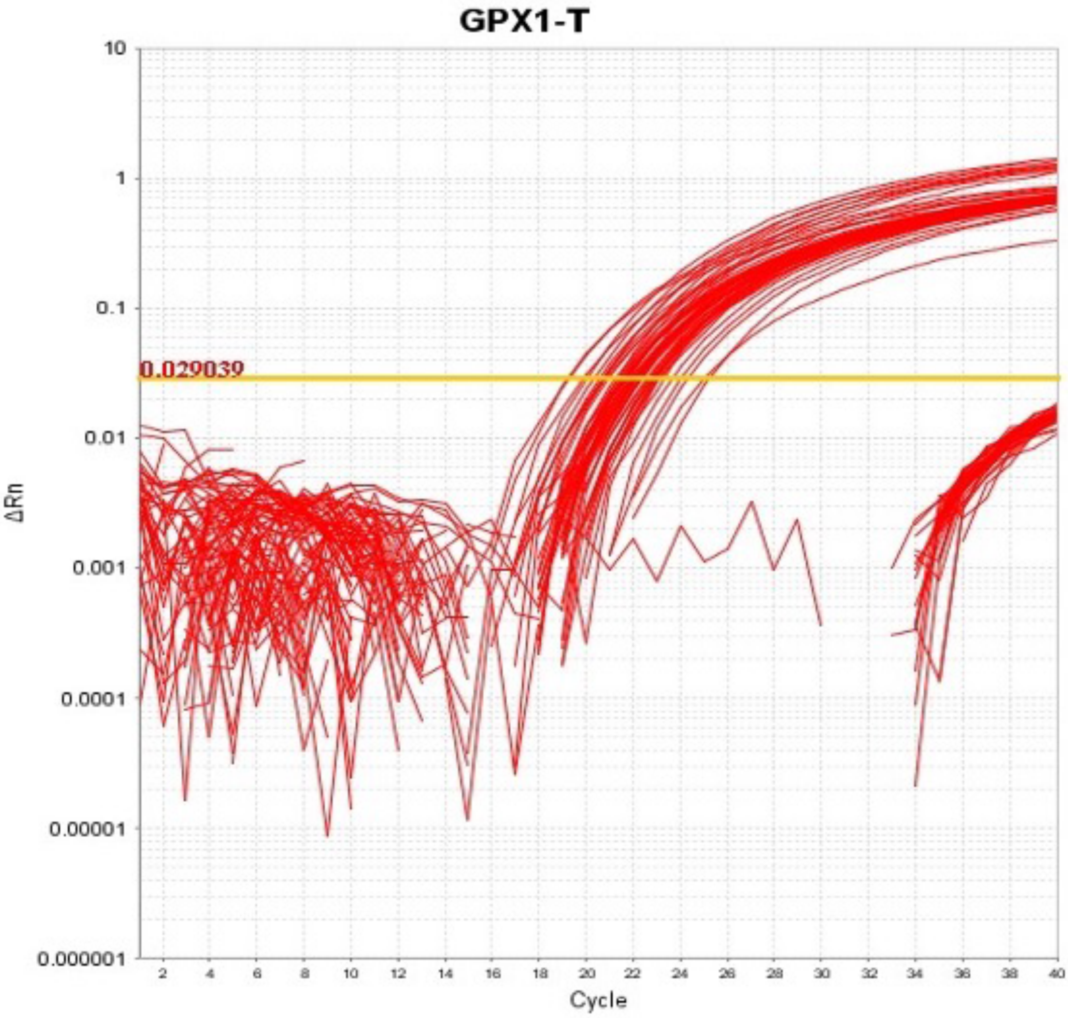

Figure 9.GPX1 gene amplification plot for allele T.

5. DISCUSSION

Gynecological malignancies pertain to tumors affecting the complicated network of female reproductive systems, encompassing disorders in the cervix, ovaries, vagina, vulva, and uterus [56]. Within the area of gynecological malignancies, among these cancer forms, ovarian cancer entails the most enormous burden in terms of death and morbidity [57]. In 2020, around 207,252 fatalities were expected globally owing to ovarian cancer [58].

Among Ovarian tumors, Epithelial ovarian cancers (EOC) constitute the main malignant subtype, representing roughly 90% of occurrences [59]. As the most prevalent histological subgroup within EOCs, high-grade serous ovarian cancer (HGSOC) is usually detected at an advanced stage [59]. In our investigation, we analyzed a cohort of 90 individuals diagnosed with ovarian cancer, and Epithelial cancer accounted for precisely 90% of all instances. Among these EOC instances, High-grade serous lesions accounted for 55%, making them the most prevalent subtype. Following that, we discovered Mixed epithelial tumors in 15% of cases, mucinous tumors in 10%, endometriosis in 7.5%, and Clear cell tumors in 2.5% of cases.

Numerous biomarkers have been investigated in ovarian cancer involving the tumor marker proteins. One of these indicators is (CA125); The specificity of CA-125 is 97% (62). However, it is essential to highlight that this marker exhibits limited sensitivity when it comes to diagnosing ovarian cancer in its early stages [60]. Moreover, high biomarkers of CA125 levels have also been documented in several other clinical or physiological circumstances, including pregnancy, menstruation, and inflammatory disorders of the peritoneum [61]. Our study indicated that approximately 84% of ovarian Patients showed an elevated amount of CA-125, making it the most frequent tumor marker. The second most prevalent marker was CA15-3, which revealed increased levels in 64.5% of individuals. Conversely, CEA had the lowest expression, with just 14.3% of ovarian patients expressing high levels.

Some epidemiological studies examine the connections between age and ovarian cancer development in occurrence, histology, and death among ovarian cancer patients. Meanwhile, it has been observed that the prevalence of ovarian cancer is raised with aging., reaching its greatest point at roughly 50-60 years old [63]. In our demographic investigation of ovarian cancer, the age average was 53.31 years.

Based on demographic data analysis, we noticed that roughly 7.8% of patients were alcoholics, whereas 21.1% of the control group acknowledged alcohol intake. The statistical investigation generated a significant P-value of 0.00002, demonstrating a negative relationship between ovarian cancer and consuming alcohol. These findings align with previous research that likewise demonstrated no association between drinking alcohol and ovarian cancer progression [64].

Menopausal status plays a critical element in ovarian cancer development [65]. Our investigation found a significant variance between the two parties concerning menopausal state, the data suggested that being a postmenopausal woman was associated to an elevated likelihood of having ovarian cancer, with 79.5% of postmenopausal women impacted.

The vast majority of patients, 64.2% in our investigation, had no family history of cancer, whereas 35.8% had a cancer history in their family. Numerous prior research has underlined an apparent relationship between the progress of cancer ovaries and a family history of cancer, specifically a breast cancer history [66].

A comprehensive investigation has been performed to analyze the association between pregnancy and ovarian cancer risk. The development of pregnancy initiates a condition of anovulation and limits the release of pituitary gonadotropic hormones, and This accords with the beliefs known as the 'incessant ovulation' and 'gonadotropin' hypotheses [67]. Based on this hypothesis, Women who have had one or more pregnancies are 30-60% less likely than women who have never been pregnant to develop OC [68]. Moreover, the likelihood of developing ovarian cancer declines by approximately 15% with the occurrence of each succeeding full-term pregnancy [69]. In a comparable manner, parous women have about a 20% lower chance of having serous carcinoma than nulliparous women, whereas they have a 50%–70% reduced risk of developing clear cell and endometrioid carcinomas [70][39].

Our research found a substantial difference among the patients; 77.3% had experienced numerous pregnancies, and 72.7% had a history of recurrent childbirths, whereas these values were at 43.5% for the control group in both categories. This disparity might result from variances in the quantity and composition of the research participants.

Metastasis plays a vital role in cancer's growth and can make it difficult to cure. Metastasis was identified in 76.1% of our cases. In instances of advanced ovarian cancer, the primary strategy involves debulking surgery (42.5%), which seeks to remove all visible lesions, followed by chemotherapy 72.1% [72]; however, even with these medications. Relapses are widespread in advanced ovarian cancer patients [71] and are estimated to occur in 47.8% of cases.

Other risk factors that exist within the range of modifiable risk factors for ovarian cancer are cigarette smoking, nutritional value, overweight or obesity, and hormone treatment (HRP) [73]. Our investigation emphasizes an essential finding: Women with ovarian cancer demonstrated a markedly higher BMI (30.11 ± 5.89) compared to healthy controls (23.761 ± 4.30) with a P-value of 0.043 [74].

Approximately 58% of women diagnosed with ovarian cancer were identified at an advanced stage, (III or IV). This delayed diagnosis seems to be connected with poor prediction, as evidenced by survival data, demonstrating a 5-year survival rate of 27% for stage III ovarian cancer, which further reduces to 13% for stage IV ovarian cancer [75]. Likewise, in our analysis, a substantial majority (55.0%) of ovarian cancer patients were detected at stages III or IV."

The glutathione peroxidase (GPX) family contains critical antioxidant enzymes, and within this family, GPX1 rates as the most prevalent variation generated by the GPX1 gene found on chromosome 3 in humans. Its principal activity includes detoxifying hydrogen peroxide [76]. Despite the prevalence of multiple single nucleotide polymorphisms (SNPs) in the GPX1 gene's DNA, the Pro198Leu polymorphism (also known as rs1050450) has gained substantial scientific interest.

Prior studies have disclosed a relationship between lowered circulating GPX1 levels and an increased susceptibility to cancer, stated in numerous cancer types, including breast [77], lung [78], prostate [52], and colorectal malignancies [79].

In light of this, our research has investigated the potential function of the GPX1 (rs1050450) polymorphism in the susceptibility to ovarian cancer, and it is noteworthy that the research we conducted represents the first attempt to investigate within the Turkish population.

The exceedingly high P-value reported for the GPX1 gene (0.001) reflects a reasonably strong and statistically meaningful link between the GPX1 gene and ovarian cancer initiation. There is a reduced risk of ovarian cancer among individuals who have homozygous genotype CC at the GPX1 gene, as indicated by an odds ratio (OR) of 0.304 (95% Confidence Interval [CI] 0.161 - 0.577). As opposed to this, those with the TT polymorphism at the GPX1 gene showed an elevated ovarian cancer risk, with an OR of 3.308 (95% CI 1.024 - 10.682), with a statistically significant p-value of 0.036.

Additionally, A noteworthy attention refers to a considerable reduction in ovarian cancer risk related to the C allele (p-value = 0.036). Conversely, for the T allele, the odds ratio (OR) of 3.286 among patients with ovarian cancer implies they are nearly three times more prone to ovarian cancer than those in the control group without cancer.

In analogous published studies conducted on bladder cancer within the Turkish population by Canan Kucukgergin et al.., it has been revealed that TT genotype GPX1 (rs1050450) polymorphism (OR of 1.67 (95% CI = 1.17–2.40, P = 0.005) displayed a significant correlation with a promoted susceptibility to bladder cancer than CC genotype GPX1 (rs1050450) [80][14]. Moreover, the work done by Duminda Ratnasinghe et al. focused on the effect of the GPX1 (rs1050450) polymorphism on lung cancer [78]. Their findings indicated a significant link, evidenced by an odds ratio (OR) of 1.9 and 95% CI, 1.02-3.58, with a P-value of less than 0.001[78]. Compared to earlier research, our results precisely fit this known paradigm, demonstrating an impressive synergy within our conclusions.

Recently, there has been a surge of studies looking into the role and processes of GPX1 in connection to altered expression and activity within the cancer setting. Wei et al. evaluated the levels of GPX1 expression in several human malignancies. Their investigation suggested that GPX1 is typically raised in several malignancies, exhibiting considerably larger tumor tissue levels than nearby normal samples. These constitute ovarian serous cystadenocarcinoma, endometrial cancer, and cutaneous melanoma [81]. Conversely, the prospective functions of GPX1 might reveal various consequences among diverse cancer types. For instance, a study by Cullen, Joseph J et al. revealed a progressive reduction in glutathione peroxidase 1 level from normal pancreatic cells to chronic pancreatitis and ultimately to pancreatic cancer. The reduced expression of GPX1 in pancreatic cancer cells [82] suggests that this might be one of the genes that may play a role in suppressing tumors in these situations. This suggests that GPX1 may assist in preventing cancer cells from developing and spreading [82].

Other Functional enrichment analysis has highlighted the vital role of GPX1 in various essential biological processes. These include the manipulation and modulation of reactive oxygen species (ROS), engagement in iron metabolism (ferroptosis), stimulation of cell growth signaling, regulation of glutathione metabolism, and participation in metabolic pathways under the control of the p53 protein [81].

While our present study effectively demonstrates the positive relationship between the genotype GPX1 (rs1050450) and ovarian cancer, future studies are essential to offer a more detailed explanation and refinement of the clinical effects of GPX1. Moreover, a crucial requirement is knowing the molecular processes and corresponding functional networks that drive GPX1's role in carcinogenesis. This fundamental insight will pave the way for revolutionary approaches to precision cancer therapy.

6. CONCLUSION

Gynecological malignancies, particularly ovarian cancer, affect the female reproductive organs and severely impact morbidity and death. Epithelial cancers (EOC) are the most frequent malignant form; high-grade serous tumor (HGSOC) is the most prevalent histological subtype within EOCs and is often identified at an advanced stage.

Biomarkers such as CA125 have been studied as potential approaches for diagnosing ovarian cancer, with 84% of patients having high levels. Age is a determinant of ovarian cancer development, with its incidence escalating. The menopausal state seems to be a significant factor affecting ovarian cancer development, with a lower risk seen among pregnant and parous women.

Our work analyzes the prospective relevance of the GPX1 (rs1050450) polymorphism in developing ovarian cancer risk among the Turkish population.

The present study explores the potential impact of the GPX1 polymorphism on the possibility of establishing ovarian cancer. The statistical analysis yielded a notable and statistically significant connection, as exemplified by the low P-value of 0.001 for the GPX1 gene. Furthermore, it was clear that the TT and CT genotypes are more prevalent among women diagnosed with cancer of the ovaries compared to those in the unaffected group. Conversely, the CC genotype is more typically detected in the control group than in ovarian cancer patients, suggesting a potential protective effect. Additionally, the T allele appears more frequent among patients with ovarian cancer, while the C allele is more prevalent among controls, supporting a potential preventive effect against ovarian cancer.

Ultimately, this study's reach is confined to a single group, and future research on different populations is needed to validate our conclusions and observations. Understanding the genetic components contributing to ovarian cancer risk can lead to better prediction, early detection, and individualized treatment choices. Future studies should focus on unraveling the underlying processes related to these genetic differences to better understand ovarian cancer progression and boost therapeutic results for affected patients.

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8. APPENDIX

8.1. Raw Material

Crosstabs

Output Created		19-JUL-2023 19:55:13
Comments		
Input	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	180
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=Type BY GPX1 CC TT CT C T /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.00
	Dimensions Requested	2
	Cells Available	524245

[DataSet3]

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Type * GPX1	180	100.0%	0	0.0%	180	100.0%
Type * CC	180	100.0%	0	0.0%	180	100.0%
Type * TT	180	100.0%	0	0.0%	180	100.0%
Type * CT	180	100.0%	0	0.0%	180	100.0%
Type * C	180	100.0%	0	0.0%	180	100.0%
Type * T	180	100.0%	0	0.0%	180	100.0%

Type * GPX1

Crosstab

			GPX1			
			TT	CT	CC	Total
Type	C	Count	4	42	44	90
		% within Type	4.4%	46.7%	48.9%	100.0%
		% within GPX1	25.0%	42.4%	67.7%	50.0%
		% of Total	2.2%	23.3%	24.4%	50.0%
	P	Count	12	57	21	90
		% within Type	13.3%	63.3%	23.3%	100.0%
		% within GPX1	75.0%	57.6%	32.3%	50.0%
		% of Total	6.7%	31.7%	11.7%	50.0%
Total		Count	16	99	65	180
		% within Type	8.9%	55.0%	36.1%	100.0%
		% within GPX1	100.0%	100.0%	100.0%	100.0%
		% of Total	8.9%	55.0%	36.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	14.411[a]	2	.001
Likelihood Ratio	14.785	2	.001
N of Valid Cases	180		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 8.00.

Type * CC

Crosstab

			CC N	CC Y	Total
Type	C	Count	45	45	90
		% within Type	50.0%	50.0%	100.0%
		% within CC	39.5%	68.2%	50.0%
		% of Total	25.0%	25.0%	50.0%
	P	Count	69	21	90
		% within Type	76.7%	23.3%	100.0%
		% within CC	60.5%	31.8%	50.0%
		% of Total	38.3%	11.7%	50.0%
Total		Count	114	66	180
		% within Type	63.3%	36.7%	100.0%
		% within CC	100.0%	100.0%	100.0%
		% of Total	63.3%	36.7%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	13.780[a]	1	.0002		
Continuity Correction[b]	12.656	1	.0004		
Likelihood Ratio	14.021	1	.0002		
Fisher's Exact Test				.000	.000
N of Valid Cases	180				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 33.00.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	95% Confidence Interval Upper
Odds Ratio for Type (C / P)	.304	.161	.577
For cohort CC = N	.652	.515	.826
For cohort CC = Y	2.143	1.397	3.287
N of Valid Cases	180		

Type * TT

Crosstab

			TT		
			N	Y	Total
Type	C	Count	86	4	90
		% within Type	95.6%	4.4%	100.0%
		% within TT	52.4%	25.0%	50.0%
		% of Total	47.8%	2.2%	50.0%
	P	Count	78	12	90
		% within Type	86.7%	13.3%	100.0%
		% within TT	47.6%	75.0%	50.0%
		% of Total	43.3%	6.7%	50.0%
Total		Count	164	16	180
		% within Type	91.1%	8.9%	100.0%
		% within TT	100.0%	100.0%	100.0%
		% of Total	91.1%	8.9%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.390[a]	1	.036		
Continuity Correction[b]	3.361	1	.067		
Likelihood Ratio	4.576	1	.032		
Fisher's Exact Test				.064	.032
N of Valid Cases	180				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 8.00.
b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Type (C / P)	3.308	1.024	10.682
For cohort TT = N	1.103	1.005	1.209
For cohort TT = Y	.333	.112	.995
N of Valid Cases	180		

Type * CT

Crosstab

			CT		
			N	Y	Total
Type	C	Count	49	41	90
		% within Type	54.4%	45.6%	100.0%
		% within CT	59.8%	41.8%	50.0%
		% of Total	27.2%	22.8%	50.0%
	P	Count	33	57	90
		% within Type	36.7%	63.3%	100.0%
		% within CT	40.2%	58.2%	50.0%
		% of Total	18.3%	31.7%	50.0%
Total		Count	82	98	180
		% within Type	45.6%	54.4%	100.0%
		% within CT	100.0%	100.0%	100.0%
		% of Total	45.6%	54.4%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.734[a]	1	.017		
Continuity Correction[b]	5.040	1	.025		
Likelihood Ratio	5.766	1	.016		
Fisher's Exact Test				.024	.012
N of Valid Cases	180				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 41.00.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Type (C / P)	2.064	1.137	3.749
For cohort CT = N	1.485	1.067	2.067
For cohort CT = Y	.719	.546	.947
N of Valid Cases	180		

Type * C

Crosstab

			C N	C Y	Total
Type	C	Count	4	86	90
		% within Type	4.4%	95.6%	100.0%
		% within C	25.0%	52.4%	50.0%
		% of Total	2.2%	47.8%	50.0%
	P	Count	12	78	90
		% within Type	13.3%	86.7%	100.0%
		% within C	75.0%	47.6%	50.0%
		% of Total	6.7%	43.3%	50.0%
Total		Count	16	164	180
		% within Type	8.9%	91.1%	100.0%
		% within C	100.0%	100.0%	100.0%
		% of Total	8.9%	91.1%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.390[a]	1	.036		
Continuity Correction[b]	3.361	1	.067		
Likelihood Ratio	4.576	1	.032		
Fisher's Exact Test				.064	.032
N of Valid Cases	180				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 8.00.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	95% Confidence Interval Upper
Odds Ratio for Type (C / P)	.302	.094	.976
For cohort C = N	.333	.112	.995
For cohort C = Y	1.103	1.005	1.209
N of Valid Cases	180		

Type * T

Crosstab

			T		
			N	Y	Total
Type	C	Count	45	45	90
		% within Type	50.0%	50.0%	100.0%
		% within T	68.2%	39.5%	50.0%
		% of Total	25.0%	25.0%	50.0%
	P	Count	21	69	90
		% within Type	23.3%	76.7%	100.0%
		% within T	31.8%	60.5%	50.0%
		% of Total	11.7%	38.3%	50.0%
Total		Count	66	114	180
		% within Type	36.7%	63.3%	100.0%
		% within T	100.0%	100.0%	100.0%
		% of Total	36.7%	63.3%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	13.780[a]	1	.00020552		
Continuity Correction[b]	12.656	1	.00037446		
Likelihood Ratio	14.021	1	.00018076		
Fisher's Exact Test				.000	.000
N of Valid Cases	180				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 33.00.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Type (C / P)	3.286	1.733	6.230
For cohort T = N	2.143	1.397	3.287
For cohort T = Y	.652	.515	.826
N of Valid Cases	180		

8.2. Ethic Committee Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2339 **14/04/2022**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Baker Alsheikh)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu Tıp Fakültesi Kadın Hastalıkları ve Doğum AD.Prof. Dr. Rukset Attar'ın sorumlu araştırmacı olduğu, **"GPX1 Gen Polimorfizimi İle Yumurtalık Kanseri Arasındaki İlişki"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2387)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **13.04.2022** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No: 1592)**

Prof. Dr. Turgay ÇELİK

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