T.C
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCE
DEPARTMENT OF MOLECULAR MEDICINE

DETERMINATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) POLYMORPHISM IN PATIENT WITH PROSTATE CANCER

Master Thesis

Gülce YAVAŞ

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Assoc. Prof. Gülsüm Seda GÜLEÇ YILMAZ

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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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DECLARATION

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

1.12.2021

Gülce YAVAŞ

DEDICATION

To best version of me

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LIST OF SYMBOLS AND ABBREVIATIONS

µl:	Mikroliter
ADT:	Androgen Deprivation Therapy
BHT:	Butylatedhydroxy Toluene
BMI:	Body Mass Index
BRCA2:	Breast Cancer2
CHECK2:	Checkpoint kinase 2
CST®:	ChargeSwitch®
CT:	Computed Tomography
CYP17:	Cytochrome P450 17
DNA:	Deoxyribonucleic Acid
DRE:	Digital Rectal Examination
ds-DNA:	double-strand Deoxyribonucleic Acid
EBV:	Epstein Barr Virus
EDTA:	Ethylenediaminetetraacetic Acid
EGF:	Epidermal Growth Factor
EGFR:	Epidermal Growth Factor Receptor
ELAC2:	Zinc Phosphodiesterase ELAC Protein 2
ErbB:	Epidermal Growth Factor Receptor Family
FISH:	Fluorescence in situ Hybridization
GDF15:	Growth Diferrentiation Factor 15
GLOBOCAN: The Global Cancer Observatory	
Her2:	Human Epidermal Growth Factor Receptor2
HHPV-8:	Human Herpes Virus 8
HPV:	Human Papilloma Virus
HTLV-I:	Human T Lymphotropic Viruses I
HTLV-II	Human T Lymphotropic Viruses II
IARC:	International Agency for Research on Cancer
kD:	kilo Dalton

mg:	Miligram
mL:	mililiter
MRI:	Magnetic Resonance Imaging
MSR1:	Macrophage Scavenger Receptor 1
ng:	nanogram
nm:	nanometer
OD:	Optical Density
OGG1:	Oxoguanine glycosylase1
PC:	Prostate Cancer
PCR:	Polymerase Chain Reaction
PET:	Positron Emission Tomography
pH:	Power of Hydrogen
PON1:	Paraoxonase 1
PSA:	Prostate Spesific Antigen
RNaseL:	2-5A-dependent ribunuclease
RTK:	Receptor Tyrosine Kinase
SNP:	Single Nucleotide Polymorphism
ss-DNA:	single-strand Deoxyribonucleic Acid
TPA:	Tetradecanoylphorbol Acetate
USA:	United States of America
UV:	Ultraviolet
WHO:	World Health Organisation

ABSTRACT

Yavas, G. (2021). Determination of Epidermal Growth Factor Receptor (EGFR) Polimorphism In Patient With Prostate CancerYeditepe University, Institute of Health Science, Department of Molecular Medicine MSc thesis, İstanbul.

Cancer isassociated with increased mortality ratioworldvide while, prostate cancer (PC),possess the most prevalentdeath ratio among male population. The metastatic form of PC is the second leading cause of cancer-related death. Epidermal Growth Factor (EGF) receptor (EGFR), is one of a member of ErbB family of receptor tyrosine kinases (RTKs) that encodes 170 kD glycoprotein. Studies have revealed that single nucleotide polymorphisms (SNPs) occurring in the EGFR gene cause different types of carcinomas, such as prostate adenocarcinomas and gliomas [1]. For better understanding of how EGFR polymorphism lead to cancer development, case-control study has been conducted in Turkish male population with 61 patient and 62 healty individuals. Genotype analysis was performed with Real-Time PCR method for both patient and control groups and statistical data were obtained from SPSS. Based on the eveluated results, CC homozygous genotype 24 (38.7%), CT heterozygous genotype 53.2(%33), TT homozygous genotype 5 (8.1%) has been detected in patient group. On the other hand, CC genotype 30 (49.2%), CT genotype 26 (42.6%) and TT genotype 5 (8.2%) has been recorded in control group. Consequently, there is a non-significant association between patient and control groups depend on genotype analysis (p=0.475). This study aims to detect EGFR rs1468727 SNP in patient with prostate cancer is Turkish population and improve new aspects to clinical trials.

Key words: Cancer, prostate cancer, EGFR

ÖZET

Yavas, G. (2021). Prostat Kanseri Hastalarında Epidermal Büyüme Faktörü Reseptörü Polimorfizminin İncelenmesi,Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Departmanı,Master Tezi, İstanbul.

Kanser, dünya çapında artan ölüm oranı ile ilişkilendirilirken, prostat kanseri (PC), erkekler arasında en yaygın maligniteye sahiptir. PC'nin metastatik formu, kansere bağlı ölümlerin ikinci nedenini olarak gösterilmektedir. Epidermal Büyüme Faktörü (EGF) reseptörü (EGFR), 170 kD'lık glikoproteini kodlayan ErbB reseptör tirozin kinaz (RTK) ailesinin bir üyesidir. Çalışmalar, EGFR genindeki tek nükleotid polimorfizmlerinin (SNP'ler), prostat adenokarsinomları ve gliomalar gibi farklı tipteki karsinomların ilerlemesine yol açtığını ortaya çıkarmıştır[1]. EGFR polimorfizminin kanser gelişimine nasıl yol açtığının daha iyi anlaşılması için Türk erkek populasyonunda 61 hasta ve 62 sağlıklı birey ile vaka kontrol çalışması yapılmıştır. Hem hasta hem de kontrol gruplarına Real-Time PCR yöntemi ile genotip analizi yapılmış ve SPSS'den istatistiksel veriler elde edilmiştir. Değerlendirilen sonuçlara göre hasta grubunda CC homozigot genotip 24 (%38.7), CT heterozigot genotip 53.2(%33), TT homozigot genotip 5 (%8.1) tespit edildi. Kontrol grubunda ise CC genotip 30 (%49.2), CT genotip 26 (%42.6) ve TT genotip 5 (%8.2) olarak kaydedildi. Sonuç olarak, genotip analizine bağlı olarak hasta ve hasta bireyleri içermeyen kontrol grupları arasında anlamlı bir ilişki ortaya konmadı (p=0.475). Bu çalışma, Türk populasyonunda prostat kanserli hastalarda EGFR rs1468727 SNP'yi saptamayı ve klinik araştırmalara yeni yönler kazandırmayı amaçlamaktadır.

Anahtar kelimeler: Kanser, prostat kanseri, EGFR

1. INTRODUCTION AND PURPOSE

Nowadays, cancer is releated with increased human mortality in all over the world. One of the most hazardous type of this disease, prostate cancer (PC),possesses the highest malignancy ratio among male population in worldvide. In particular, the metastatic form is shown as the second cause of cancer-related deaths. Several various types of remedies such as radiotheraphy, surgery and anti-androgen therapy (ADT) are frequently used inclinic in order to treat PC. Researchers could not have higlighted the molecular mechanism of prostate cancer progression completely, however, several findings indicated that various pathways including growth factor receptors have a crucial role in PC progression.

The ErbB family, also known as EGF receptor family composed of four members; epidermal growth factor receptor (EGFR) or with other word ErbB1/Her1, ErbB2/Her2, ErbB3/Her3, ErbB4/Her4, respectively. ErbB1 is the first discovered member of the ErbB family and the first to be found in the development of cancer.Because of that, ErbB1 has the most comprehensivelyinvestigated member compared with other Receptor Tyrosine Kinases (RTKs). ErbB1 is a 170-kd glycoprotein that consist of N-terminal extracellular ligand-binding domain, a single transmembrane helix, and a cytoplasmic part including a tyrosine kinase part followed by a C-terminal regulatory domain[1]. EGFR also acts in many biological events including cell proliferation, initiation of signals that controls the epithelial cells'behavior and solid malignanicies of epithelial cell origin. ErbB2, also known as Her2 and ErbB3 are closely associated with the EGFR/ErbB1, however, ErbB2 receptor is lack of ligands whereas, ErbB3 can not act as tyrosine kinase [2]. Several researchs estimated that overexpression of ErbB4/Her4 is linked with breast cancer [3] . All members of the ErbB family can form homodimers, heterodimers, and higher oligomers via a subset of growth factor ligands.[4].

Particularly, the EGFR or ErbB1/Her1 is overexpressed or mutated in progression of PC, thus, more aggressive clinical outcomes have to be found.

Pre-clinical researches have shown that the pathway of EGFR signallingmaytrigger the androgen receptor in androgen deprivation. In this thesis, it is aimed to determine the EGFR rs1468727 polimorphism in patient with prostate cancer to contribute the literature.

2. LITERATURE REWIEV

2.1 Cancer

Cancers have found in multicellular organisms for more than 200 million years. Researchers have been discovered the evidence of this disease in the ancestors of modern human which survived over a million years [5] .

One of the most considerable threat for human health, cancer, can be identified as abnormall and uncontrolled proliferation of various cell types in the body [6]. As a result of the different cell types, there are also many distinct cancer cells, that can be categorized by their behavior, property and feedback to treatment**.**

About one century before, in early 1920s, Otto Warbung highlighted that there are considerable differences between normal and cancer tissues in terms of metabolic process and features. He revealed that, even under aerobic conditions several types of cancers had the tendency to convert high level of glucose to lactate, defined as the Warbung effect [7].

There are several distinct factors act in the development of cancer, and they are mainly categorized as endogenous and exogenous [8]. Endogenous factors are composed of destruction of immune system, inflammation, age, physiological status, balance in endocrine system and hereditary inheritance [8].

The second subdivision, exogenous factorsincludes nutrition routine such as preperation and conservation of foods, lifestyle, physical compounds (e.g radiation), chemical substances (classified as synthetic and non-synthetic), socio-economic level and biological agents. According to current global cancer data, viruses cause approximately 15% of all cancer types worldwide. In humans, both DNA and RNA viruses have ability to cause cancer [9]. Hepatistis B and C viruses (related with liver diseses and primary hepatocellular carcinomas), Epstein Barr viruses (EBV) and Human Herpes Virus 8 (HHPV-8) (main reason for nasopharyngeal carcinomas and Burkitt’s

lymphoma), Human Papilloma Virus (HPV) (related with cervical cancer), Human T Lymphotropic Viruses I and II (HTLV-I and HTLV-II) (associated withadult T-cell leukemia) are the examples of biological agents which has ability to cause human cancer [10-13]. Besides, growth factors, some bacteria types (e.g *Helicobacter pylori*, responsible for gastric cancer) and parasites are associated with urinary bladder cancer[14].

Some unhealthy habits such as using too much alcohol, smoking, eating certain foods and their contamination with mycotoxins play an important role in cancer death rates [8].

Several research findings haverevealed that all cancer cells mainly have seven metabolic abilitiesgained during their progression: consistent proliferative signaling, activation of oncogenes, loss of apoptosis function, escaping from tumor supressors, becoming immortal, stimulating angiogenesis and triggering invasion and metastasis [15].

Moreover, later evidences have showed that reprogrammed energy metabolism and loss of immune-mediated disruption function have alsoessential role in cancer development [15].

Accumulated cancer incidence studies indicated that carcinogenesis shows differences between societies and those differences are related with several factors (lifestyle, daily routines etc). Furthermore, migration of populations leads to the development of certain cancer types in specific geographical regions [8].

In addition, spontenous genetic erros take place during the cell division. It is assumed that these genetic errors occur with a frequency of about $10^{-5}$to 10^{-6}, through change in nucleotides during cell division.Cancer development risk will increase, when the errors occur in gene which causes neoplasmic progression [8].

Neoplasia is classified as benign or malign according to its characteristics.The main properties of a malignant neoplasia are shown in Table2.1-1 [15]. Besidesmalign neoplasias, benign neoplasias increase much more slowly. Additionally, they have any effect on normal tissue funtion, unless they supress vital molecules[6]. The recent neoplasia studies lead to better understanding of carcinogenesis, however, it can not be sufficient to detect more minor variations such as molecular alterations [8].

Table 2.1-1. Malignant cell properties [8]

Neoplasic Cell	
1- Escaping apoptosis	5- Blockingangiogenesis
2- Invasion and metastasis	6- Expression of telomerase
3- Unlimited replication possibility	7- Self-competence in growth signals
4- Neoplasic diversification	8- Lack sensitivity to anti-growth signals

2.2 Stages Of Carcinogenesis

The result of accumulated *in vitro* animal experiments has established that neoplasia formation is a highly complex biological mechanism that may be divided into three main phases. These are initiation, promotion and progression, respectively [8].

In addition, alterations in structure of genome take place in these three stages. During promotion, many biologic processess take place such as generation of pre-neoplastic cell, variations in gene expression and selective proliferation of initiated cells. While intracellular apoptosis and cell proliferation sustain in a balance, they may begin to occur at different rates. During the progression phase, this balance changes and this causes an increase in malignancy. (Fig. 1) [8].

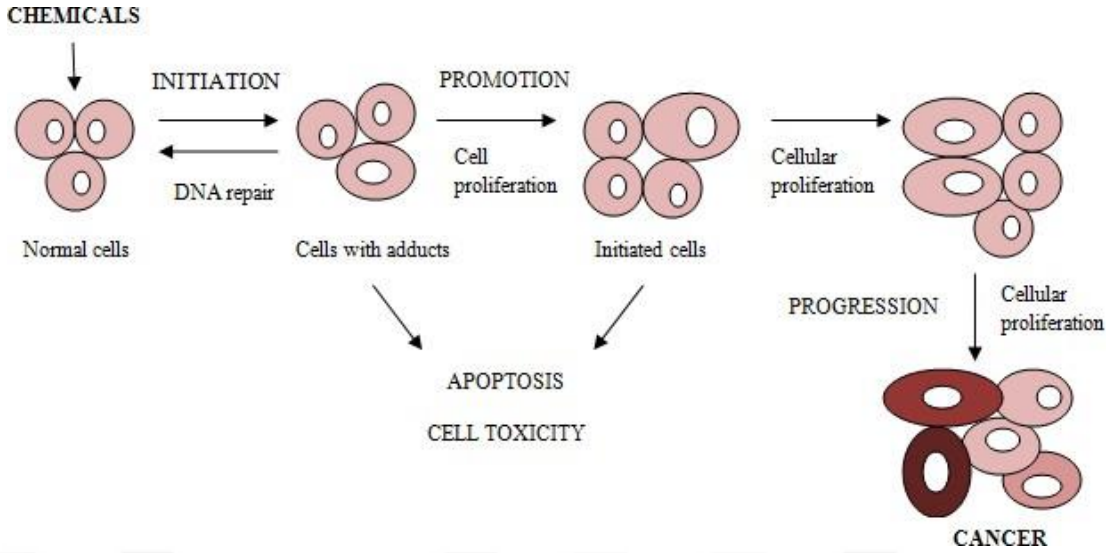

Fig. 2.1-1 Chemical carcinogenesis stages [8]

2.2.1 Molecular Characteristics of Initiation

Results from multiple experiments indicated that tumorigenesis leads to irreversible genetic modifications and that susceptible healthy cells become immortal and malignant[8]. Tumor supressor genes are transcriptionally silenced by DNA methylation of promoter regions of genes during initiation phase[16]. As a result of chemical carcinogens, genetic errors take place by altering the molecular structure of DNA that can give rise to mutation along DNA synthesis [8].

Consequently, mutation appears and causes proliferation, however, it does not induce the differentiation [8].

2.2.2 Molecular Characteristics of Promotion

The biological mechanism of promotion became clear when the association with low carcinognic activity and chemichal matters were figured out [17].

First of all, it has explained with epigenetic alterations, however, recent studies showed that promotion stage comprises genetic changes as well [8,15]. Moreover,

genotoxical and mutational processes are not essential for promotion stage and mitogenesis is the most significant biologic mechanism occured by promoters [18]. Promotion define as reversible phase and after the vanishing of promoters proliferation may take place by apoptosis. [19-21].Many chemically identified promoting compounds exert their effect throughout receptor molecules, some examples of this agents are represented in Table 2.2.2-1 .

Table 2.2.2-1 Some Promoter-Target Tissue Associations in Carcinogenesis

Promoting Agents	Target Tissue	Reference(s)
Polypeptide trophic hormones and growth factors (EGF, prolactin, glucagon)	Liver Skin Mammary gland	[22]
Tetradecanoylphorbol acetate (TPA)	Skin	[23]
Sex steroids (androgens and estrogens)	Liver, mammary tissue	[24]

In chemical carcinogenesis experiments with high use of all promoter compounds and with prolonged exposure, neoplasias were seen to form without an initiation stage. [18, 21, 25]. Sustain with phenobarbital, benzene, asbestos, TPA, butylatedhydroxy toluene (BHT), arsenic give rise to the progression of neoplasias even without exertion of initiation compounds [25, 26].There are two possible theories that can be used to explain this contradiction; firstly, the genotoxic effect cannot be detected by mutagenicity, and secondly, the automatic emergence of initiated cells.

Additionally, not all cells tend to be promoters, only cells that are induced to divide, undifferentiated, and do not maintain apoptosis can cause malignant neoplasia by destabilizing the mechanism of growth and natural cell death[25].

2.2.3 Molecular Characteristics of Progression

The lesions detected between initiation and promotion stage by using histopathological methods are defined a preneoplastics and/ or benign neoplasias [21]. The final step of carcinogenesis is the conversion into malign lesions and particularly it is noted as progression stage [27, 28]. Epigenetic and genetic pathways are required to obtain a neoplasic phenotype in stage of progression [29]. Without being any stimuli, cell proliferation undergoes during progression stage [21,30]. Growth faster, invasion, irreversibility, metastasis, genetic instability and alterations in the metabolical, biochemical properties of cells are the main characteristics of progression [18, 21, 27, 31, 32, 33]. Furthermore, angiogenesis occured by epigenetic mechanisms is fundemental for progression of neoplasias.

Consequently, there are several reasons which have fundemental role in the formation of carcinogenesis.

2.3 Causes of Cancer

Anything can lead to normal healthy cell to transform malignant cancer cell. Several reasons may perform to generate abnormalities in cells and have been associated to development of cancer. Researchers have not higlighted the reasons of some cancer types, besides that, other cancers are affected by environmental or chemichal substances or derived from one or more than one established reason. Furthermore, genetic tendency has a key role in development of cell abnormalities that causes cancer. In some cases, cancer take place because of the combination of many factors in human [34].

Experimental animal studies show that carcinogens which lead to cancer progression have been determined in humans. Since the mechanism of malignancy is referred as complicated and multistage process, there are several factors that account for

the development of cancer and it would be quite simple to mention that cancer development arise from not only one cause[6].

Ionizing radiation and many other chemichal carcinogens perform by damaging deoxyribonucleic acid (DNA), thus, mutation appears in cells. Since the stimulation of mutation in main target genes are thought to be the pioneer case that give rise to the progression of cancer, those carcinogenes are mostly termed as initiating agents. Solar ultraviolet (UV) radiation, smoking and aflotoxin which is a potent carcinogenparticularly for liver are counted as initiating agents and they are related with the cancer development [6].

Another types of carcinogens cause to progression of cancer by inducing cell proliferation instead of initiating mutations. Those substances are called tumor promoters, that induce to cell proliferation during the early stages of tumor progression [35].

Moreover, biologic agents such as hormones are also one of major reason of cell abnormalities. Particularly estrogens perform in progression of cancer as tumor promoter. It can be given as an well-known example that proliferation of uterine endometrium cells are induced by estrogen and excess amount of estrogen dramatically rises the risk of endometrial cancer in woman [36].

Recent evidences show that, another risk factors have been also foundin order to increase prostate cancer risk. Especially beef, obesity, lack of exercise, chronic inflammation, processed meats, stress and red meat are among the important causes that contribute to the progression of cancer [37].

2.4 Types Of Cancer

Presence of cancer can be the consequence of abnormall proliferation of different kind of cells. In this case, the most significant point in pathology of cancer is the difference between malignant and benign tumors. A benign tumor such as fibromas, adenomas and lipomas continues bound to its original zone, however, it does not perform to invade adjacent normal tissue and these tumors do not have any tendency to spread other different body parts unlike malignant tumors. On the other hand, the malignant tumors are both able to invade surrounding other normal tissues and spread to distant part of body by using lymphatic systems called metastasis. In order to diagnose tumors as cancer, cells must be classified as malignant. Although benign tumors are generally abducted from body by using surgical procedures, the gaining of metastasize property becomes them more resistant to the medical therapies [38].

Moreover, both malign and benign tumors are categorized based on the cell type from that they take place. Generally cancer types are seperated into three main sub-groups: sarcomas, leukemias or lymphomas and carcinomas. Additionally, there are other different ways to distinguish tumors such as determination of the tissue origin and cell types (e.g. adenocarcinomas arise from adenomas) [6] .

According to the currentvalues of World Health Organisation (WHO), it has been given below a table composed of number of new cases in 2020 for both sexes and all ages in Turkey (Table 2.4-1).

Table 4-1: Number of new cases and deaths, both sexes and all ages in Turkey, 2020[WHO]

Cancer Site	Number of New Cases	Number of Deaths
Lung	41 264 (17.6%)	37 070
Breast	24 175 (10.3%)	7 161
Colorectum	21 191 (9.1%)	10 723
Prostate	19 444 (8.3%)	5 464
Thyroid	13 682 (5.9%)	795
Other cancers	114 078 (48.8%)	65 22

2.5 Noval Treatment Of Cancer

Cancer represents several different symptoms derived from localization, size and growth pattern of tumor. Although some patients have more awareness than others, there are incredible variations in diagnosis durations of cancer.Recently, imaging and biomarkers are frequently used in cancer diagnosis to provide the most appropriate treatment to patients. There is a marked increase in the use of computed tomography (CT) and other diagnose tool called magnetic resonance imaging (MRI) to achieve great results on detailed tumor structure and cancer anatomy. Besides these methods, there is an another tool using in cancer treatment named as positron emission tomography (PET). In this method, substances are marked with a radioactive molecule and it is an obviously beneficial way toprovide the detection of whole body biochemistry [39].

Biomarkers, in other words biologic alterations are created by the existence of tumor progression. They can be synthesized directly by cancer cells (e.g prostat spesific antigen, PSA) as well as exhibit complex alterations in higher organ systems. Cancer patients are more advantageous than others if the disease is in the initial stage, but the most appropriate treatment should be started as soon as possible according to the condition, size and type of the tumor. Treatment of cancer requires the collaboration of many different study fields following that pharmacology, oncology and molecular biology. The treatments used against cancer are shown in Table 2.5-1 below and a brief explanation is given about them[40].

Table 2.5-1 Main cancer therapies and their functions

Treatment	Explanation
1- Surgery	To abduct cancer or as much of cancer as possible.
2- Hormone Therapy	Cancer can be cured by blocking some hazardous effects of many hormones, best-known exmples are used in breast and prostate cancer
3- Targeted Drug Therapy	Focuses on specific variations
4- Bone marrow transplant	Chemotherapy, adittionally reffered as stem cell transplant
5- Clinical Trials	New approaches to explore new procedures to fight with cancer
6- Chemotherapy	Using the combination of different drugs to kill cancer cells
7- Immunotherapy	Usage of patients's immune system and its members to fight with cancer
8- Cryoablation	Eliminate cancer cells by using cold, reperated more than one time
9- Radiofrequency ablation	Leading to cancer cells to die by heating
10- Radiation Therapy	Destroying cancer cells by usage of high-powered energy beams

Recent cancer investigations reported that molecular biology also may be used as a tool for decreasing the hazardous effects of treatments and provide direct therapy to specific targets [41].

The existence of different types of cancer in humans was mentioned before, and following that information about prostate cancer will be given.

2.6 Prostate Cancer

2.6.1 General Information About Prostate Cancer

Prostate cancer (PC), the fifth cause of death worldwide, is one of the most prevalent cancer diagnoses in men. [42-43]. PC is the type of cancer with the highest incidence after lung cancer in the world. [42]. According to WHO's current values in 2020, 19 444 people were diagnosed with prostate cancer and 13,980 survived in Turkey [44]. In developed countries, prostate cancer constitutes %33 of cancers among men [45]. The incidence of prostate cancer differs from country to country, with the highest rates occurring in developed countries such as the United States, Scandinavia, and Canada, while China and other Asian countries have the lowest rates.These differences are derived from several factors, genetic tendency, exposure to environmental risk factors, social life conditions, quality of health care systems or the combination of them can be given as the best examples [45]. Interestingly, prostate cancer has a lower incidence in white men than in African-American men.The main reasons of this inequility have been assumed to variations depending on genetic susceptibility, social or external factors [42].

Symptoms of prostate cancer mostly may not appear at the early stages, remains its progression indolently, thus, treatment can not be necesary or less may be sufficient. However, patients with prostate cancer often face with frequent urination and nocturia as a result of prostatic hypertrophy.In the advanced stages of prostate cancer, patients complain of urinary retention and back pain. Also, another dangerous feature of prostate cancer is that the axis skeleton is the body part most common to metastasize to bone.

In general, the diagnosis of prostate cancer is made by the plasmatic level of prostate specific antigen (PSA, glycoprotein > 4 ng/mL) [42]. After the application of PSA, the incidence of the prostate cancer undeniably rised [45]. Normally, prostate tissue synthesize PSA. Tissue biopsy is widely used in medicine to confirm the presence of cancer, because PSA can also be found in patients without cancer, which can lead to some diagnostic errors.

In addition, androgen deprivation therapy (ADT), radiotherapy, chemotherapy, ablative treatments, and immunotherapy are classified as non-surgical methods used to treat patients with PC. Depending on the patient's history, these treatments can be used alone or in combination [46].

In the generation and progression stages of prostate cancer, nutritional habits physical exercies and adopting no stationary lifestyle has a crucial role.In particular, dietary differences are associated with ethnicity in the observed worldwide incidence differences of PC [42].

2.6.2 Epidemiology

It has been rarely observed that people under than 50 years old are suffered from prostate cancer (< 0-1% of all patients). Approximately people over 65 years constitue the %85 of patients while the average age of this disorder between 72 to 74 years. The total risk for patients with prostate cancer at 85 years ranks between 0.5% to %20 all over the world (statistics are obtained from Cancer statistics in Sweden 1999 and 2001) [47].

The incidence rate of disease varies across the ethnic groups and regions.

According to the GLOBOCAN's 2020 data, the frequency of prostate cancer differs from one ethnic origin to another shown in Figure 2.6.2-1.

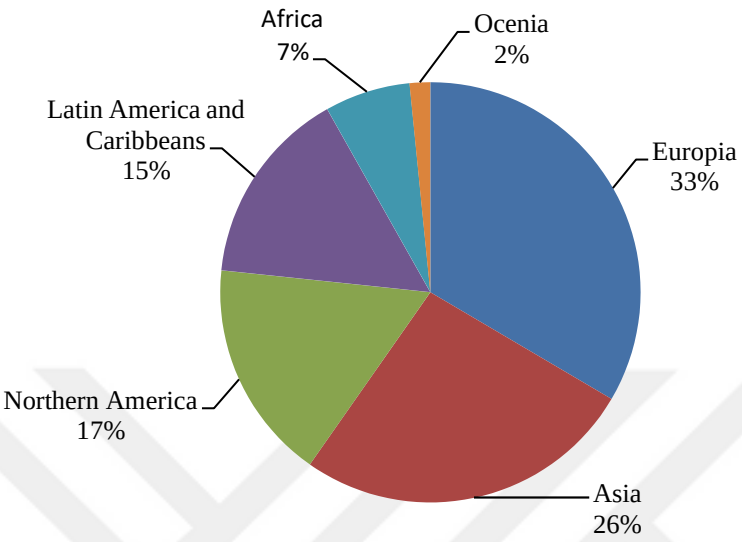

Figure 2.6.2-1 Incidence of prostate cancer across main regions in the worldvide

Additionally, incidences of prostate cancer shows differences across ages.According to noval researches, prostate cancer is rare in men under 50 ages, however, the incidence rate is steadily increasing between the ages of 50 and 59[48]. Approximately 60% incidence rate is constitued by men over the ages of 65 depending on 2016 National Cancer Institue statistics.

2.6.2.1 Mortality

Mortality rates show obvious regional variations, as do prostate cancer incidence.Central America had the highest mortality ratios in patient with prostate cancer at 10.7 per 100,000 people reported in 2018. After that, Central America is followed by other developed countries; Australia, New Zealand and Western Europe, respectively (Figure 2.6.2.1-1).

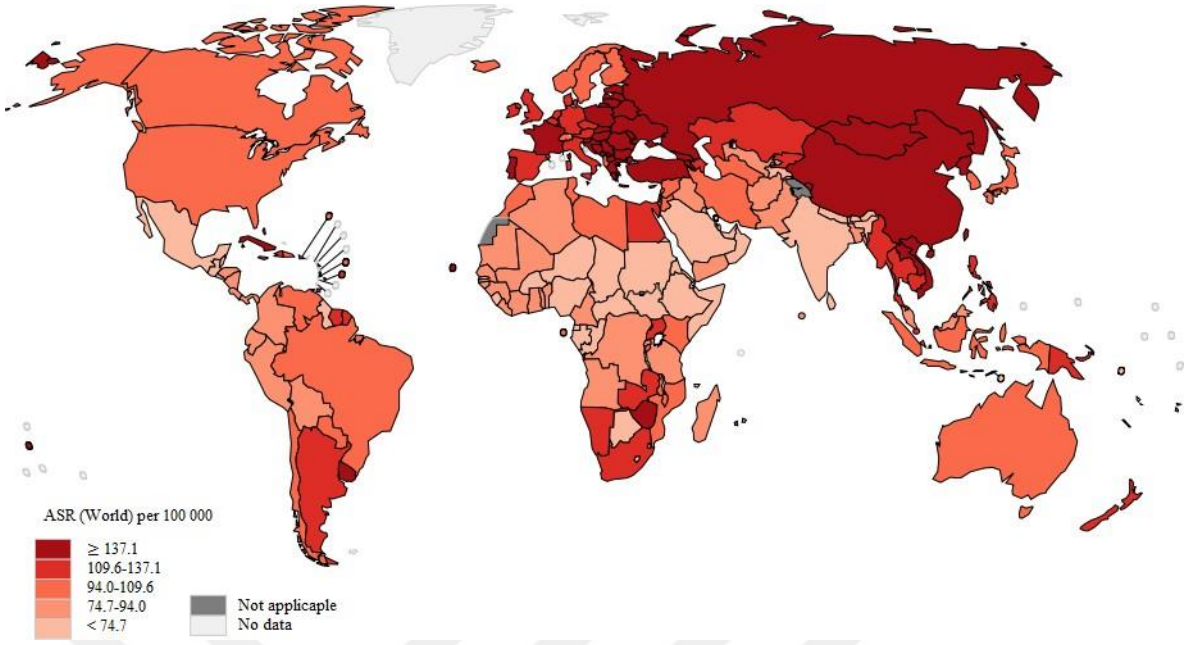

Figure 2.6.2.1-1. Age-standardized all ages mortality ratios in patient with prostate cancer in 2020 worldwide, produced by gco.iarc.fr. Data achieved from GLOBOCAN 2020.

By using PSA screening method, mortality rate decreased in patients with prostate cancer in men aged 55-69 has been noted by USA Preventive Task Force (USPSTF, 2018) [49].

Besides, prostate cancer data for men more than 70 ages are still high [50] .Among all races, Afro-American men are placed at the top of the mortality rate pyramide from prostate cancer. It is not only associated with that Afro-American men can have many particular genes which tend to undergo mutations in order to generate prostate cancer, however generally that those alterations are related with a more invader kind of malignancy [42].

2.6.2.2 Survival

The survival rate can differ from region to region. While it is around 76% in Eastern countries, it is around 88% in Southern and Central European countries.Because of prostate cancer awareness with common use of PSA, survival is increasing in all European countries[43].

2.6.3 Risk Factors of Prostate Cancer

There are many different types of established risk factors which perform to increase the existance of prostate cancer in human body are represented below Table 2.6.3-1.

Table 2.6.3-1 Risk Factors of Prostate Cancer

Risk Factors	
1- Age	5- Androgens
2- Ethnicity	6- Diet and Exercise
3- Familial and genetic risk	7- Lifestyle
4- Infection and Inflammation	

2.6.3.1 Age

The risk to have prostate cancer is considerable higher in men after 50 ages compared to younger and they have lower survival ratio. Based on this evidence, medical doctors consider the most appropriate treatment according to the age of patients. In other words, age performs to detect treatment type [52].

Men under the ages of 50, have low risk to diagnosed with prostate cancer, this constitues less than 0.1% of all cases. Mostly prostate cancer is detected in 65 years old men, and this group accounts for approximately 85% of all affected patients.All over the world, the risk of developing prostate cancer in 85 years old men varies between 0.5% and 20%. [53]. Besides, microscopic prostate cancer particles were detected in 30% of

men in their forties, 50% of men in their fifties, and more than 75% of men in their eighties in autopsy examinations [54].Due to the significant effectof age on prostate cancer development, PSA screening and digital rectal examination (DRE) are highly recommended, especially for men after the age of 50 [52,55**]**. Prostate cancer researches reported that only one PSA measurement between the ages of 44 to 50 years has capability to predict prostate cancer untill 25 years after current date [56].

Men aged 75 years have a lower risk of developing prostate cancer with a PSA level as low as 0.5 ng/ml compared to men of other ages. On the other hand, patients with a PSA level of 0.5 to 1.0 ng/ml have a two-and-a-half times greater risk of prostate cancer than men with PSA level of up to 0.5 ng/ml. Furthermore, men who have between 2.0 to 3.0 ng/ml PSA level has more than 19 times risk compared to men who have up to 0.5 ng/ml PSA to develop prostate cancer [57].

2.6.3.2 Ethnicity

Prostate cancer incidence shows variations between different ethnicities and regions. Asia has the minimum ratio of prostate cancer incidence, particularlyin China, Japan and India. On the other hand, while the USA has 161 new recorded patients per 100,000 population in a year, African-Americans have the maximum incidence of PC with 272 new recordedpatients per 100,000 population [58]. Researches on immigration revealed that when people originally Japanese emigrate to the USA, their PC risk increase dramatically. This evidence higlight that unclear external risk factors also have crucial role to the progresion of prostate cancer [52].

2.6.3.3 Familial And Genetic Risk

One of the most well-known and researched risk factors for prostate cancer is family history[59]. The Health Professional Follow-up Study conducted on 3,695 prostate cancer cases between 1986 and 2004 revealed a 2.3-fold greater risk in patients with brother's history of prostate cancer (95% confidence interval (CI) = 1.76–3.12).Moreover, the same research showed that the risk was 1.95 to 2.16 times higher

for those who had a brother or father diagnosed with PC at 60 ages or before 60 ages. [60].The relationship between familial PC and the risk of its occurrence is shown in Table 2.6.3.3-1

Table 2.6.3.3-1 Association Between Relative and Absolute Risks and Familial History [61]

Familial History	Relative Risk	Absoulte Risk (%)
No history	1	8
Brother or Father	2	15
Brother or Father being patient under the age of 60	3	20
Both father and brother	4	30
Hereditary PC	5	35-45

Sporadic, familial and hereditary are the main phenotypes of prostate cancer.If one or more first-degree relatives of a person diagnosed with PC have been diagnosed with PC, it is called familial type of prostate cancer. One sub-group of familial prostate cancer, hereditary prostate cancer points a pattern which is coherent with Mendelian inheritence [62]. Sporadic prostate cancer phenotype is approximately constitue 85% of all prostate cancers, besides hereditary type is account for nearby 43% of at all early age disese(early age refers at 55 years or younger than 55 years) [63].

Using complex segregation analysis, many different types of susceptibility genes responsible for prostate cancer, which show a dominant pattern in inheritance, have been identified[64].

RNASEL was the first gene locus discovered using genome-wide screening methods in patients with familial PC that encodes an enzyme called 2-5A-dependent ribonuclease (RNaseL). It is responsible for regulating the biological impacts of interferons, especially those that respond to certain viral infections.

There are also another candidate genes were determined such as*ELAC2*, *MSR1*, *OGG1*, *CHEK2*, *BRCA2*, *PON1*, and *GDF15*. However, mutations that occur in many of these loci are associated with a low frequency of prostate cancer risk.

Furthermore, some of those genes encode proteins which play a crucial role in defense system against oxidative stres and infection. Existance of structural damages in these proteins may causeincreased tendency in cancer development [65].

Although scientists detected other different several genetic variants that responsible for PC, association among these variants and aggresive behavior of this disease is stil not fully highlighted yet[66].

In addition, hypermethylation of cytosine residues in DNA at promoter CpG islands perform in prostate cancer development. In other word, hypermethylation at multiple locus are related with pathological and clinical properties of prostate carcinogenesis [67].

Current discoveries revealed that there are fusion oncogenes (*TMPRSS2–ERG* and *TMPRSS2–ETV)* perform in progression of PC. With investigation of those fusion oncogenes, its role in this disease has been confirmed and can be used as diagnostic tool. In addition, they can be performed in treatment stages of prostate cancer in the future [68]. *TMPRSS2-ERG* fusion leads to more higher stages of prostate cancer and metastasis to lymph nodes in comparison with patients in absence of the gene [69]. Several investigations about fusion oncogenes are stil maintained in order to use them as biomarkerto detect prostate cancer in early stages [70].

2.6.3.4 Infection and Inflammation

With the highlight of the molecular and genetic knowledges, researchers have found that hyperproliferation and inflammation (particularly infection-associated) lead to the generation of several distinct cancer types such as colon and bladder [71]. The prostate is exposed to agents that cause inflammation through sexual activity and urine. Based on this information, the hypothesis is that those agents can trigger inflammatory mechanishms and cause to malignancy in the prostate.

Despite several epidemiological studies about association between prostate cancer and inflammation, there is still inconsistencies, however strong evidences indicates that infectious agents can cause the PC pathogenesis.

Moreover, there is a strong relationship between the well-known susceptibility genes responsible for prostate cancer and the defense mechanism that combats oxidative damage and inflammation. The existance of any functional damage of these genes can give rise to disruption in prevention of tumor formation. The protein products of these genes have many different functions shown in Table 2.6.3.4-1.

Table 2.6.3.4-1 Risk FactorGenes For Prostate Cancer

Gene	Chromosome	Function of Protein
ELAC2	17p11	Generating 3' termini of tRNAs
RNASEL	1q24-25	Innate immunity against viruses and other pathogens
MSR1	8p22-23	Homeostasis by clearing modified lipids and proteins
OGG1	3p26.2	Base exicision repair
CHEK2	22q12.1	Regulates cell divison and controlls check points
BRCA2	13q12.3	DNA repair and tumor supressor
PON1	7q21.3	Antioxidant and anti-inflammatory
GDF15	17p13	Growth and differentiation, still not completely clear
TMPRSS2-ERG	21q22.3; 21q22.2	Fusion oncogene
TMPRSS2-ETV1	21q22.2; 7p21.2	Fusion oncogene

2.6.3.5 Androgens

It has been proved that androgens have a vital role in maturation, protection anddevelopment of prostate. In addition, it performs in proliferation and differentiation processes in the luminal epithelium. Several studies have indicated that there are two major androgen types in adult men named as testosterone and dihydrotestosterone. Testosterone is categorized as the main circulating type of androgen in adult men, however dihydrotestosterone is basicly seen in tissues. Principally in peripheral tissues (e.g prostate gland and skin) dihydrotestosterone is produced from testeosterone by the catalitic activity of the 5α reductase isoenzymes type 1 located in mostly hair and skin and type 2 found in the genital skin and prostate [72].Androgen receptor proteins contribute to maintain activity of androgen in prostate cells.These proteins connect with DNA for triggering DNA synthesis and transcription of genes that able to remain the cellular proliferation process.

There are numerous studies involving the function of serum androgens in prostate malignancies. Besides these numerous studies only one research has indicated that the presence of excess testosterone serum levels increases the risk of PC progression [72]. Excess plasma levels of testosterone and lower amount of globulin (sex-hormone binding type) have been seen as great risk for progression of prostate cancer under the condition of both marker's existance simultenously. Interestingly, epidemiological studies revealed no differences in circulating hormone levels between men who produce PC and live without it, using eight prospective meta-analyzes [73]. Moreover, current meta-analysis results obtained from all over the world provedanyassociation with the risk of PC and sex hormone levels containing total amount of androstenediol, free estradiol, estradiol, dihydrotestosterone, testosterone,free testosterone anddehydroepiandosterone[74]. In other words, the obtained data mostly do not directlyprovide better-understandingandrogen role in progression ofPC. However, researchers do not completely prove anassociation between androgen-exposure situation and generation of PC.

Recent findings indicate that polymorphism in the SRD5A2 gene, which encodes the type 2 isozyme of 5α reductase, is associated with high-grade prostate cancer and poor prognosis.On the other hand, emergence of substitution at codon 49 (A49T) lead to the five-fold better enzymatic activity, besides it has been linked with poor prognosis [75]. Noval studies obtained from meta analysis indicates that variant allele of SRD5A2 gene (49T) is not directly associated with the risk of PC[76].

In addition, another type of SRD5A2 polymorphism that occurs at codon 89 (V89L) is related with aggressive clinical consequences of this disease, such as increased PSA level and disease stage, and is seen in men who experience the first stages of the disease [77].

Current evidence has proven that mutant genes play a role in the anabolism and catabolism mechanism of testosterone and thus are involved in progression of PC. Cytochrome P450 17 (CYP17) is responsible for the two main steps of testosterone synthesis. In addition, recent findings have demonstrated an increased risk of prostate

cancer with the CYP17A1 polymorphism [78-79].Also, prostate cancer patients who carry at least one SRD5A2 89L allele are at extremely high risk[80].

2.6.3.6 Diet and Exercise

It has been emphasized that dietary components and nutritional habits may cause an increase in the risk of PC [81].

Mortality and incidence of PC correlate with the average fat consumption such as unsaturated fats worldwide [81]. Insuline resistance and inflammation have a significant role in the carcinogenesis mechanisms appeared by obesity. Insulin resistance-associated oxidative stres, circulating inflammatory-linked factors (e.g leptin, tumor necrosis factor and interleukin 6, contribute to tumor growth) are rised during the progression of prostate cancer [82-83]. Based on *in vivo* and *in vitro* experimental results, a correlation was indicated between the reduction in consumption of fat and the absence of proliferative prostate malignancies [84-86].

Although obesity is recognized as one of the most common risk factors for PC, data on the correlation between obesity and tumor growth are still inconsistent. It is categorized as risk factor in aggressive PC cases due to changes in circulating metabolic and sex hormone levels[87]. Additionally, the only explanation for the low risk of obese men for prostate cancer is the complexity and difficulties in diagnostic processes [88,89].

Eventually, obese men have lower possibility to possess an elevated PSA, biopsy ratio is lower and because of that diagnose of prostate cancer is lower. Furthermore, obese men have a larger prostate compared with normal-weight men, thus, there is much more difficultyin detecting cancer for biopsy [89]. Clinicians apply Body Mass Index (BMI) in order to eveluate the concentration of PSA because obesity is a major risk and it causes a lower diagnose of PC. Application of BMI as like other methods which are recently included (race, ethnicity, origin, familial history) is used as risk calculator for prostate cancer detection, also it enhances the diagnosis performance [90].

In addition, high uptake of red meat is also known as a significant risk for the progression of PC[91,92].With other words, red meat has been counted as a risk for PC due to the high-temperature preparation process that leads to the production of carcinogenic agents such as heterocyclic amines.

Numerous researches have indicated a preservative function of vitamin D against the progression of prostate tumors. Depending on the experimental studies, vitamin D blocks cell growth in prostate cancer by stimulating apoptosis mechanism, inhibiting the cell-cycle process and decreasing the metastatic activity [93].

2.6.3.7 Lifestyle

Based on International Agency for Research on Cancer (IARC) findings carcinogenes are categorized depending on the degree of hazard, and tobacco products include over 4,000 chemical substances that over 60 of them are categorized as class 1 or class 2 carcinogenes [94].

Smoking also has an impact on hormonal alterations in human body. Smoker men were detected to possess increased levels of circulating testosterone and androsterone. This finding demonstrated that smoking contributes to progression of prostate cancer and increases the risk [94-96].

Many studies have revealed that alcohol consumption is another important factor affecting prostate cancer as in other types of cancer.Alcohol consumption and its continuous use increase the risk of occurrence for all types of cancer, including prostate [97]. It has been proved that alcohol consumption has an effect on polymorphisms in genes encoding enzymes such as alcohol dehydrogenase for ethanol metabolism, folate metabolism, and the DNA repair system [98].

In addition, sexual activity exert infectious agents to prostate. It is stated that the risk of prostate cancer is high especially in men who have many and different sexual

partners and are sexually active from an early age.Despite all these findings, results are still not consistent among other studies [99,100].

2.6.4 Diagnosis and Treatment

Prostate cancer has been seen commonly all over the world.Although it is an indolent disease, the third cause of male death worldvide.Early-stage diagnosis and proper treatment for patients have a vital role in their survival without cancer.

First, tissues are obtained by needle biopsy for the diagnosis of prostate cancer, then microscopic examination is performed on the obtained tissues. The prostate biopsy is maintained by the usage of transrectal untrasound to acquire between 10 to 12 samples of prostate tissue in a grid-like design. The tissue samples are examined by a pathalogist, then the pathologist extracts a primary Gleason grade for the detection of pre-dominant histological pattern. The secondary Gleason grade is then determined and both degrees should be between 1 and 5 depending on the structure of the microscope and the presence of cells.Clinicians define prostate cancer as low, intermediate, and high risk, and this diagnosis is made based on the outcome of Gleason patterns, circulating PSA levels, and disease stage. Because of the presence of heterogeneity in each risk group, much more specific and seperator insturments have been improved and confirmed [101]. The Comprehensive Cancer Network risk classification is an example of a 5-layer system where groups are divided into low and high riskconfirmed [101].

Many different types of new biomarkers have been found, such asConfirmMDx, 4Kscore, prostate cancer antigen 3 test, Prostate Health Index,which contribute to detecting possible negative outcomes.

Testing for serum PSA variants is available to determine the probability of prostate cancer in patients with a previous negative biopsy [37,102].On the other hand, there is an epigenetic assay exerted to prostate biopsy tissue, which exhibits similar discrimination ability and can measure DNA methylation [103].

In addition, new imaging tools are introduced to improve diagnostic performance.One of these imaging tools is magnetic resonance imaging, briefly called MRI is generallyused in clinics in order to detect PC in early stages[104].

It has a vital role to apply an appropriate treatment followed by diagnosis of prostate cancer. Treatment of this disease can be divided into two main subgroups, depending on whether it is localized or metastatic.

If the patient has a localized type of prostate cancer, in other words, is not showing a visible lymph node or metastatic behavior, they can choose one of two main treatment options: surgery and radiation therapy.

The surgery and radiation are counted as a powerful treatments for patients who have more advanced prostate cancer (e.g. if the patient has more than 10 ng/mL PSA level and nodules are visible obtained by digital rectal examination). Radiation theraphy has technologic advantages among other therapy methods. Similar with surgery, intensity-modulated radiation therapy has generally substituted another type of treatment called 3-D conformal radiation. While both cross-sectional imaging and computerized software are applied in planning methods, intensity modulated radiation therapy emits inhomogeneous radiation beams suitable for not regularly shaped organs.Therefore, it reduces the radiation transmission to the surrounding tissues and the subsequent toxicity that may occur in the urine and intestines[105, 106].

Another major type of PC treatment, androgen deprivation theraphy (ADT) is applied to metastatic prostate cancer patients. Unfourtunately, researchers have proven there is an undeniable associationamongADT and toxicity. Some metabolic changes, sexual failure, hot flashes, heart failure, and cognitive impairment have been noted, as well as proven adverse effects such as decreased bone mineral density [107,108]. A meta-analysis result showed that here is no association between ADT and increased mortality from cardiovascular failures, while the result of a post hoc analysis claimed that heart failure can be present in patients with pre-existing heart problems [107,109].

Based on all these concerns about usage of ADT, intermittent type ADT was found and became more prevelant. A meta-analyis declerad a link between the intermittent ADT and non-inferiority, compared with continuous ADT and progression of prostate cancer, cancer-related survival and all survival [110].

Furthermore, multimodal theraphy and precision medicine have a vital role particularly in future advances to treat metastatic form of PC [111].

In summary, developments in PC diagnosis and treatment provide advantages into classifying patients according to risk and allowing medical doctors to suggest treatment according to the prognosis of the cancer and the patient's choice. Initial treatment combined with chemotherapy increases the survival rate compared to ADT. In addition, abiraterone, enzalutamide, and other chemical carcinogens may help to the progression of metastatic PC which is resistant to homone therapy.

2.7 Epidermal Growth Factor Receptor

First of all, it has been demonstrated that growth factor has a crucial role in several biologic mechanisms for instance development, growth and many others. They are essential for several biologic mechanisms such as cell-cell communications, fate determination, cell homeostasis, apoptosis, migration of cells and specilization. The main function of growth factor receptors is to transmit signals directly to the nucleus through stimulation of messengers or receptor translocation.

ErbB family of receptor tyrosine kinases are composed of four sub-group; epidermal growth factor (EGF) receptors (EGFR), additionally named as ErbB-1 or HER1, HER2/neu (ErbB2), HER3 (ErbB3) and HER4 (ErbB4), respectively. Among other subgroups, EGFR is one of the most comprehensivelyexamined members of the ErbB family in cancer progression.Those receptors are also categorized as single-chain transmembrane glycoproteins which composed of an extracelullar ligand-binding ectodomain, a transmembrane domain, a short juxtamembrane section, lastly a tyrosine kinase domain and C-terminal tail including tyrosine (Figure 2.7-1).

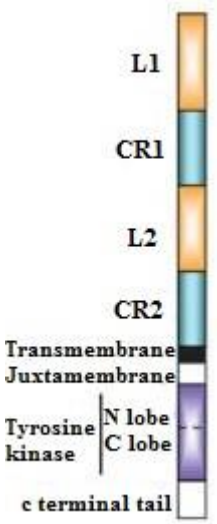

Figure 2.7-1. Linear schema of ErbB receptor domains. An ecto N terminal domain includes four different subdomains. L1 and L2 (leucine rich subdomains) connect with ligand directly. CR1 (cysteine-rich subdomain) includes the dimerization loop which has role in receptor-receptor connection. A short transmembrane and a juxtamembrane domain interact with ectodomain to divided into two lobes; tyrosine kinase domain and the C terminal tail.

Considerable amount of N-linked oligosaccharide and a single polypeptide chain of 1186 amino acid residues built a mature EGFRform. The ligand binding ecto domain is separated from a cytoplasmic domain responsible for encoding an EGF-regulated tyrosine kinases by a single hydrophobic membrane sequence [112-114]. The most conspicuous property of the receptor's cytoplasmic protein is sequencing of the tyrosine kinase domain.On the other hand, the ligand-binding ectodomain is less conserved among the other four receptors, so it has proven to have distinct properties for ligand binding [115].

Hemodimerization or heterodimerization which have a crucial role in tyrosine kinase activation are stimulated by ligand binding. In other word, the ligand binding promotes dimerization of receptors.Stimulation of tyrosine kinase is fundemantal and constitue the first step in the transduction pathway of EGF signal [116].

First of all, the ligand binds to the appropriate receptor. After binding, it triggers several signal pathways [117-120].

Much evidence indicates that the development and pathogenesis of different types of carcinoma involves EGFR.In human carcinomas, EGFR and other EGF-like peptides are overexpressed. Based on *in vivo* and *in vitro* studies, these protein compounds stimulate the transformation of cells. Thus, cases of cancer are diagnosed if a mutation occurs in the tyrosine kinase domain of Epidermal Growth Factor Receptor and also gene amplification ofEGFR is seen[121].

Activation of EGFR causes the progression and development of many different types of cancer through multiple complex pathways which are still not fully known.

Prostate cancer is frequently seen in male population and the members of ErbB family act in progression and development of it. Although the mechanisms by which members of the ErbB family contribute to disease progression are still not fully elucidated, the aberrant behavior of EGFR has been related with castration-resistant disease development, possibly due to defects in androgen signaling [122].

3. MATERIALS AND METHODS

3.1 Sample Selection and Definition

In this study, patients diagnosed with prostate cancer (n=61) and a control group (n=62) were examined. The patient group was consist of patients who were diagnosed by urology department, while a control group was composed of healthy individuals. Ethical approval was achieved from the Ethics Committee of Yeditepe University for this thesis work (Ethics committee decision no:)

3.1.1. Control group: The control group contained individuals age between 40 to 70 who were not diagnosed with prostate cancer by clinical studies.

3.1.2. Patient Group: The patient group included individuals obtained from Yeditepe University Hospital. This group consisted of individuals age between 40-70 who were suffer from prostate cancer.

3.2. Materials and Devices Used in the Experiment

3.2.1. Materials Used in DNA Isolation

DNA isolation was carried out from peripheric venous blood samples. Those samples were stored in tubes at +4°C till the experiment was started. EDTA (Ethylenediaminetetraacetic acid) contained tubes were used in order to prevent blood clotting. For the DNA isolation, DNA Isolation Robot (IPrep pure link, Invitrogen and the Thermo Fischer Scientific Inc) system was applied.

3.2.2. The Equipment Used in the Experiment

In this study, DNA Isolation Robot (IPrep Purelink, Invitrogen, Thermo Fischer Scientific Inc), Nanodrop 2000 (Thermo Fischer Scientific Inc), Real Time PCR (Fast Real Time 7500, Applied Biosystems), 7500 Fast Real-Time PCR Instrument, Plate

Centrifuge (Hettich), Centrifuge (Centrifuge 22R-Beckman Coulter), +4°C Refrigerator (Haier), -20°C Refrigerator (Haier), Ultra Pure Water (Pure Lab Option Q,EICT), Vortex (V.I Plus Biosan) and a Pipette Kit (Thermo Fischer Scientific Inc) were utilized.

3.3. Methods

3.3.1. Genomic DNA Isolation From Blood

All venous blood of patient and control group samples were collected in the EDTA-containing tubes volumed of 5 mL. Until the DNA isolation was started, all blood samples were stocked in refrigerator at +4°C. A robot of iPrep DNA extraction (Invitrogen) and from the genomic DNA isolation Kit with iPrep was used for DNA isolation. By using this system, DNA can be isolated from 350 µL of peripheral blood, therefore this system allows to run 13 blood samples simultaneously.One cartridge is used for each of the samples, and these cartridges are shaken for a while to ensure that the magnetic beads bind effectively with the DNA before placing the samples in their cartridge.

iPrep robot operates according to ChargeSwitch® (CST®) technology, acting as a calibrated extraction procedure. This procedure provides an advantage to isolate high amount of genomic DNA from the samples. In this procedure, elevated amounts of pure genomic DNA may be obtained from samples by utilizing paramagnetic particles. A DNA-binding surface encloses those particles. ChargeSwitch® (CST®) extraction procedure is unique indeed compared with other extracion methods such as silica-based DNA extraction procedure. The pH of enclosing bufferof particles alters the charge of beads. Under low pH conditions, the DNA backbone is negatively charged, then binds to positively charged beads. By the utilization of low salt buffer which has higher pH for DNA elution, those charged beams beconme neutralized. After the nucleic acid molecules pass into the washing buffer, the DNA samples become ready for the experiment. As a final stage of the experiment, aqueous DNA samples were acquired and stored in the refrigerator at +4°C.

3.3.2. Measurement of DNA Purity

Uv spectroscopy, in other word NanoDrop, measure the ultraviole (UV) absorbance of nucleic acids at 260 nm. Particularly in that spectrophotometric method, cuvettes or caprillaries are not demanded. Nanodrop is used in order to measure DNA concentrations of both OD260/OD280 and OD260/230 equations. Not only the purity, also the concentration of nucleic acids such as DNA is measured by NanoDrop. Because of the utilization of NanoDrop for the measurement of nucleic acids, this device is not capable of separete many molecules such as RNA, double-stranded DNA (ds-DNA), nucleotides and single-stranded DNA (ss-DNA) [123].

In this thesis, the concentration equation was obtained by using the NanoDrop 2000 (Thermo Fischer Scientific Inc). DNA sample were used for 1,5 µL for each individuals. Before the measurement of DNA samples, they were diluted with the 1/100 proportion. To measure the DNA concentration, each sample was placed on open arm, and then the arm of the instrument was closed. At the end of each sample quantification, distille water was used for cleaning the teritory, and this situation provides a clean and safe zone for another experiments.

One Optical Density (OD) Unit corresponds to 50 µg / mL of ds-DNA at 260 nm wavelength. By quantifying the OD260 / OD280 equation, the purity of all DNA samples was analyzed. The appropriate OD260 / OD280 proportion ranges between 1.7 to 1.9 while working on genotyping [125].

DNA concentration was measured at 260 nm by using NanoDrop. The formula in Figure 3.3.2-1 was applied to obtain the concentration.

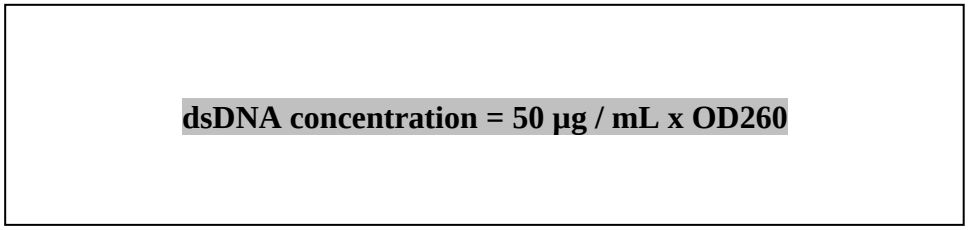

Figure 3.3.2-1 Formula of DNA concentration at 260 nm

3.3.3 Detection of EGFR Polymorphism by Using Real-Time PCR

7500 Fast-Real-Time Polymerase Chain Reaction (Applied Biosystems) instrument with Real-Time PCR was used for genotype analysis.

To detect the single nucleotide polymorphisms (SNPs) by using Real-Time PCR, fluorescence dyes probes were used. This device has a unique working mechanism that can genotyping by detecting and reading the fluorescent radiations. There are two different types of TaqMan probes named as FAM and VIC. The fluorescent dye-linked probes of DNA connects with the amplified region. Taq polymerase enzyme is performed to hydrolyze the probes. The detection of fluorescent signals can be seen obviously. In Real-Time PCR method, the fluorescence dye probes are utilized and they have two distinguish wavelengths which is specific for allelles referred as mutant and wildtype.

The primer sequence of EGFR is represented below. This primer was designed depending on the sequence of EGFR gene in human, then utilized in this study. Based on this procedure, the polymorphism-contained region was amplified by using 5'GATCCAGAAATATTTAGGAGC3' called Forward primer and 5'TTTCATCACCTTGCCTCT3' called Reverse primer. By genotyping, a target region of gene was produced, and this situation leads to the analyzing of EGFR (rs 1468727) polymorphism. The target region of gene was rs 1468727 which is particular for EGFR gene. TaqMan Genotyping Assays were used for both primers specific for the regions and the probe sets. 7500 Fast Real-Time PCR device pointed out the discrimination of allelles. There is a table shows the primers of EGFR below.

Table 3.3.3-1EGFR Primers [124]

SNP	Primers	Annealing Temperature ℃	Product Length (bp)
rs1468727	Forward: 5’GATCCAGAAATATTTAGGAGC3’ Reverse: 5’TTTCATCACCTTGCCTCT3’	56	207

3.3.4. Real Time PCR Procedure

The reagents used in Real-Time PCR and reaction mixtures have been indicated in Table below. The total volume for each sample was detected according to Real-Time PCR protocol. The reaction mixtures have been represented in Table 3.3.4-1 below for Real Time PCR protocol.

Table 3.3.4-1 The mixtures of Real-Time PCR reaction

The Agent	Quantity of Agent
Distiled Water	3,75 µL
Template DNA	1 µL
TaqMan Genotyping Assay	0,25 µL
Master Mix	5 µL

Real Time PCR steps were organized; After a waiting time of 10 minutes at 95 ° C, the denaturation process was continued for 15 seconds per cycle at 92 ° C and finally elongation to 60 seconds for each cycle at 60 ° C.As can be seen in the Table3.3.4-2, denaturation and elongation / connecting processes were applied as 40 cycles. The conditions required for Real-Time PCR are comprehensively showed in the Table 3.3.4-2.

Table 3.3.4-2 Real-Time PCR Conditions

	Temperature ° C	**Duration / Period of Time**
Waiting	95 ° C	10 minutes
40 cycle **Denaturation**	92 ° C	15 seconds
Connection / Elongation	60 ° C	60 seconds

3.4 Statistical Analysis

To obtain the numeric values, the student's t-test was applied in this thesis. After all informations was obtained by genotyping, this information was assessed in SPSS 25.0 with Fisher's Exact Tests and Chi-square tests to complete the statistical analysis. Chi-square and Fischer's Exact Tests were applied to comment the genotypes dispersion and alleles in groups. If P-values could be lower than 0,05, it shows statistically significance.

4. RESULTS

4.1 Demographic Characteristics

Extensive demographic results of 61 individuals diagnosed with prostate cancer and 62 healthy individuals as control has been represented in Table 4-1 .

Both the patient and control groups consist of male participants.

Table 4-1 Demographic Characteristic of Individuals

Parameter	**Prostate cancer (n=61)**	**Control (n=62)**	***p value***
Age (years), mean±SD*	66.91±7.39	67.00±9.08	0.969
Body mass index (kg/m2), mean±SD	78.86±11.35	78.47±9.62	0.895
Smoking (pack years), mean±SD	30.32±18.72	27.42±17.85	0.608
PSA (ng/ml),mean±SD	43.39±105.80	3.18±2.78	0.038
Family history of cancer, (%)			
Yes	37 (%29.8)	-	-
No	41(%33.1)	-	-
Gleason score, mean±SD	7.66±1.24	-	-
Pathological T-stage, n (%)			
T2a	7 % 5.6	-	-
T2b	10 % 8.1	-	-
T2c	24 %19.4	-	-
T3a	11 %8.9	-	-
T3b	9 %7.3	-	-
Clinical T-stage n (%)			
cT1c	25 %20.2	-	-
cT2	31 %25.0	-	-
cT3	5 %4	-	-

4.2 Statistical Assesment of Real-Time PCR Findings

By using the 7500 Fast-Real Time PCR instrument, allele types of each individuals were examined in both patient and control groups. The allelic discrimination plot that was acquired from this device has been represented below. The allelic discrimination were determined via the software of this tool automatically. Explication and readings of fluorescence irradiation carried out with the contribution of dyes contained in the probes. Despite, some samples could not be discriminated and interprated. In this experiment, two different dyes, which correspond to FAM colour blue and VIC colour green, were used. ROX corresponds a reference colour in order to compare FAM and VIC dyes. Allelic discrimination was eveluated by examining and interpreting the radiancee curves.

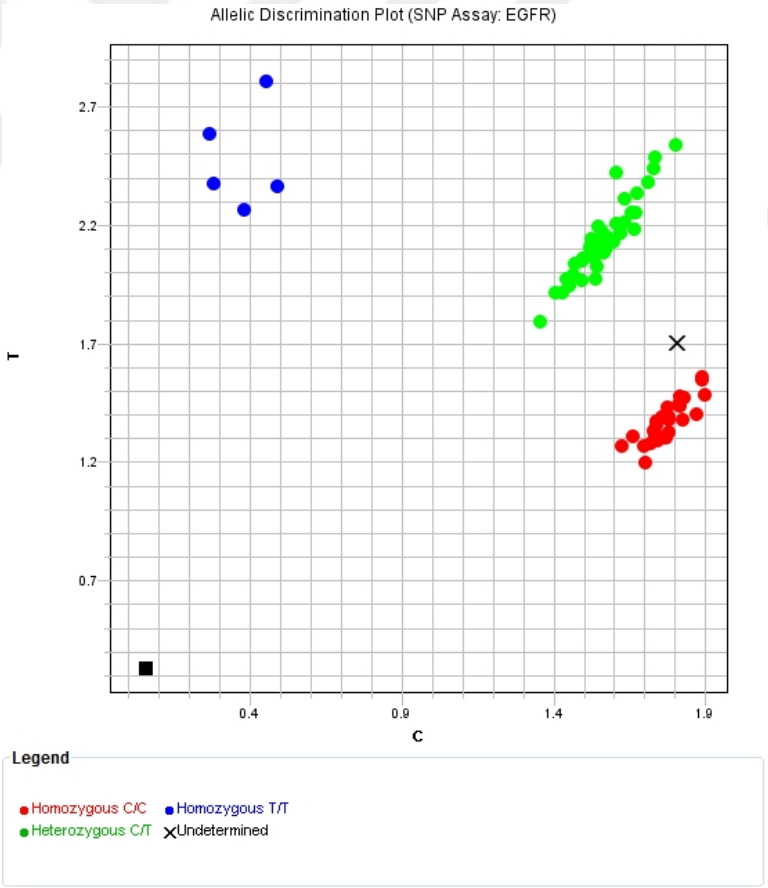

Figure 4.2 - 1 Allele Discrimination FindingsCC: Homozygote Wild Type CT: Heterozygote TT: Homozygote Mutant Type

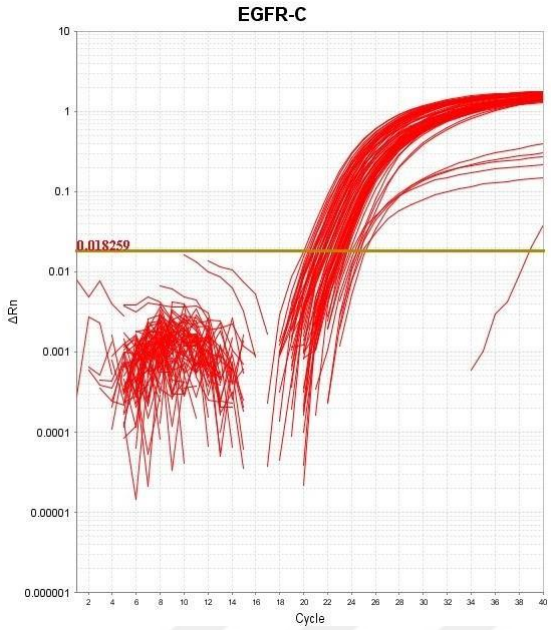

Figure 4.2- 2 Allele C Amplification Plot

The plot represented above indicates amplification plots of Allele C. Yellow line is correspond to Threshold value (0.1825).

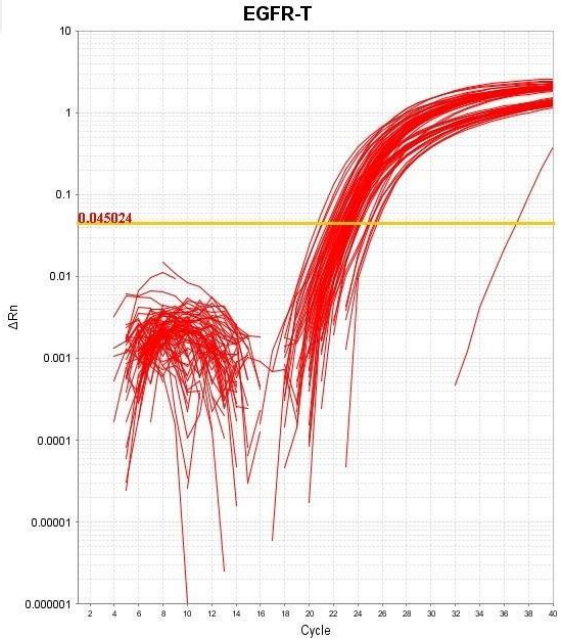

Figure 4.2-3 Allele T Amplification Plot

The figure represented above shows amplification plot of Allele T. The yellow line is correspond to Threshold value (0.0450).

4.3 Genotype and Allele Analysis Within Patient and Control Groups

In this thesis, EGFR polymorphism was analyzed in patient with prostate cancer and healthy individuals named as control group.Not significant results have been revealed from this study between control and patient groups (p=0.475). It has been obvious that there is a undeniable major relationship seen in table 4.3-1below among these two groups. The calculated value of homozygote mutant genotype corresponds to 0.320, heterozygote genotype is 0.748 and homozygote wild-type genotype is 0.242. Based on the results found in this thesis, rates of homozygote wild type (CC), heterozygote type (CT), and homozygote mutant type (TT) are determined as 38.7%, 53.2%, 8.1% in control group respectively. On the other hand, homozygote wild type (CC), heterozygote type (CT) and homozygote mutant type (TT) was calculated respectively as 49.2%, %42.6, 8.2% in PC patient group. When the control and patient groups were compared for the C allele, it was lower in the control group (81) than in the patient group (86) with the C allele. Interestingly, as seen in the Table below, the T allele was higher in the control group(43) than in the patient group (36).

Based on the assessment of properties of these two groups, homozygote wild type genotype (CC) corresponds to 38.7% frequency in control group while the patient group has 49.2%. In addition, the frequency of heterozygous genotype (CT) was 53.2% in control group, 43.6% in PC patient group. Homozygous mutant type (TT) genotype was seen with a frequency of 8.1% in the control group and 8.2% in the patient group.When statistically obtained values were evaluated, each homozygous CC wild type (p = 0.242), heterozygous CT type (p = 0.748), and homozygous TT mutant type (p = 0.320) showed a very large difference between the two groups showed in Table 4.3-1.

C alleles were 86 in patient group, besides T alleles were counted as 36. The p value of C allele corresponds to 0.976. Besides, C allele were noted as 81 in control group, however T allele were 43. The p value was determined as 0.176 for the T allele (Table4.3-1). Consequently, in comparison of EGFR gene polymorphism accorging to the genotype and allelic frequencies, not major association was recorded between control and patient groups (p=0.475) (Table 4.3-2).

Table 4.3-1 Comparison of EGFR genotype between prostate cancer patients and healthy individuals.

EGFR Genotypes	Control Group (n=62)	PC Patients (n=61)	*p Value*	Odd Ratio (OR)	Confidence Interval (%)
CC Genotype	38.7% (n=24)	49.2% (n=30)	0.242 NS	1.532	0.749-3.136
CT Genotype	53.2% (n=33)	42.6% (n=26)	0.748 NS	0.800	0.204-3.133
TT Genotype	8.1% (n=5)	8.2% (n=5)	0.320 NS	0.698	0.343-1.419
Allelic Distributions					
C Allele	81	86	0.976 NS	0.982	0.298-3.133
T Allele	43	36	0.176 NS	0.609	0.297-1.251

** (NS)= not significantly different (p >0.05), S= significant (p<0.05). *O.R (Odds Ratio), X± SD (Mean ± Standard Deviation), n (number of sample). The independent sample student t-test was used to determine the differences between two groups.

In patient with prostate cancer, homozygous (CC) genotype represented less protective behaviour (OR=1.532) than compared with heterozygous (CT) genotype and homozygous TT genotype. In other words, having C allele does not show any advantage in patient with prostate cancer. In addition, according to statistical analysis findings, heterozygous type (CT) (OR: 0.800; p: 0.748) does not carry a risk of prostate cancer as shown in the Table above. Also, possessing the C allele obviously enhances the risk. Even possessing the C allele in heterozygous form (CT) constitue a risk factor.

5. DISCUSSION AND CONCLUSION

Nowadays, cancer is one of the most dangerous disease that originate from internal and external factors and constitutes one of the greatest dangers to human life.Cancers are also characterized as a combination of diseases that are also detected by uncontrolled cell growth and abnormal spread of the cell. If there is a spread of cancer which lead to metastasis, it can be cause to death. When the cells in the body become unable to control growth mechanism, cells gain the ability to divide rapidly, thus, cancer becomes visible [126].

Generation of cancer, in other words named as carcinogenesis is complex biological mechanism which is seperated into three stages; initiation, promotion and progression, respectively [21, 25, 127-129].

There are various internal and external causes give rise to the appearance of cancer cells. While ionizing radiation, smoking tobacco and excess alcohol consumption are categorized as external factors, some hormones, tumor promoters and genetic tendency are counted as internal reasons for cancer progression [6].

Cancers are mainly classified as malign and benign tumors. Metastasis is the biological event in which malignant tumors invade surrounding tissues and spread to another body parts.Unlike malign tumors, benign tumors are not capable of invading adjacent tissues and metastasis. However, both malign and benign tumors are classified depend on the type of originated cell [6].Lung, prostate, liver and cervical cancers can be given as examples of cancers affected on an organ basis.Cancer cells show distinct symptoms that arise from the location, growth and the size of the tumor. There are several different diagnosis and treatment methods which aim to cure cancer. Biomarkers (such as PSA), imaging, MRI and PET are commonly used to diagnose cancers in clinics. Early-stage diagnosed cancer patients have much more advantage to remain their life healthy [39].

In addition, there are many types of treatment applied according to characteristics of the tumor. Immunotherapy, cryoablation, radiofrequency ablation, hormone therapy, targeted drug therapy, bone marrow transplant, surgery, chemotherapy and radiation therapy are commonly used in cancer tratment. [40].

Prostate cancer is categorized as one of the most dangerous and aggressive type of cancer all around the world. Although it is very common, it ranks fifth in cancer-related deaths in the world [42-43].

There are differences in PC frequency from one country to another.Incidence, mortality and survival rates in prostate cancer show different frequencies in different regions.These differences can be caused by many reasons such as external risk factors, genetic predisposition, living standards, as well as combinations of these factors[45].

Moreover, patients with PC are suffered from urination and nocturia as symtopms. Prostate spesific antigen (PSA) level is one of the most prevalentway to detect prostate cancer [42].

Additionally, many risk factors increase the generation of PC such as age, androgens, ethnicity, familial history and genetic tendency, life standarts and habits, infection and inflammation [52].

The first stage of PC diagnosis is depend on the evaluation of prostate tissue under microscope. By using transrectal ultrasound, prostate biopsy is conducted and this method is used in order to achieve 10-12 samples in a pattern. After examining these tissue samples, primary Gleason grade is applied for the pre-dominant histological pattern and a secondary grade for the highest pattern. They both have rank a value between 1 to 5 which depends on the presence of cells and microscopic evaluation [101]. The Comprehensive Cancer Network risk classification is an example of a 5-layer system where groups are divided into low and high riskconfirmed [101]. There is a Table 5-1 represents the risk stratification of prostate cancer below.

Table 5-1 Risk Stratification of Prostate Cancer [101]

	Very Low Risk	Low Risk	Intermediate Risk	High Risk	Very High Risk
Clinical Stage	T1c	T1-T2a	T2b-T2c	T3a	T3b-T4
Gleason Score	≤ 6	≤6	7	8-10	Primary Gleason Pattern 5 orbigger than 4 biopsy cores with 8-10 Gleason Score
PSA level	≤10 ng/mL	≤10 ng/mL	10-20 ng/mL	≥20 ng/mL	-

The scoring system (0-10) depends on the some variables; age, stage of disease, level of PSA, Gleason pattern 4 or 5, the percentage of biopsy samples included PC. According to the knowledges about that system, the rank between 0-2 is called low risk, 3-5 is named as intermediate and 6-10 is mentioned as high [131].

After the accurate biopsy and diagnosis, treatment should be performed in order to remain the life of patient cancer-free. New imaging technologies (such as MRI, PET, CT) and molecular biomarkers (such as PSA) enhance the diagnostic performance of PC. After the diagnosis of prostate cancer, the treatment method is performed by clinicians and decision of patient. For the localized PC tumors, radiation and surgery have been performed, while androgen deprivation theraphy is used for metastatic form of PC [101].

Several studies have investigated that, besides external factors some internal factors (i.e growth factors) can also in charge of PC progression. Epidermal Growth Factor Receptor (EGFR) is one member of ErbB family of tyrosine kinase receptors which is responsible for regulating development of epithelial tissue and remain homeostasis [133]. It is also involved in signal transduction into cells induced by an EGFR ligand[113,134]. This gene has a location on chromosome 7p12-13 and in charge

of encoding 170 kD transmembrane RTK located on the facial of epithelial cells [135]. It has an important role in some molecular mechanisms such as increased tumor growth, metastasis and invasion in malignancies [136].According to the requirements, cell proliferation is regulated by EGFR in normal tissues and this process is called homeostasis.In malignancies, on the other hand, EGFR is permanently induced due to the continuous production of EGFR ligands in the environment around the tumor[137, 138].

Overexpression of EGFR from tumor cells usually leads to a much more aggressive phenotype. Therefore, EGFR is currently used as one of the most preferred molecules to develop therapeutics[139].

Several investigations have been carried out the overexpression of EGFR in PC, but its act in the generation of PC is not compeletely understood yet. Researchers have been investigated the relationship between EGFR and PC progression with androgen independence and the results were found consistent with a model in which ErbB1 expression rises during the progression of the androgen-independent state[140].

In addition, in a different study, the importance of overexpression of EGFR and addition of gene copy number in PC was investigated using the “Fluorescent in situ hybridization (FISH) method. As a result of this study, substantial amount of EGFR was noted in 18% cases and they have been claimed that there is a relationship between poor prognosis and overexpression of EGFR [141].

The prostate cancer risk was for the first time investigated in the EGFRrs1468727 gene in Turkish population with case-control study. Moreover, by the prognosis of EGFR gene in Turkish patient with prostate cancer, a single nucleotide polymorphism (SNP) relation was investigated.

Depite many case-control studies have indicated the alterations in EGFR may have a potential risk for PC, the association among those alterations of EGFR and prostate cancer in Turkish population was not stil fully-understood.

Based on the results of this study, the rs1468727 CC homozygous genotype was found to be more common in the patient group than in healthy individuals (p=0.242). In addition, it has been showed that the frequency of C allel is more frequent in patient group than healthy individuals (p=0.976). Consequently, this thesis indicates the relationship of SNP in the EGFR gene with the potential risk of PC. Higher C allel frequency (in EGFR rs1468727) in the patient group shows increased risk for prostate cancer.

Polymorphism in EGFR rs1468727 gene has been investigated for glioma in Chinese population before, however, it is the first time that a study has worked on EGFR rs1468727 polymorphism and prostate cancer in Turkish population. Because of that, there was not found any previous study conducted on Turkish population in patient with prostate cancer.

As a result, CC homozygous genotype and C allele may be given as risk factor in Turkish population with PC. By new insights on this subject, the polymorphism in EGFR rs 1468727 gene and prostate cancer will be deeply investigated in different populations thus, the mechanism will be fully-understood.

As a consequence,

The polymorphism of EGFR rs1468727 gene in Turkish patient with PC has not been deeply studied before. According to the findings obtained from this study, it was determined that this polymorphism may have a action in PC progression.Depend on the evaluated results, having the CT heterozygous type can be considered as an advantage for the patients. The T allele can be counted as protective by itself, and its p value is not significant. However, having CT in patients was considered an advantage and it was observed that this type can contribute to decrease the risk of PC.

Examination of TT-carried patients have been claimed that, having TT homozygous wild type may not be considered as potential risk factor for prostate cancer with not significant p value (0.320) and 0.698 Odd Ratio (OR). On the other hand, having CC homozygous genotype may be noted as risk factor (O.R= 1.532 p=0.242) for prostate cancer progression. Also carrying C allele alone may constitue a potential risk with 0.982 OR.

Based on all these findings, it may be claimed that, patients carry CC homozygous genotype has much more increased risk for prostate cancer and carrying C allele alone is also considered as a disadvantage for this disease. However, having the CT heterozygous genotype may be protective for prostate cancer, and carrying the T allele alone has the same protective effect as the CT genotype. Although the T allele has a protective effect, having the TT allele is not important in predicting prostate cancer progression.The results obtained from this study is considered as statistically insignificant. As a result, it has been found that polymorphism of EGFR rs1468727 gene rises the risk of prostate cancer.

6. REFERENCES

1. Kovacs E, Zorn JA, Huang Y, Barros T, Kuriyan J, A structural perspective on the regulation of the epidermal growth factor receptor, *Annu Rev Biochem*,2015; 84:739–764

2. Citri A, Skaria KB, Yarden Y, The deaf and the dumb: the biology of ErbB-2 and ErbB-3. Exp Cell Res. 2003.10;284(1):54-65

3. Wheeler, D. L., & Yarden, Y. (Eds.),*Receptor Tyrosine Kinases: Family and Subfamilies*, 2015

4. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, et al. "Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha", 2002. *Cell*. 110 (6): 763–773

5. Hausman, D. M,What Is Cancer? *Perspectives in Biology and Medicine,* 2019. 62(4),778784

6. G.M. Cooper, ed.,*The Cell: A Molecular Approach. 2nd edition*, the USA, 2000

7. Dang CV. Cancer Metabolism: the Known, Unknowns. Biochim Biophys Acta Rev Cancer. 2018;1870(1):1

8. Oliveira PA, Colaço A, Chaves R, Guedes-Pinto H, De-La-Cruz P LF, Lopes C, Chemical carcinogenesis. *An Acad Bras Cienc*. 2007;79(4):593-616

9. zur Hausen H. Viruses in human cancers. *Science*. 1991; 22;254(5035)

10. Fattovich G, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology*. 1997 ;112(2):463-72

11. Borza CM, Hutt-Fletcher LM. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat Med*. 2002;8(6):594-9

12. Wallin KL, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med*, 1999;341(22):1633–1638

13. Gallo RC, et al. Association of the human type C retrovirus with a subset of adult T-cell cancers. *Cancer Res.* 1983;43(8):3892–3899

14. Vennervald, B.J. and Polman, K, Helminths and malignancy. *Parasite Immunol.* 2009; 31, 686–696

15. Hanahan D andWeinberg RA. The Hallmarks of Cancer. *Cell*. 2000. 7;100(1):57-70

16. Jones PA, Baylin SB, The fundamental role of epigenetic events in cancer. *Nat Rev Genet.* 2002;3(6):415-28.

17. Berembulum I and Shubik P, The role of croton oil applications, associated with a single painting of a carcinogen, in tumor induction of the mouse's skin. *Br J Cancer*, 1947. 1: 379–382

18. Pitot H.C and Dragan Y.P, Facts and Theories Concerning the Mechanisms of Carcinogenesis. *FASEB* J, 1991. 5: 2280–2286

19. Yuspa S.H and Poirier M.C, Chemical Carcinogenesis: From Animal Models To Molecular Models in One Oecade. *Adv Cancer Res*, 1988. 50: 25–70

20. Scott R.E, Willie JR JJ, Wier ML, Mechanisms For the Initiation and Promotion of Carcinogenesis: A Review and A New Concept. *Mayo Clin Proc*, 1984. 59: 107–117

21. Gutierrez JB and Salsamendi AL.. Fundamientos de ciência toxicológica. *Diaz de Santos, Madrid*, 2001. p. 155–177

22. Dao TL, Chan PC, Hormones and Dietary Fat As Promoters In Mammary Carcinogenesis. *Environ Health Perspect*. 1983 ;50:219-25

23. Ashendel CL, The Phorbol Ester Receptor: A Phospholipid-regulated Protein Kinase. *Biochim Biophys Acta*. 1985; 9;822(2):219-42

24. Yager JD Jr, Yager R, Oral Contraceptive Steroids as Promoters of Hepatocarcinogenesis in Female Sprague-Dawley Rats,*Cancer Res.* 1980; 40(10):3680-5

25. Trosko JE, Commentary: Is The Concept Of "Tumor Promotion" A Useful Paradigm? *Mol Carcinog*, 2001; 30: 131– 137

26. Melnick R.L, Kohn MC, Portier CJ, Implications For Risk Assessment Of Suggested Non-Genotoxic Mechanisms Of Chemical Carcinogenesis, *Environ Health Perspect*, 1996; 104: 123–134.

27. Klaunig J.E, Kamendulis L.M and Xu Y, Epigenetic Mechanisms Of Chemical Carcinogenesis, *Hum Exp Toxicol*, 2000; 19: 543–555

28. Williams G.M, Mechanisms of Chemical Carcinogenesis And Application To Human Cancer Risk Assessment, *Toxicology*,2001; 161: 3–10

29. Shacter E and Weitzman S.A, Chronic Inflammation and Cancer. *Oncology*, 2002;6: 217–226

30. Lutz W.K, A True Threshold Dose in Chemical Carcinogenesis Can not Be Defined For A Population, Irrespective of The Mode of Action, *Hum Exp Toxicol*, 2000; 19: 566–568

31. Butterworth BE, Templin MV, Constan AA, Sprankle CS, Wong BA, Pluta LJ, et al. Long-term Mutagenicity Studies With Chloroform and Dimethylnitrosamine in Female lacI Transgenic B6C3F1 Mice,*Environ Mol Mutagen,* 1998; 31: 248–56

32. Loeb LA, Cancer Cells Exhibit A Mutator Phenotype,*Adv Cancer Res,* 1998;72: 25–56

33. Dixon K and Kopras E, Genetic Alterations and DNA Repair in Human Carcinogenesis, *Semin Cancer Biol,* 2004; 14: 441–448.

34. Precision Medicine in Cancer Treatment, National Cancer Institute the United States

35. Rakoff-Nahoum S, Why cancer and inflammation?,*Yale J Biol Med*. 2006;79(3-4):123-30

36. Rodriguez AC, Blanchard Z, Maurer KA, Gertz J, Estrogen Signaling in Endometrial Cancer: a Key Oncogenic Pathway with Several Open Questions. *Horm Cancer*. 2019;10(2-3):51-63

37. Aykan NF, Red Meat and Colorectal Cancer. *Oncol Rev.* 2015 28;9(1):288

38. Bethesda MD, *National Institutes of Health (US); Biological Sciences Curriculum Study,* National Institutes of Health (US); 2007

39. Price P. and Sikora K. Ed, *Treatment of Cancer 7th Edition*, 2020

40. Litin S, *Mayo Clinic Family Health Book 5th Edition,* the USA, 2018

41. de Castro Sant' Anna C, Junior AGF, Soares P, Tuji F, Paschoal E, Chaves LC, et al. Molecular Biology As a Tool For The Treatment Of Cancer, *Clin Exp Med*. 2018;18(4):457-464

42. Rawla P. Epidemiology of Prostate Cancer,*World J Oncol*, 2019;10(2):63-89

43. Litwin, M. S., & Tan, H.-J, The Diagnosis and Treatment of Prostate Cancer, *JAMA*, 2017. 317(24), 2532

44. Internation Agency for Research on Cancer, World Health Organisation, 2020 December

45. Crawford E.D, Epidemiology of prostate cancer, *Urology*, 2003, 62(6):1

46. Evans, A. J, Treatment effects in prostate cancer. *Modern Pathology*,2018, *31*, S110–121

47. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, et al. Cancer incidence in five continents,*Vol VII. Lyon*: *IARC Sci Publ*, 1997

48. Perdana NR, Mochtar CA, Umbas R, Hamid ARActa,The Risk Factors of Prostate Cancer and Its Prevention: A Literature Review. *Med Indones. 2016; 48(3):228-238*

49- Force USPST, Grossman DC, Curry SJ, Owens DK, Bibbins-Domingo K, Caughey AB, et al. Screening for prostate cancer: US Preventive Services Task Force Recommendation Statement. *JAMA.* 2018;319(18):1901–1913

50- Negoita S, Feuer EJ, Mariotto A, Cronin KA, Petkov VI, Hussey SK et al. Annual Report to the Nation on the Status of Cancer, part II: Recent Changes in Prostate Cancer Trends and Disease Characteristics. *Cancer.* 2018;124(13):2801–2814

51- De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, Pierannunzio D, et al. Cancer survival in Europe 1999-2007 by Country and Age: Results of EUROCARE—5-a population-based study. *Lancet Oncol.* 2014;15(1):23–34

52- Howlader N, Noone AM, Krapcho M, et al (eds). SEER CancerStatistics Review, 1975-2010, National Cancer Institute

53- Grönberg H., Prostate Cancer Epidemiology, *Lancet.* 2003; 8;361(9360):859

54- Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD, The Frequency of Carcinoma and Intraepithelial Neoplasia of the Prostate in Young Male Patients,*J Urol*. 1993;150(2 Pt 1):379-85

55- Vickers AJ, Cronin AM, Bjork T, et al. The Concentration of Prostate Specific Antigen at Age 60 Predicts Lifetime Risk of Metastasis And Death From PCa, *BMJ*. 2010;341:1-8

56- Potosky AL *et al*. The Role of Increasing Detection in the Rising Incidence of Prostate Cancer. *JAMA*, 1995; 273: 548–552

57- Lilja H et al. Long-term Prediction of Prostate Cancer up to 25 Years Before Diagnosis of Prostate Cancer Using Prostate Kallikreins Measured at Age 44 to 50 Years, 2007;*J Clin Oncol* 25: 431–436

58- Quinn M and Babb P, Patterns and Trends in Prostate cancer Incidence, Survival, Prevalence and Mortality Part II: Individual Countries, *BJU Int,* 2002; **90**: 174–184

59- Addo BK, Wang S, Chung W, et al. Identification of Differentially Methylated Genes in Normal Prostate Tissues from African American and Caucasian Men, *Clin Cancer Res,*2010;16:3539-47

60- Perdana NR, Mochtar CA, Umbas R, Hamid AR, The Risk Factors of Prostate Cancer and Its Prevention: A Literature Review. Acta Med Indones, 2016;48(3):228-238

61- Bratt O, Hereditary Prostate Cancer: Clinical Aspects,*J Ur*ol, 2002;168(3):906-13

62- Carter BS et al.Hereditary Prostate Cancer: Epidemiologic And Clinical Features. *J Urol*, 1993; **150**: 797–802

63- Carter BS et al. Mendelian Inheritance of Familial Prostate cancer. *Proc Natl Acad Sci USA*, 1992;**89**: 3367–3371

64- Gillanders EM et al. Combined Genome-Wide Scan For Prostate Cancer Susceptibility Genes. *J Natl Cancer Inst,*2004 **96**: 1240–12474

65- Klein EA and Silverman R, Inflammation, Infection, and Prostate Cancer. *Curr Opin Urol,* 2008*;* **18**: 315–319

66- Xu J et al. Association of Prostate Cancer Risk Variants With Clinicopathologic Characteristics of the Sisease. *Clin Cancer Res*, 2008*;* 14: 5819–5824)

67- Ellinger J et al.CpG Island Hypermethylation At Multiple Gene Sites in Diagnosis and Prognosis of Prostate Cancer. *Urology*,2008; **71**: 161–167

68- Tomlins SA et al. Recurrent Fusion of *TMPRSS2* and *ETS* Transcription Factor Genes in Prostate Cancer, *Science*,2005; **310**: 644–648

69- Perner S et al.*TMPRSS2*:*ERG* Fusion-Associated Deletions Provide Insight into The Heterogeneity of Prostate Cancer, *Cancer Res*,2006; **66**: 8337–8341)

70- Morris DS et al.The Discovery and Application of Gene Fusions in Prostate Cancer. *BJU Int*, 2008;**102**: 276–282

71- Coussens LM and Werb Z, Inflammation and cancer. *Nature*, 2002;420: 860–867

72- Hsing AW, Hormones and Prostate Cancer: What's Next? *Epidemiol Rev*, 2001;**23**: 42–58

73- Eaton NE et al. Endogenous Sex Hormones and Prostate Cancer: A Quantitative Review Of Prospective Studies, *Br J Cancer*, 1999; **80**: 930–934

74- Roddam AW et al. Endogenous Sex Hormones and Prostate Cancer: A Collaborative Analysis Of 18 Prospective Studies, *J Natl Cancer Inst*,2008; **100**: 170–183

75- Jaffe JM et al. Association of *SRD5A2* Genotype and Pathological Characteristics of Prostate Tumors. *Cancer Res*,2000; **60**: 1626–1630

76- Pearce CL et al.No Association Between the *SRD5A2* gene A49T Missense Variant and Prostate Cancer Risk: Lessons Learned, *Hum Mol Genet*,2008; **17**: 2456–2461

77- Scariano JK et al. The *SRD5A2* V89L Polymorphism is Associated With Severity of Disease in Men With Early onset Prostate Cancer, *Prostate*, 2008; **68**: 1798–1805).

78- Setiawan VW et al. CYP17 Genetic Variation and Risk of Breast and Prostate Cancer From the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). *Cancer Epidemiol Biomarkers Prev*,2007;**16**: 2237–2246

79. Sarma AV et al.Genetic Polymorphisms in CYP17, CYP3A4, CYP19A1, SRD5A2, IGF-1, and IGFBP-3 and Prostate Cancer Risk in African-American men: the Flint Men's Health Study. *Prostate*, 2008;**68**: 296–305

80- Neslund-Dudas C et al.*SRD5A2* and *HSD3B2* Polymorphisms are Associated With Prostate Cancer Risk and Aggressiveness. *Prostate*, 2008;**67**: 1654–1663)

81- Bostwick DG et al. Human Prostate Cancer Risk Factors. *Cancer*,2004;**101**: 2371–2490

82- Kaaks R et al.Plasma Androgens, IGF-1, Body Size, and Prostate Cancer Risk: a Synthetic Review. *Prostate Cancer Prostatic Dis*,2000; **3**: 157–172,

83- Nelson WG et al. The Role of Inflammation in the Pathogenesis of Prostate Cancer. *J Urol* **172**,2004; (5 Suppl): S6–S11)

84- Clinton SK et al.The Combined Effects of Dietary Protein and Fat Intake During the Promotion Phase of 7,12-dimethylbenz(a)anthracene-induced Breast Cancer in Rats. *J Nutr*,1988; **118**: 1577–1585,

85- Wang Y *et al*. Decreased Growth of Established Human Prostate LNCaP tumors in Nude Mice Fed a Low-Fat Diet. *J Natl Cancer Inst*,1995; **87**: 1456–1462

86-Aronson WJ et al. Decreased Growth of Human Prostate LNCaP tumors in SCID Mice Fed a Low-fat, Soy Protein Diet With Isoflavones, *Nutr Cancer*,1999; **35**: 130–136)

87- Mcbride RB, *Obesity and Aggressive Prostate Cancer Bias and Biomarkers*, Columbia University, 2012

88- Allott EH, Masko EM, Freedland SJ, Obesity and Prostate Cancer: Weighing the Evidence. Euro Urol. 2013;63:800–9

89. Freedland SJ, Wen J, Wuerstle M, Shah A, Lai D, Moalej B et al. Obesity is a Significant Risk Factor For Prostate Cancer At the Time of Biopsy, *Urol*, 2012;72:1102–5

90- Parekh N, Lin Y, Dipaola RS, Marcella S, Yao GL, Obesity and PCa Detection: Insights From Three National Surveys,*Am J Med*. 2010;123:829-35

91- Giovannucci E et al. A Prospective Study of Dietary Fat and Risk of Prostate Cancer. *J Natl Cancer Inst*,1988,**85**: 1571–1579

92- Hayes RB et al. Dietary Factors and Risks for Prostate Cancer Among Blacks And Whites in the United States, *Cancer Epidemiol Biomarkers Prev*, 1999,**8**: 25–34

93- Oakley-Girvan I *et al*. Risk of early-onset Prostate Cancer in Relation to Germ line Polymorphisms of the Vitamin D Receptor. *Cancer Epidemiol Biomarkers Prev*,2004;**13**: 1325–1330

94- Huncharek M, Haddock S, Reid R, Kupelnick B, Smoking As a Risk Factor For Prostate Cancer: A Metaanalysis of 24 Prospective Cohort Studies, *Am J Public Health*. 2010;100:693–701

95- Nock NL, Liu X, Cicek MS, et al. Polymorphisms In Polycyclic Aromatic Hydrocarbon Metabolism and Conjugation Genes, Interactions With Smoking and Prostate Cancer Risk, *Cancer Epidemiol Biomarkers Prev*. 2006;15(4):756–61

96- Gutt R, Cox DG, Dostal L, et al. Statin Use and Risk of Prostate Cancer Recurrence in Men Treated With Radiation Therapy, *J Clin Oncol*, 2010;28:2653-9

97- Rizos CH, Papassava M, Golias CH, Charalabopoulos K, Alcohol Consumption and Prostate Cancer A Mini Review, *Exp Oncol*, 2010;32(2):66–70

98- Rohrmann S, Linseisen J, Key TJ, et al. Alcohol Consumption and the Risk for Prostate Cancer in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol BiomarkersPre,*. 2008;17:1282-7

99- Ewings P and Bowie C, A Case–Control Study of Cancer of the Prostate in Somerset and east Devon, *Br J Cancer* 74: 661–666,

100- Giles GG et al.Sexual Factors And Prostate Cancer. *BJU Int*, 2003;92: 211–216

101- Mohler JL, Armstrong AJ, Bahnson RR, et al. Prostate Cancer, version 1.2016.J *Natl Compr Canc Netw*, 2016;14(1):19-30.- 14

102- Scattoni V, Lazzeri M, Lughezzani G, et al. Head-to-head Comparison of Prostate Health Index and Urinary PCA3 for Predicting Cancer at Initial or Repeat Biopsy, *J Urol*, 2013;190(2):496-501

103- Partin AW, Van Neste L, Klein EA, et al. Clinical Validation of an Epigenetic Assay to Predict Negative Histopathological Results in Repeat Prostate Biopsies, *J Urol*,2014;192(4):1081-1087

104- Weinreb JC, Barentsz JO, Choyke PL, et al. PI-RADS prostate imaging—Reporting and Data System: 2015, version, *2. Eur Urol*. 2016;69(1):16-40

105- Wortel RC, Incrocci L, Pos FJ, et al. Acute Toxicity After Image-guided Intensity Modulated Radiation Therapy Compared to 3D Conformal Radiation Therapy in Prostate Cancer Patients, *Int J Radiat Oncol Biol Phys*. 2015;91(4):737-744.

106- Viani GA, Viana BS, Martin JE, Rossi BT, Zuliani G, Stefano EJ, Intensity-Modulated Radiotherapy Reduces Toxicity With Similar Biochemical Control Compared with 3-dimensional Conformal Radiotherapy for Prostate Cancer: A Randomized Clinical Trial, *Cancer.* 2016;122(13):2004-201

107- Nguyen PL, Alibhai SM, Basaria S, et al. Adverse Effects of Androgen Deprivation Therapy and Strategies to Mitigate Them. *Eur Urol.* 2015;67 (5):825-836**.**

108- Nead KT, Gaskin G, Chester C, Swisher-McClure S, Leeper NJ, Shah NH. Association between androgen deprivation therapy and risk of dementia. JAMA Oncol. 2017;3(1):49-55

109- D'Amico AV, Chen MH, Renshaw A, Loffredo M, Kantoff PW, Long-term Follow-up of a Randomized Trial of Radiation With or Without Androgen Deprivation Therapy for Localized Prostate Cancer, *JAMA*, 2015;22-29;314(12):1291-3.

110- Magnan S, Zarychanski R, Pilote L, et al. Intermittent vs Continuous Androgen Deprivation Therapy For Prostate Cancer: a Systematic Review And Meta-analysis, *JAMA Oncol*. 2015;1(9):1261-1269

111- James ND, Spears MR, Clarke NW, et al; STAMPEDE Investigators. Failure-Free survival and Radiotherapy In Patients With Newly Diagnosed Nonmetastatic Prostate Cancer: Data From Patients In the Control Arm of The STAMPEDE trial. , *JAMA Oncol*, 2016;2(3):348-357)

112- A. Ullrich, L. Coussens, J. S. Hayflick et al., Human Epidermal Growth Factor Receptor cDNA Sequence and Aberrant Expression Of The Amplified Gene in A431 Epidermoid Carcinoma Cells, *Nature*,1984; 309(5967): 418–425,

113- G. Carpenter, L. King Jr., and S. Cohen, "Epidermal growth factor stimulates phosphorylation in membrane preparations *in vitro*," *Nature*, vol. 276, no. 5686, pp. 409–410, 1978.

114- H. Ushiro and S. Cohen, Identification of Phosphotyrosine As A Product Of Epidermal Growth Factor-activated Protein Kinase in A-431 Cell Membranes, *The Journal of Biological Chemistry*, 1980; 255(18):8363–8365

115- Yarden Y, Sliwkowski M.X, Untangling the ErbB Signalling Network, *Nat. Rev. Mol. Cell Biol.* 2001; 2: 127-137

116- W. S. Chen, C. S. Lazar, M. Poenie, R. Y. Tsien, G. N. Gill, and M. G. Rosenfeld, Requirement For intrinsic Protein Tyrosine Kinase in the Immediate And Late Actions Of The EGF receptor, *Nature*, 1987; 328(6133):. 820–823, 1987

117- R. A. Perugini, T. P. McDade, F. J. Vittimberga Jr., and M. P. Callery, Pancreatic Cancer Cell Proliferation Phosphatidylinositol 3-kinase dependent, *Journal of Surgical Research*, 2000; 90(1): 39–44

118- K. M. Nicholson and N. G. Anderson, The Protein kinase B/Akt Signalling Pathway in Human Malignancy, *Cellular Signalling*, 2002; 14(5): 381–395

119- W. Wang, J. L. Abbruzzese, D. B. Evans, L. Larry, K. R. Cleary, and P. J. Chiao, The Nuclear Factor-*κ*B RelA Transcription Factor is Constitutively Activated in Human Pancreatic Adenocarcinoma cells, *Clinical Cancer Research*, 1999: 5(1); 119–127.

120- N. Douziech, E. Calvo, J. Laine, and J. Morisset, Activation of MAP Kinases in Growth Responsive Pancreatic Cancer Cells, *Cellular Signalling*, 1999: 11(8); 591–602

121- R.I Nicholson, J.M.W Gee, M.E Harper, EGFR And Cancer Prognosis, *European Journal of Cancer*, 2001;37(4); 9-15

122- Kathleen C. Day, Lorenzatti HilesG, HER2 and EGFR Overexpression Support Metastatic Progression of Prostate Cancer to Bone, *Cancer Res*, 2017; (77):(1);74-85

123-Olson ND, Morrow JB, DNA Eextract Characterization Process For Microbial Detection Methods Development and Validation, *BMC Res Notes*, 2012;5:668

124- Green M. and SambrookJ., *Isolation and Quantification of DNA*, Cold Spring Harb Protoc; 2018

125- Wang X, Zhang H, Wang D, Li X, Association of Genetic Polymorphisms of EGFR With Glioma in A Chinese Population, *Genet Test Mol Biomarkers*, 2015;19(1):59-62

126- Garima Mathur, Sumitra Nain and Pramod Kumar Sharma, Cancer: An Overview, *Academic Journal of Cancer Research*, 2015:8 (1): 01-09

127- Foulds L, The Experimental Study of Tumor Progression: AReview, *Cancer Res*,1954;14: 327–339

128- Grisham JW, Kaufmann WK, Kaufmann DG, The Cell Cycle And Chemical Carcinogenesis, *Surv Synth Patho Res,* . 1984; 1: 49–66

129- Cohen SM, Analysis of Modifying Factors in Chemical Carcinogenesis, *Prog Exp Tumor Res,* . 1991; 33: 21–40

130- Mehta R,The Potential For The Use of Cell Proliferation And Oncogene Expression As Intermediate Markers During Liver Carcinogenesis, *Cancer Lett*, 1995;93: 85–102

131- Hasegawa R, Futakuchı M, Mizaguchi Y, Yamaguchi T, Shirai T, Ito N, D Lijinsky W, Studies Of Initiation And Promotion Of Carcinogenesis By N-nitroso Compounds, Cancer Lett, 1998;123: 185–191

132- Cooperberg MR, Pasta DJ, Elkin EP, et al. The University of California, San Francisco Cancer of the Prostate Risk Assessment Score: a Straightforward And Reliable Preoperative Predictor of Disease Recurrence After Radical Prostatectomy, *J Urol,*2005;173(6):1938-1942

133- Sara Sigismund, Emerging Functions of the EGFR in Cancer, *Molecular Oncology*, 2018; Pages 3-20

134- I. Alroy and Y. Yarden, The ErbB Signaling Network In Embryogenesis And Oncogenesis: Signal Diversification Through Combinatorial Ligand-receptor Interactions," *FEBS Letters*, 1997; 410(1): 83–86

135- Sara Sigismund, Emerging Functions of the EGFR in Cancer, *Molecular Oncology*, 2018; Pages 3-20

136- N. Normanno, A. de Luca, C. Bianco et al., Epidermal Growth Factor Receptor (EGFR) Signaling In Cancer, *Gene*, 2006; 366(1): 2–16

137- N. E. Hynes and H. A. Lane, ERBB Receptors And Cancer: The Complexity Of Targeted Inhibitors, *Nature Reviews Cancer*, 2005;5(5): 341–354

138- J. Mendelsohn and J. Baselga, The EGF Receptor Family As Targets For Cancer Therapy, *Oncogene*, 2000;19(56):. 6550–6565

139- Y. Umekita, Y. Ohi, Y. Sagara, and H. Yoshida, Co-expression Of Epidermal Growth Factor Receptor And Transforming Growth Factor-*α* Predicts Worse Prognosis In Breast Cancer Patients, *International Journal of Cancer*, 2000; 89(6): 484–487

140- Shah RB, Ghosh D, Elder JT. Epidermal Growth Factor Receptor (ErbB1) Expression in Prostate Cancer Progression: Correlation With Androgen Independence, *Prostate*, 2006;15;66(13):1437-44

140- Schlomm T, Kirstein P, Iwers L, Daniel B, Steuber T, Walz J, ET AL. Clinical Significance Of Epidermal Growth Factor Receptor Protein Overexpression and Gene Copy Number Gains in Prostate Cancer, *Clin Cancer Res*, 2007;15;13(22 Pt 1):6579-84

7. APPENDICES

7.1 SPSS Results

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotip_rs1468727 * grupp	123	99,2%	1	0,8%	124	100,0%
rs1468727_CC * grupp	123	99,2%	1	0,8%	124	100,0%
rs1468727_TT * grupp	123	99,2%	1	0,8%	124	100,0%
rs1468727_CT * grupp	123	99,2%	1	0,8%	124	100,0%
rs1468727_C * grupp	123	99,2%	1	0,8%	124	100,0%
rs1468727_T * grupp	123	99,2%	1	0,8%	124	100,0%

Genotip_rs1468727 * grupp

Crosstab

			grupp		
			kontrol	hasta	Total
Genotip_rs1468727	CC	Count	24	30	54
		% within Genotip_rs1468727	44,4%	55,6%	100,0%
		% within grupp	38,7%	49,2%	43,9%
		% of Total	19,5%	24,4%	43,9%
	CT	Count	33	26	59
		% within Genotip_rs1468727	55,9%	44,1%	100,0%
		% within grupp	53,2%	42,6%	48,0%
		% of Total	26,8%	21,1%	48,0%
	TT	Count	5	5	10
		% within Genotip_rs1468727	50,0%	50,0%	100,0%
		% within grupp	8,1%	8,2%	8,1%
		% of Total	4,1%	4,1%	8,1%
Total		Count	62	61	123
		% within Genotip_rs1468727	50,4%	49,6%	100,0%
		% within grupp	100,0%	100,0%	100,0%
		% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1,489[a]	2	,475
Likelihood Ratio	1,492	2	,474
Linear-by-Linear Association	,831	1	,362
N of Valid Cases	123		

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 4,96.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Interval by Interval	Pearson's R	-,083	,090	-,911	,364[c]
Ordinal by Ordinal	Spearman Correlation	-,092	,090	-1,018	,311[c]
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value
Odds Ratio for Genotip_rs1468727 (CC / CT)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

rs1468727_CC * grupp

Crosstab

			grupp		
			kontrol	hasta	Total
rs1468727_CC	YOK	Count	38	31	69
		% within rs1468727_CC	55,1%	44,9%	100,0%
		% within grupp	61,3%	50,8%	56,1%
		% of Total	30,9%	25,2%	56,1%
	VAR	Count	24	30	54
		% within rs1468727_CC	44,4%	55,6%	100,0%
		% within grupp	38,7%	49,2%	43,9%
		% of Total	19,5%	24,4%	43,9%
Total		Count	62	61	123
		% within rs1468727_CC	50,4%	49,6%	100,0%
		% within grupp	100,0%	100,0%	100,0%
		% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,369[a]	1	,242		
Continuity Correction[b]	,977	1	,323		
Likelihood Ratio	1,371	1	,242		
Fisher's Exact Test				,278	,162
Linear-by-Linear Association	1,358	1	,244		
N of Valid Cases	123				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 26,78.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for rs1468727_CC (YOK / VAR)	1,532	,749	3,136
For cohort grupp = kontrol	1,239	,859	1,788
For cohort grupp = hasta	,809	,568	1,152
N of Valid Cases	123		

rs1468727_TT * grupp

Crosstab

			grupp		
			kontrol	hasta	Total
rs1468727_TT	YOK	Count	57	57	114
		% within rs1468727_TT	50,0%	50,0%	100,0%
		% within grupp	91,9%	93,4%	92,7%
		% of Total	46,3%	46,3%	92,7%
	VAR	Count	5	4	9
		% within rs1468727_TT	55,6%	44,4%	100,0%
		% within grupp	8,1%	6,6%	7,3%
		% of Total	4,1%	3,3%	7,3%
Total		Count	62	61	123
		% within rs1468727_TT	50,4%	49,6%	100,0%
		% within grupp	100,0%	100,0%	100,0%
		% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,103[a]	1	,748		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,103	1	,748		
Fisher's Exact Test				1,000	,510
Linear-by-Linear Association	,102	1	,749		
N of Valid Cases	123				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 4,46.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Interval by Interval	Pearson's R	-,029	,090	-,318	,751[c]
Ordinal by Ordinal	Spearman Correlation	-,029	,090	-,318	,751[c]
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	95% Confidence Interval	
Value	Lower	Upper

Odds Ratio for rs1468727_TT (YOK / VAR)	,800	,204	3,133
For cohort grupp = kontrol	,900	,488	1,661
For cohort grupp = hasta	1,125	,530	2,389
N of Valid Cases	123		

rs1468727_CT * grupp

Crosstab

			grupp		
			kontrol	hasta	Total
rs1468727_CT	YOK	Count	29	34	63
		% within rs1468727_CT	46,0%	54,0%	100,0%
		% within grupp	46,8%	55,7%	51,2%
		% of Total	23,6%	27,6%	51,2%
	VAR	Count	33	27	60
		% within rs1468727_CT	55,0%	45,0%	100,0%
		% within grupp	53,2%	44,3%	48,8%
		% of Total	26,8%	22,0%	48,8%
Total		Count	62	61	123
		% within rs1468727_CT	50,4%	49,6%	100,0%
		% within grupp	100,0%	100,0%	100,0%
		% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,989[a]	1	,320		
Continuity Correction[b]	,663	1	,416		
Likelihood Ratio	,990	1	,320		
Fisher's Exact Test				,369	,208
Linear-by-Linear Association	,981	1	,322		

N of Valid Cases	123				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 29,76.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Interval by Interval	Pearson's R	-,090	,090	-,990	,324[c]
Ordinal by Ordinal	Spearman Correlation	-,090	,090	-,990	,324[c]
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for rs1468727_CT (YOK / VAR)	,698	,343	1,419
For cohort grupp = kontrol	,837	,589	1,190
For cohort grupp = hasta	1,199	,836	1,721
N of Valid Cases	123		

rs1468727_C * grupp

Crosstab

			grupp kontrol	grupp hasta	Total
rs1468727_C	YOK	Count	6	6	12

		% within rs1468727_C	50,0%	50,0%	100,0%
		% within grupp	9,7%	9,8%	9,8%
		% of Total	4,9%	4,9%	9,8%
	VAR	Count	56	55	111
		% within rs1468727_C	50,5%	49,5%	100,0%
		% within grupp	90,3%	90,2%	90,2%
		% of Total	45,5%	44,7%	90,2%
Total		Count	62	61	123
		% within rs1468727_C	50,4%	49,6%	100,0%
		% within grupp	100,0%	100,0%	100,0%
		% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,001[a]	1	,976		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,001	1	,976		
Fisher's Exact Test				1,000	,607
Linear-by-Linear Association	,001	1	,976		
N of Valid Cases	123				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,95.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Interval by Interval	Pearson's R	-,003	,090	-,029	,977[c]

Ordinal by Ordinal	Spearman Correlation	-,003	,090	-,029	,977[c]
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for rs1468727_C (YOK / VAR)	,982	,298	3,232
For cohort grupp = kontrol	,991	,547	1,797
For cohort grupp = hasta	1,009	,556	1,832
N of Valid Cases	123		

rs1468727_T * grupp

Crosstab

			grupp		
			kontrol	hasta	Total
rs1468727_T	YOK	Count	23	30	53
		% within rs1468727_T	43,4%	56,6%	100,0%
		% within grupp	37,1%	49,2%	43,1%
		% of Total	18,7%	24,4%	43,1%
	VAR	Count	39	31	70

	% within rs1468727_T	55,7%	44,3%	100,0%
	% within grupp	62,9%	50,8%	56,9%
	% of Total	31,7%	25,2%	56,9%
Total	Count	62	61	123
	% within rs1468727_T	50,4%	49,6%	100,0%
	% within grupp	100,0%	100,0%	100,0%
	% of Total	50,4%	49,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,831[a]	1	,176		
Continuity Correction[b]	1,371	1	,242		
Likelihood Ratio	1,835	1	,175		
Fisher's Exact Test				,205	,121
Linear-by-Linear Association	1,816	1	,178		
N of Valid Cases	123				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 26,28.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Interval by Interval	Pearson's R	-,122	,089	-1,352	,179[c]

Independent Samples Test

Ordinal by Ordinal	Spearman Correlation	-,122	,089	-1,352	,179[c]
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for rs1468727_T (YOK / VAR)	,609	,297	1,251
For cohort grupp = kontrol	,779	,537	1,130
For cohort grupp = hasta	1,278	,898	1,819
N of Valid Cases	123		

T-Test

Group Statistics

	grupp	N	Mean	Std. Deviation	Std. Error Mean
tanıyaş	hasta	61	66,9180	7,39661	,94704
	kontrol	17	67,0000	9,08983	2,20461

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
tanıyaş	Equal variances assumed	2,175	,144	-,038	76	,969	-,08197	2,13475	-4,33369	4,16975
	Equal variances not assumed			-,034	22,248	,973	-,08197	2,39941	-5,05483	4,89090

T-Test

Group Statistics

	grupp	N	Mean	Std. Deviation	Std. Error Mean
kiloo	hasta	61	78,8689	11,35998	1,45450
	kontrol	17	78,4706	9,62495	2,33439

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
kilo	Equal variances assumed	,916	,342	,132	76	,895	,39826	3,02161	-5,61980	6,41633
	Equal variances not assumed			,145	29,643	,886	,39826	2,75045	-5,22173	6,01826

T-Test

Group Statistics

	grupp	N	Mean	Std. Deviation	Std. Error Mean
sigarapaketyıl	hasta	50	30,3200	18,72948	2,64875
	kontrol	14	27,4286	17,85719	4,77253

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
sigarapaketyıl	Equal variances assumed	,082	,776	,515	62	,608	2,89143	5,60899	-8,32078	14,10363
	Equal variances not assumed			,530	21,696	,602	2,89143	5,45829	-8,43758	14,22044

T-Test

Group Statistics

	grupp	N	Mean	Std. Deviation	Std. Error Mean
psaa	hasta	61	43,3967	105,80870	13,54742
	kontrol	17	3,1882	2,78700	,67595

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
psaa	Equal variances assumed	8,220	,005	2,101	90	,038	40,03543	19,05902	-2,17137	77,89949
	Equal variances not assumed			2,953	60,167	,004	40,03543	13,55682	12,91930	67,15156

Frequencies

Statistics

ailedekanser

N	Valid	78
	Missing	46
Mean		,4744
Std. Deviation		,50257

ailedekanser

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yok	41	33,1	52,6	52,6
	var	37	29,8	47,4	100,0
	Total	78	62,9	100,0	
Missing	System	46	37,1		
Total		124	100,0		

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
gleasonskoru	62	7,6613	1,24062	,15756

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference Lower	Upper
gleasonskoru	48,625	61	,000	7,66129	7,3462	7,9763

Frequency Table

		Statistic
patolojikevre		
N	Valid	124
	Missing	0

patolojikevre

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		46	37,1	37,1	37,1
	pT2a	7	5,6	5,6	42,7
	pT2b	10	8,1	8,1	50,8
	pT2c	24	19,4	19,4	70,2
	pT3a	11	8,9	8,9	79,0
	pT3b	9	7,3	7,3	86,3
	NA	17	13,7	13,7	100,0
	Total	124	100,0	100,0	

Frequencies

Statistics

evre

N	Valid	61
	Missing	63
Mean		1,6721
Std. Deviation		,62507

evre

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	cT1c	25	20,2	41,0	41,0
	cT2	31	25,0	50,8	91,8
	cT3	5	4,0	8,2	100,0
	Total	61	49,2	100,0	
Missing	System	63	50,8		
Total		124	100,0		

7.2 ETHICAL APPROVALFORMS

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1947 **10/09/2020**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Gülce Yavaş)

Yeditepe Üniversitesi Moleküler Tıp ABD Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu Üroloji A.D. Prof. Dr. Faruk Yencilek'in sorumlu araştırmacı olduğu **"Prostat Kanseri Hastalarında Epidermal Büyüme Faktörü Reseptörü Polimorfizminin İncelenmesi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1939)** kayıtlı Numaralı KAEK Başvuru Dosyası , Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **09.09.2020** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu,yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No:1279)**

Prof. Dr. Turgay Çelik

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

Yeditepe Üniversitesi 26 Ağustos Yerleşimi, İnönü Mahallesi Kayışdağı Caddesi 34755 Ataşehir / İstanbul
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7.3 CASE REPORT FORM

	YEDİTEPE ÜNİVERSİTESİ OLGU RAPOR FORMU

ÇALIŞMANI ADI: Prostat Kanseri Hastalarında Epidermal Büyüme Faktörü Reseptörü Polimorfizminin İncelenmesi

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Grupları için;

- Gönüllü Olma
- Prostat kanseri olma
- 40 – 70 yaş aralığında olma
- Yukarıda belirtilenler haricinde bir hastalığa sahip olmama

Kontrol Grubu için ;

- Gönüllü olma
- Sağlıklı olma (yukarıda belirtilenlerde dahil olmak üzere hiçbir hastalığa sahip olmama)
- 40 – 70 yaş aralığında olma

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü olmama
- 40 – 70 yaş aralığı dışında olma
- Yukarıda belirtilenler dışında bir hastalığa sahip olma