T.C.
YEDİTEPE UNIVERSITY
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THE RELATION BETWEEN VITAMIN D RECEPTOR (rs2228570) POLYMORPHISM AND PROSTATE CANCER

MASTER OF SCIENCE THESIS

Parinaz Khademi

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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 the Administrative Board of the Institute with a decision dated ..... and numbered .....

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Director of the Institute of Health Science

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.

Parinaz Khademi

DEDICATION

I dedicate my thesis to my lovely family, who supported me at all stages.

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LIST OF SYMBOLS AND ABBREVIATIONS

PC	Prostate cancer
VDR	Vitamin D receptor
HCC	Hepato cellular cancer
DBP	Vitamin D binding protein
Nm	Nanometer
µg	Microgram
OD	Optical density
OR	Odd ratio
CI	Confidence interval
CT	Computed tomography
pH	Potential of hydrogen
RT-PCR	Real-Time polymerase chain reaction
PXR	Pregnane X Receptor
CAR	Constitutive Androtane Receptor
SNP	Single nucleotide polymorphism
UV	Ultraviolet light
X-ray	X-radiation
DNA	Deoxyribose nucleic acid
SPSS	Statistical Package For The Social Sciences

ABSTRACT

Khademi, P. (2022). The Relation between vitamin D receptor (rs2228570) polymorphism and prostate cancer. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. MSc thesis, Istanbul.

AIM: To investigate the association between the vitamin D receptor (VDR) gene rs2228570 polymorphism and prostate cancer (PC) and Reduce risk in the Turkish population.

MATERIAL and METHODS: The SNPs of the VDR gene rs2228570 will be genotyped using Taqman^{R} SNP Genotyping Assays and BioScience (ABI) 7500 real-time PCR system. The statistical analysis was carried out using SPSS version 26.0. This research project will be a case-control retrospective study comprising 69 patients with prostate cancer and a control group which consisted of 63 healthy individuals.

RESULTS: In this study, VDR gene (rs2228570) polymorphism was analyzed in patients with PC and control group. There was no significant difference obtained from this study regarding genotype analysis (p=0.475). In the patient group, the genotype frequency of homozygous wild type (AA) was 4.6%, heterozygous genotype (AG) frequency was 41.7%, and homozygous mutant genotype (GG) was 53.9%. In the control group, the genotype frequency of homozygous wild type (AA) was 8.3%, heterozygous genotype (AG) frequency was 40%, and homozygous mutant genotype (GG) was 51.7%.

CONCLUSION: It is the first study that investigating the relation between VDR gene variaton (rs2228570) with prostate cancer susceptibility in Turkish populations. The limited sample size could be a limitation for the study. Thus, new studies with larger populations should be conducted with the VDR polymorphism to show the effect of VDR on PC susceptibility.

Keywords: Vitamin D, Prostate cancer, (VDR) gene (rs2228570) polymorphism

ÖZET

Khademi, P. (2022). D vitamini reseptörü Geni (rs2228570) polimorfizmi ile prostat kanseri arasındaki ilişkinin Arastirilmasi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Yüksek lisans tezi, İstanbul.

AMAÇ: Bu calışmanın amacı Türk popülasyonunda vitamin D reseptör (VDR) geni rs2228570 polimorfizmi ile prostat kanseri (PK) duyarlılığı arasındaki ilişkiyi araştırmaktır.

GEREÇ ve YÖNTEMLER: VDR geni rs2228570'in SNP'leri, TaqmanR SNP Genotyping Assays ve BioScience (ABI) 7500 gerçek zamanlı PCR sistemi kullanılarak genotiplendirilmiştir. İstatistiksel analiz SPSS versiyon 26.0 kullanılarak yapılmıstır. Bu araştırmaya prostat kanserli 69 hasta ve 63 sağlıklı kişiden oluşan bir kontrol grubunu içeren kişiler dahil edilmiştir.

BULGULAR: Bu çalışmada PK'li hastalarda ve kontrol grubunda VDR geni (rs2228570) polimorfizmi analiz edildi. Genotip analizi açısından bu çalışmadan elde edilen anlamlı bir fark yoktu (p=0,475). Hasta grubunda homozigot yabani tip (AA) genotip sıklığı %4,6, heterozigot genotip (AG) sıklığı %41,7 ve homozigot mutant genotip (GG) %53,9 idi. Kontrol grubunda homozigot yabani tip (AA) genotip sıklığı %8,3, heterozigot genotip (AG) sıklığı %40 ve homozigot mutant genotip (GG) %51,7 idi.

SONUÇ: Bu çalışma, Türk popülasyonunda VDR gen varyasyonu (rs2228570) ile prostat kanseri duyarlılığı arasındaki ilişkiyi araştıran ilk çalışmadır. Sınırlı örneklem büyüklüğü çalışma için en büyük dezavantaj olduğu kanaatindeyiz. Bu nedenle, VDR'nin PK duyarlılığı üzerindeki etkisini göstermek için VDR polimorfizmi ile daha geniş popülasyonlu yeni çalışmalar yapılmalıdır.

Anahtar Kelimeler: D vitamini, Prostat kanseri, (VDR) geni (rs2228570) polimorfizmi

1. INTRODUCTION AND PURPOSE

Prostate Cancer (PC) is one of the most common types of cancer in older men across the globe. also known as PC, is the progression of cancer in the prostate that is a gland places in the male reproductive system. Most of the PC grow slowly, but relatively some of them grow quickly. In the metastatic stage cancer cells can spread from the prostate and reach other parts of the body, especially lymph nodes and bones. The symptoms of the disease may not show at first. In more advanced stages, it can cause problems with urination, blood in the urine, or pain in the pelvis and lower back when urinating. The prostate cancer incidence is increasing worldwide while ethnic origin, family history, hormonal status, dietary structure, age and low vitamin D levels are risk factors for PC. (1,2). Furthermore, studies have reported the association between Vitamine D receptor (VDR) polymorphism with an increased risk of prostate cancer (1,3). However, there has been contradiction in the results of numerous studies (4,5). Most of the studies found no association between VDR polymorphism and prostate cancer. Prostate cancer is diagnosed through Tests and biopsies of suspected cancer tissue are performed. For people suspected of having cancer who have early symptoms with common cancer tests, which generally include blood tests, X-rays, CT scans and endoscopy, etc. Be sure whether the patient has or does not have cancer. Medical imaging may also be done later to determine if the cancer has spread to other body parts (6).

There may not be any special symptoms. Obstructive symptoms usually occur earlier and include frequent urination, urinary retention, decreased urine diameter and pressure of urine flow and end dropping, which is similar to the symptoms of benign prostatic hyperplasia. Bone metastases can cause pain. If you see the following symptoms, Disruption in the beginning of urine, Frequent urine, especially at night, Urine drainage in a drop-down manner, Urine withdrawal with low pressure, Presence of blood or blood clots in urine, Pain during ejaculation, Pain during urination, Pain in the lower back and pelvis if intermittent, Loss of weight or appetite unintentionally. (7).

Other genes that may increase the risk of PC include HPC1, HPC2, HPCX, CAPB, ATM, FANCA, HOXB13, and mismatch repaire genes. However, the direct effect of none of them in PC has not been proven. (7).

Most mutations responsible for prostate cancer are Autosomal Ghalib and Autosomal Recessed. In autosomal dominant inheritance, only one copy of the mutated gene increases the chance of developing cancer. In this case, a person's parents may show the effects of the mutated gene or just carry the mutated gene. (6,7).

In the case of autosomal recessive inheritance, a person only faces an increased risk of cancer if they inherit both copies of the mutated gene from their parents. In this case, parents who carry a mutated copy and a healthy copy of the gene do not have an increased risk of cancer. (6).

Normal healthy prostate epithelium progresses to localized PC, then to metastatic PC, and finally to androgen-independent cancer. Proposed early genetic events leading to localized prostate cancer are germline changes, methylation changes, loss of heterozygosity (LOH) of chromosome 8p and changes in the number of androgen receptor CAG repeats Proposed early genetic events leading to localized prostate cancer are germline change. loss of glutathione-s transferase pi gene the third form of cancer predisposing gene heredity is X-chromosome-dependent inheritance. While men only have one copy of the X-chromosome inherited from their mother, women have two copies of the X-chromosome, each inherited from one of their parents. A woman with a predisposing mutation of recessive cancer on one of her X chromosomes does not face an increased risk of developing cancer as a carrier. However, his son, who inherits a mutated version of the gene, faces this increased risk. (7)

However, even if a person carries a mutated version of the gene with dominant inheritance or inherits both copies of the mutated gene with recessive inheritance, or a man's X chromosome carries a mutated version of recessive inheritance, they do not necessarily develop cancer. Some mutations have imperfect influence, meaning that only some people exhibit mutation effects. Some mutations also have variable expressions, meaning that the severity of symptoms varies from person to person. (7).

Regulating vitamin D level in the blood, the amount of oral absorption, synthesis, catabolism and its effectiveness are the same as other reactions of living organisms under genetic and epigenetic control. The amount of this important vitamin in circulation is

associated with the expression of numerous genes such as CG, DHCR1, CYP2R1, VDR, CYP24A1. (8).

Although specialists believe that men should not expect supplements to fix the rapid growth of tumors, new research suggests that prostate cancer can be more invasive in men who are short of vitamin D. A study conducted on almost 200 men whose prostate was deleted showed that those with lower vitamin D levels developed the tumor more quickly than those with natural levels of this vitamin. (8).

Several studies revealed, men with a vitamin D shortage are more possible to have a more advanced illness during prostate surgery, then perhaps if they are diagnosed with prostate cancer in men, they should also be tested for the amount of vitamin D and prescribed vitamin D supplements if they are diagnosed with a deficiency. (9).

Vitamin D is a steroid hormone, which has been shown to inhibit cellular expansion and promote. It has specifically been represented to play a crucial role in the thriving of natural and malignant human prostate cells and has been involved in the pathogenesis of prostate cancer by an ecological study suggesting that prostate cancer mortality was inversely related to the geographic distribution of UV-light. This finding contributes to the hypothesis that the higher incidence of PC in aged men and black men may be associated with vitamin D exposure, which is reduced with aging skin and darker skin pigmentation. (9).

Much epidemiological research has examined the relation of serum 1&25-dihydroxy vitamin D (1,25-D) level to risk of PC, with inconsistent outcomes. Observed a decrease in PC risk with a higher level of 1,25-D among older men, but others reported no association (10). The proactive form of the vitamin D hormone exerts its impact by attaching to the vitamin D receptor (VDR), part of the steroid receiver family, which regulates gene expression. Many polymorphisms in the VDR gene have been detected. These include a Poly-A microsatellite in the 30-flanking area, a shift in intron VIII that produces Bsml and Apal constraints enzyme site, and a 50 Fokl site in exon II that alters the start codon. Given suggestive evidence for a relationship between Vitamin D receptor polymorphisms and the risk of prostate cancer, and conflicting results on the VDR polymorphisms in conjunction

with prostate cancer, we do a case-control large crowd-based study of the Bsml, Fokl, and Poly-A genetic variants concerning PC. (11,12).

Studies of VDR gene function are essential for understanding prostate cancer formation and spread, especially in states that predispose and pre-diagnose for prostate cancer. The VDR gene provides guidelines for constructing a protein called VDR which lets the body answer to vitamin D. This vitamin can be acquired from foods in the diet or made in the body with assistance from sunlight exposure. (13).

Several studies demonstrate that the VDR has a function in promoting the growth of breast cancer cells, independent of its cognate ligand, 1,25(OH)2D. This previously unrecognized function of the VDR is operational both within and outside the bone microenvironment. (14).

As VDR (rs2228570) AA has been provisionally shown to be associated with higher VDR expression than the GG genotype. This result is consistent with a protective effect for VDR in prostate cancer development. (15).

Consequently, all study results about the gene variants Vitamin D (rs2225870) and their role in prostate cancer are insufficient. Therefore, studies are needed to determine the role of Vitamin D polymorphism in association with prostate cancer. (15).

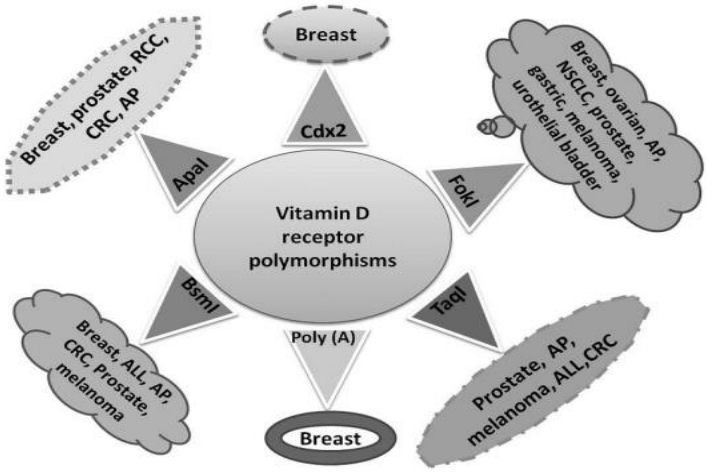

Figure 1 Appears how VDR polymorphisms are related to an expanded hazard of cancer. The vicinity of these polymorphisms is related to an expanded chance of cancer. In any case, these associations may alter depending on components counting age, color, and ethnicity, as examined within the content. (15).

Different researchers have performed a meta-analysis to make the lasting connection between VDR polymorphism and cancer risk. A meta-analysis involving 25 distinct studies totaling 34,018 people released before for CDX2 polymorphism connection with cancer conducted. (15).

Demonstrated that in the homozygous model, CDX2 leap was significantly associated with a rising cancer risk of colorectal and ovarian cancer, particularly in American – African but not in Asians or Caucasians. Data analysis of prevailing, homozygous and recessive comparison models performance a significant connection between cancer risk and CDX2 mutations in Americans – African. Stratifying the data by cancer types revealed a powerful connection between VDR CDX2 polymorphisms and the amplified risk of colorectal and ovarian cancer. (15).

Analyzed the connection between VDR polymorphisms (Bsm1, Taq1, Fok1, Cdx2, and Apa1) and various types of cancer such as ovarian cancer, thyroid cancer, renal cell cancer, sarcoma, hepato cellular cancer (HCC), skin (melanoma and nonmelanoma skin cancer), leukemia, non-Hodgkin's lymphoma, bladder cancer, oesophageal adenocancer (EAC), head and neck and oral squamous cell cancer including 79 independent studies, for a total of 52,427 cases and 62,225 controls. (15).

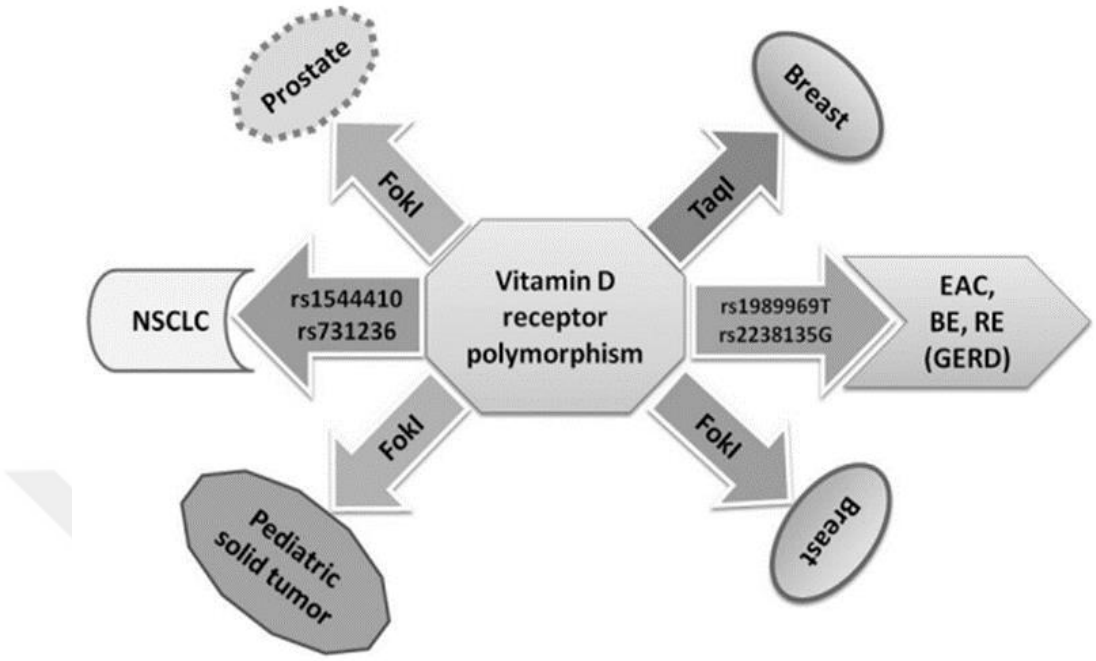

Figure 2. (VDR) polymorphisms are connected with a risk of cancer reduced. The existence of these polymorphisms is connected with a decreased risk of cancer. (15).

Table 1. Vitamin D receptor (VDR) polymotphisms and prostate cancer (TaqI).(11).

Polymorphism under study	**Number of study subjects**	Type of study	**Outcome of the study**
TaqI	27 Eligible studies included 12,276 cases and 13,506 controls	**Meta-analysis (11)**	TaqI genotypes TT and TT/Tt were significantly higher in the advanced prostate cancer and is associated with the risk in Asians.

Table 2. Vitamin D receptor (VDR) polymorphisms and prostate cancer (Fokl).(13,18,22,42).

FokI	27Case–control studies with 10,486	**Meta-analysis (13)**	Overall-no significant association. Significant
	cases and 10,400 controls		association in Caucasian population
FokI	9,720 Patients and 9,710 controls	**Meta-analysis (18)**	Increased risk of developing prostate
			cancer in Caucasian population.
	10 Studies involving 4979 cases and 4380	**Meta-analysis (22)**	
	controls (Cdx2) and 11 studies involving		not associated with prostate cancer
	2837 cases and 2884 controls (Apal)		
FokI	124-Jordanian prostate cancer	**Case–control study (42)**	No significant association

The study concluded that significant associations exist between VDR polymorphisms and prostate (Bsm1, Taq1, Fok1), breast (Fok1, Apa1, Bsm1), colorectal (Fok1, Taq1, Bsm1), and skin cancer (Bsm1, Taq1, Fok1) (Figures 1and2). (16).

Table 3. Vitamin D receptor (VDR) polymorphisms and prostate cancer (FokI,BmsI-ApaI,TaqI-rs731236, rs7975232-rs731236, rs7975232-rs3782905, rs1544410-rs2239185.(40).

FokI, BsmI	**Peripheral blood of 342 patients: 132 PC,**	**Case–control study (41)**	**VDR polymorphism has a genotype-**
ApaI, TaqI	41 BPH and 169 young healthy volunteers		phenotype association with prostate cancer.
rs731236, rs7975232,	446 AA men aged 35 to 93 years and	**Case–control study (43)**	Significant association between
rs731236, rs1544410,	379 control from AAPCA and Vitamin D and		VDR polymorphism and prostate
rs3782905, rs1544410,	Prostate Cancer Risk in AA Men Study		cancer risk in African-American men.
rs2239185			

BPH: benign prostate hypertrophy. AAPCA: African-American Sporadic Prostate Cancer Study; AA: African-American,

Table 4. Vitamin D receptor (VDR) polymorphisms and prostate cancer (Bmsl-FokI, Bsml-ApaI, and Taqαl).(40,41).

BsmI	**7,666 Patients and 8,073 controls**	**Case–control study (44)**	**BsmI polymorphism may be related to**
			prostate cancer in Caucasian descendants.
FokI, BsmI,	Blood DNA sample from 50 patients	**Case–control study (45)**	A and B alleles of the VDR ApaI and BsmI
ApaI and TaqαI	and 79 age-qualified controls		loci are associated with risk of prostate cancer.

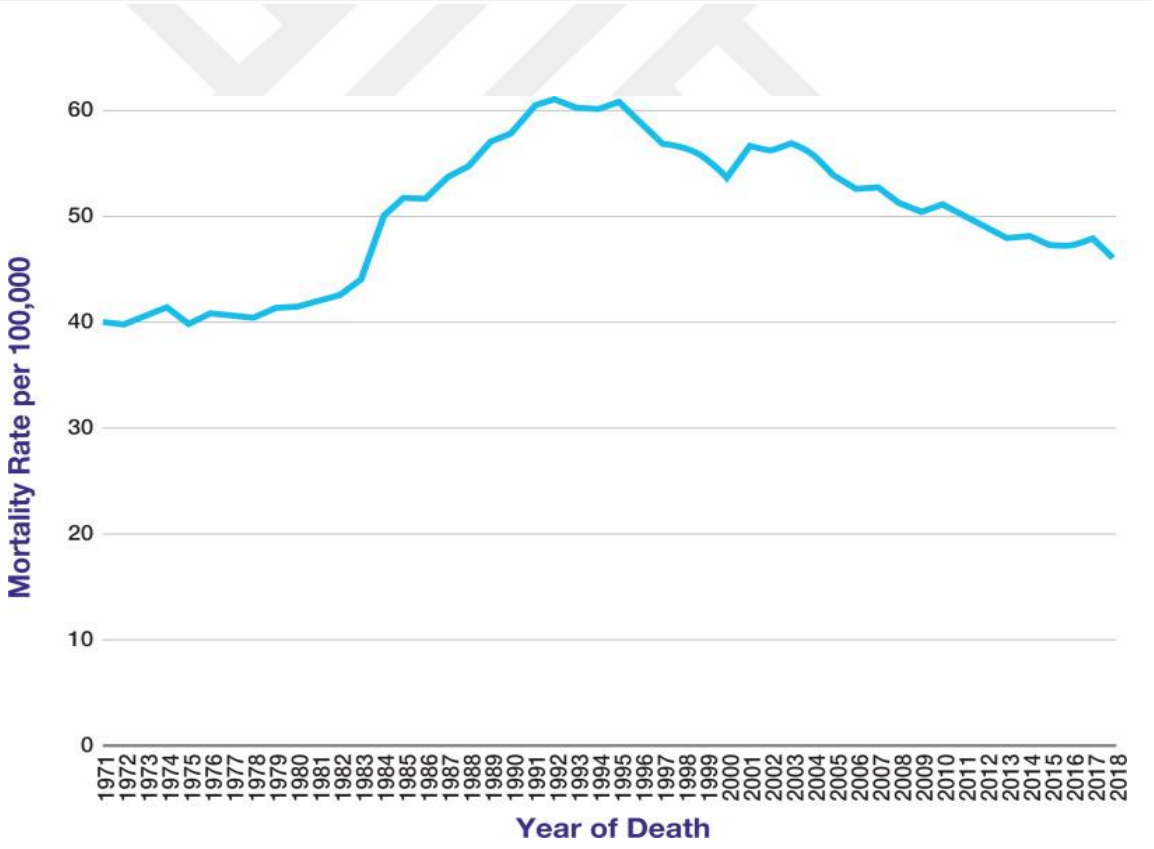

Figure.3. PC mortality Standardized mortality rate in Europe [1]

[1] Source: [accessed on 6 April-2021] www.cancerresearchuk.org.

2. LITERATURE REVIEW

2.1. Vitamin D Metabolism

Vitamin D is produced endogenous by exposure to sunlight in the body or enters the body through food containing vitamin D or dietary supplements (vitamin D). (17).

Vitamin D in the body is converted into its active form, i.e., 1 and 25 dihydroxyvitamin D (1,25(OH)2 D), a hormone that regulates phosphorus and calcium metabolism. Vitamin D deficiency disrupts bone formation, rickets in children, and osteomalasia in adults. Vitamin D and its metabolites may be classified as cholecalciferol or ergocalciferol. Cholecalciferol (vitamin D3) is a compound from the vitamin D family that is naturally produced in the skin of 7-dehydrocholesterol due to exposure to ultraviolet B protein from sunlight. Geographical location, season, age, sunscreen, and skin color affect the production of vitamin D3 by the skin. Vitamin D2 (ergocalciferol) is another compound in the family of vitamin D, which is made by radiation to ergosterols produced by yeasts. (17).

Vitamin D_2 is distinguished from vitamin D_3 by a double bond between carbon 22 and carbon 23, as well as a methyl group on carbon 24. (17).

When vitamin D or its metabolites are written without index and subtitles, they are both families. Only a limited number of foods naturally contain significant amounts of vitamin D, primarily fish liver oil, oily fish, egg yolks, and liver. Before foods were fortified with vitamin D_3 or D_2, most of the vitamin D in the body was made in the skin. In North America, a significant fraction of vitamin D is obtained by absorption from fortified foods (bread, some cereals, and milk) or vitamin D supplements. The recommended amount is 400 IU (10µg) per day. In some European countries, the recommendation for people over the age of 60 is 800 IU or (20µg). However ،some doctors in the USA have prescribed up to 2،000 IU of vitamin D daily to treat or prevent osteoporosis and ensure optimal absorption of dietary calcium. (17).

Vitamins D_3 and D_2 are metabolized in the liver by the enzyme 25 hydroxylase vitamin D, a cytochrome P450 enzyme. The concentration of vitamin D-25-hydroxylase in serum is a maximum 10-65 ng/ml or 25-162 nmd/l. The circulating half-life of vitamin D 25

hydroxylase is between 2 and 3 weeks. At a concentration of nearly 30 ng/ml of vitamin D 25 hydroxylase, dietary calcium absorption is the highest. Therefore, any reference intervals for the enzyme vitamin D 25 hydroxylase should not be confused with its healthy range and optimal. At physiological concentrations, vitamin D25 hydroxylase is biologically inactive in dietary calcium absorption and has no effect. (18).

Vitamin D_3 and vitamin D_2 to 25 and 1 dihydroxyvitamin D (OH)2 D 1,25) are metabolized as biologically active hormones, by 1-hydroxylase 25 vitamin D (cytochrome P450) in the kidneys and placenta. Its normal circulation concentrations, maximum of 15-60 pg/ml or 144-36 pmol/L, are about 25 hydroxylase vitamin D enzymes. The half-life of 1 and 25 dihydroxyvitamin D is between 4 and 6 hours. The circulating concentrations of 1 and 25 dihydroxyvitamin D are strongly regulated primarily by PTH, phosphate, calcium, and self-1 and 25 dihydroxyvitamin D (OH)2 D 1,25). PTH and hypophosphatemia increase synthesis of 1 and 25 dihydroxyvitamin D by increasing the action of vitamin D 25-hydroxy-1-hydroxylase, just as hypocalcemia indirectly increases by stimulating PTH secretion. (18).

Reduces hypercalcemia, hyperphosphatemia and 1&25 dihydroxyvitamin D, 25 hydroxyvitamin D – 1 and 25 dihydroxyvitamin D. 1-25 induces dihydroxyvitamin D, 25-hydroxyvitamin D 24-hydroxylase, an enzyme that produces 24 and 25 dihydroxyvitamin D, the most common form of vitamin D dihydroxylate in serum. This enzyme is also responsible for inactivating 1 and 25 dihydroxyvitamin D via the 24-oxidation pathway that leads to the formation of total tropic acid. (18).

In circulation, vitamin D, 1 and 25 dihydroxyvitamin D, and 25-hydroxy vitamin D are bonded to vitamin D binding protein (DBP), a high-propensity specific transport protein also known as G-C globulin or a specific compound of the serum group. DBP is part of the family – photoprotein, and albumin. In humans, DBP contains 458 amino acids with a molecular weight of 335.5 daltons, which are constantly synthesized by the liver and circulating in high amounts (about 400 µg/L) in the blood, occupying less than 5% of its vitamin D binding sites. DBP binds to vitamin D and its metabolites, especially the metabolites of vitamin D-25-hydroxylate, 24 and 25 dihydroxyvitamin D and 25&1 dihydroxyvitamin D. Only 0.03% of 25-hydroxyvitamin D and 0.4% of 1 and 25 dihydroxyvitamin D are normally free in

plasma. DBP concentration increases during pregnancy and during estrogen therapy and decreases in nephrotic syndrome. (19).

Biological Function 1 and 25 Dihydroxyvitamin D

1 and 25 dihydroxyvitamin D helps maintain levels of phosphate and calcium in the blood through its function on the small intestine, bone, kidney, and parathyroid. In the small intestine, 25 and 1 dihydroxyvitamin D stimulate calcium absorption, especially in the duodenum, and phosphate absorption in the jejunum and ileum. The three events of calcium absorption from the diet include:

Calcium entering the border brush of the cytoplasm by the epithelial transporterCa2+ or channel (CaT1 of the ECAC2 gene on Ch 7q 34)

Calcium release into a grown cell (fostered) with calbindin-D9k ،a calcium bonded cytosol protein (symbol gene S100G) located on X chromosome ،isolated from calbindin-D28k located on chromosome 8 (symbol gene CALB1). (19).

Calcium ejection from the cell via the basolateral membrane by the action of Ca ATPase (e.g., Na+/Ca 2+). CaT1 synthesis is almost 90% dependent on vitamin D, and Calbindin D synthesis is entirely dependent on vitamin D. Since knockout mice without Calbidin-D expression do not exhibit defects in calcium absorption and on the other hand, increases calcium absorption in response to vitamin D, the necessity of Calbindin-D9K for calcium absorption is questionable. A high-calcium diet reduces the expression of CalbindinD and CaT by reducing products 1 and 25 dihydroxyvitamin D. At high concentrations, 1 and 25 dihydroxyvitamin D increases reuptake, by inducing monocytic stem cells to differentiate into osteoclasts in bone marrow and by stimulating osteoblasts to produce cytokines and other factors that affect osteoclast activity. By stimulating osteoblasts, 1 and 25 dihydroxyvitamin D also increases the concentration of alkaline phosphatase and osteocalcin in circulation. In the kidneys, 1 and 25 dihydroxyvitamin D inhibits its synthesis in a very short negative physical loop and stimulates its own metabolism. (20).

Also, 1 and 25 dihydroxyvitamin D directly affect the parathyroid glands in inhibiting synthesis and secretion of PTH. In addition to the direct transcription mechanism, 25 and 1

dihydroxyvitamin D increase the concentration of calcium sensing receptors in the parathyroid gland, thus making the gland sensitive to calcium inhibition. (20).

2.2. The role of Vitamin D Receptor in prostate cancer

The study of genetic variations in VDR may elucidate the association of vitamin D levels, its metabolism, and VDR polymorphism with PC. However, you may not know that many chronic diseases are the association between low levels of vitamin D and PC has been proven throughout the world. Prostate cancer: is directly related to vitamin D shortage in the body. Prostate cancer occurs mostly in older men and, according to the American Cancer Society, the most common disease and a leading cause of death in men is cancer. Anti-proliferative effect on prostate cells is mediated by the VDR active form of vitamin D. In a case-control study, we reviewed whether the vitamin D receptor (VDR) gene (rs2228570) polymorphism could influence PC sensitivity. (21)

In target tissues, 25 and 1 dihydroxyvitamin D exerts its function through communication and binding to the specific nuclear receptor vitamin D (VDR), analog of steroid receptors for androgens, estrogens and corticosteroids. This receptor is expressed in very large amounts in tissues, and most cells respond to 25 and 1 dihydroxyvitamin D. VDR can form a heterodimer with membrane retinoid X receptors. VDR is a member of the NR1 family of receptors, including PXR (Pregnane X Receptor) and CAR (Constitutive Androstane Receptor). VDR gene is located on chromosome 21 of the Q arm (21q1). Vitamin D_3 receptor is 427 amino acids long and binds to DNA through amino acids 21 to 96 in the form of NR C4-type Zing finger at two positions 24 to 44 and 60 to 84. The Lola region contains amino acids 97 to 191. The ligand-binding area is limited to amino acids 192–427 along with the vitamin D bound area in amino acids 227 to 237 and 271 to 278. (21).

2.2.1. Receptors Positions

There is an inverse Association between the metabolic concentration of vitamin D in the blood and the prevalence of certain cancers and a variety of other disorders. These findings have led to an increased need for vitamin D testing and increased daily vitamin D intake requests. Despite a significant reduction in the spread rate of various cancers through the use of vitamin D, in clinical trials it is difficult to demonstrate. (20)

Vitamin D, as one of the basic vitamins in the body, plays a significant role in preventing cancer and stopping the growth of cancer cells. The likelihood of developing a variety of cancers in persons with less blood vitamin D levels is about 20 percent higher than in those with natural blood vitamin D (21-23).

Vitamin D is one of the most important vitamins needed by the body; its deficiency may cause various diseases. Also, based on the research, vitamin D can prevent various cancers and significantly reduce the thriving of cancer cells. Recently, studies have been conducted in Japan that have resulted in important results (24).

The results of this research showed that vitamin D consumption stops the thriving of cancer cells andecreasesse the size of cancerous tumors. Vitamin D, in addition to strengthening the immune system and maintaining bone health, plays an important role in improving neural communication and muscle function. Doctors recommend that adults must use approximately 600 IU of vitamin D on an intraday basis. As you know, sunshine is the main origin of vitamin D. (25)

As mentioned above, vitamin D is one of the most essential vitamins for maintaining health and preventing various diseases. Vitamin D, in addition to sunlight, is available in many foods such as salmon, egg yolks, and dietary supplements. As a result of the research, scientists concluded that vitamin D deficiency in the body causes a variety of cancers and leads to the rapid growth of cancer cells. (26, 27)

In Japan, researchers conducted a type of experiment on about 33,000 people with vitamin D deficiency and collected information. The subjects tested were about 40 to 69 years old. They were constantly monitored by doctors for 16 years and their physical condition was examined. After this period, about 3,301 people were diagnosed with cancer among the participants. (28,29).

Also, based on these studies, the results conclude that in individuals with high vitamin D, the risk of liver cancer decreases by about 30 to 50 percent. According to scientists and researchers in subsequent experiments, blood vitamin D concentration should be determined to determine how much vitamin D can be effective in preventing cancer. In general, it can

be said that vitamin D in the blood is very necessary, and supplements should be used in case of deficiency of vitamin D in the body. (30)

Many geographical studies conducted on different people demonstrate that the risk of PC in men who receive vitamin D daily through sunlight is much lower than in others. Some researchers also believe that deficiency of vitamin D in the blood enhances the risk of PC. While this research has never been proven and only remains the form of theory. (31,32).

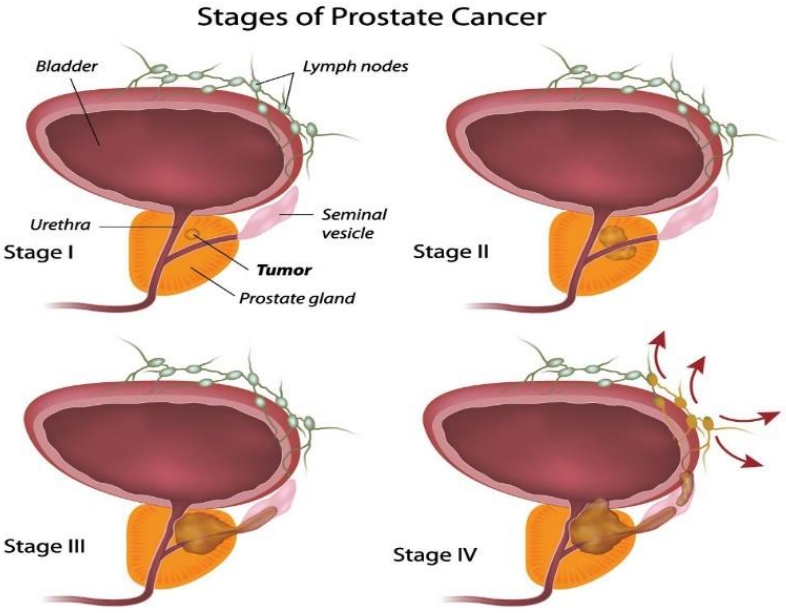

Figure 4. Since age is already having an impact on this body, prostate cancer is virtually always fatal. As white blood cells decline with age, a man begins to struggle more against germs after age 60. Prostate cancer is one of the most riskous illnesses that come with aging (25).

Scientists conducted a very extensive and large study on men who had been detected with prostate cancer a year after undergoing a blood test. The result of this study proved that high blood vitamin D levels have no relation to the rising risk of PC. Based on the obtained outcome, a higher vitamin D level can raise the risk of invasive diseases. (33)

Figure 5. universal diversity in age-standardized prostate cancer incidence rates[1]

2.3 The Impact of Vitamin D on the Reduction of Other Rare Cancers

Scientists achieved new results by analyzing data on the Impact of vitamin D intake on seven types of rare cancers. The experiment was conducted on about 12,000 men and women, and the researchers matched the participants based on the sampling date. After analyzing and analyzing the data, there was no general relationship between non-Hodgkin lymphoma cancer and inner layer cancer of the uterus, kidney, ovary, esophagus, and stomach with vitamin D. (34)

Table 5. Assessment count of accident cases from 2018 to 2040, Prostate Cancer, males, all ages

	2018	2040			
	Number	Number	Demographic Change	Change in risk	Overall Change
Africa Males (APC 0%)	80,971	178,634	97,663 (+120.6%)	97,663 (+120.6%)	97,663 (+120.6%)
Latin America and the Caribbean Males (APC 0%)	190,385	382,808	192,423 (+101.1%)	192,423 (+101.1%)	192,423 (+101.1%)
North America Males (APC 0%)	234,278	312,901	78,623 (+33.6)	78,623 (+33.6)	78,623 (+33.6)
Europe Males (APC 0%)	449,761	585,143	135,373 (+30.1%)	135,373 (+30.1%)	135,373 (+30.1%)
Asia Males (APC 0%)	297,215	597,180	299.965 (+100.9%)	299.965 (+100.9%)	299.965 (+100.9%)

[1] Source: GLOBOCAN 2018

2.4 Reducing the Risk of Cancer with the Impact of Vitamin D on body mechanisms

The mechanisms that have been altered by Vitamin D and reduce the risk of cancer are still unclear, and scientists are researching and studying this issue. The evidence obtained based on laboratory studies shows that vitamin D is one of the most important precursors and triggers of cell differentiation. Also, the results of this experiment showed that vitamin D stimulates the planned death of cells and dramatically reduces the growth and proliferation of cancer cells. (35-37).

In fact, Vitamin D binds to vitamin D receptors by entering the blood and thus affects the function of cancer cells. VDR is responsible for regulating gene transcription. (38).

Some genes regulated by vitamin D produce calbindin protein. s of proteins plays a role in calcium absorption by the intestines. Cyp3a4 gene is another gene that is regulated by vitamin D. Cyp3a4 gene products detoxify bile acid. (39).

So many scientists speculate that LCA damages the DNA of intestinal cells and makes it possible to advance and launch colon carcinogenicity. It is also possible That Vitamin D plays a protective role vs. carcinoma by stimulating the detoxifying enzyme. (40,41).

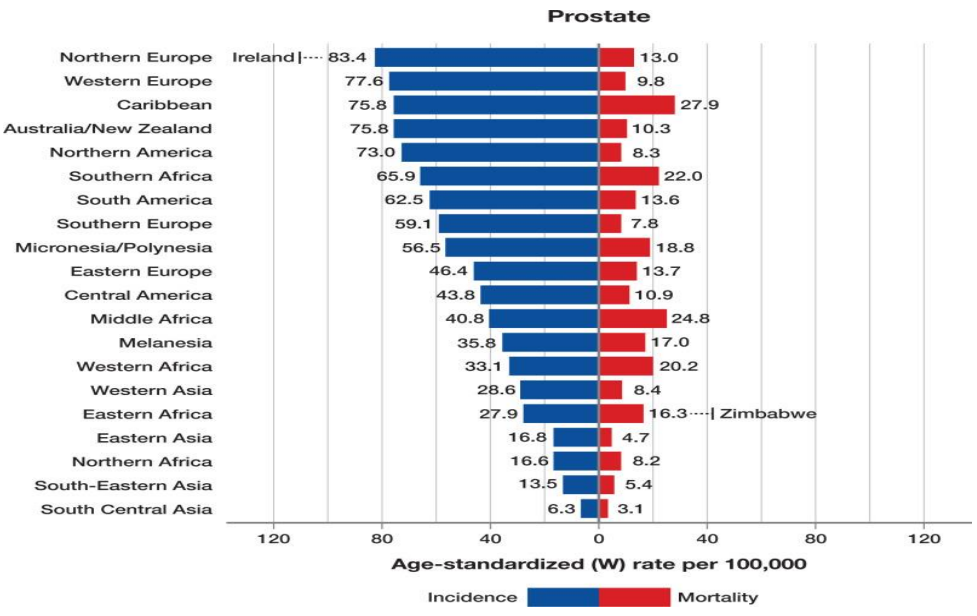

Figure 6. Region-specific occurrence and mortality. Age-standardized rates for prostate cancer in 2020.

2.5 The connection between vitamin D shortage and prostate cancer

Although experts believe that men should not expect supplements to fix the rapid growth of tumors, new research suggests that prostate cancer can be more invasive in men who are short of Vitamin D. A study conducted on almost 200 men whose prostate was deleted showed that those with lower vitamin D levels developed the tumor more quickly than those with natural levels of this vitamin (42-45).

2.5.1 Another relation between vitamin D lack and PC

In the past decade vitamin D has been shown to regulate a much wider array of cellular events than previously thought possible. This study cannot substantiate that vitamin D shortage causes invasive prostate cancer, just to show that both are related. (46). But some believe the findings of this research matter enough to encourage us to research more about the probable association of vitamin D with PC." This is a hypothesis valuable experimenting with. (47-48). Currently, however, he does not think the evidence is sufficient to advise taking vitamin D supplements to avoid prostate cancer or its invasion.

Display determines checking the connection between prostate cancer and vitamin D for a while. Many race differences were observed in this evaluation, which is proof that in black men, tumors are more invasive and have less vitamin D level than white men. (49).

According to these results, one reason black men are more susceptible to PC and more death from the same disease is due to more vitamin D deficiency in them due to sun-induced melanin obstruction and perhaps differences in their diet. However, the study failed to prove that. (50).

If a dark-skinned man develops prostate cancer or has increased PSA (prostate-specific antigen), Murphy recommended testing him for vitamin D deficiency. He believes that those with low vitamin D levels can take supplements (51,53).

Analysts found that roughly 46% of men with serious cancer had vitamin D levels that were roughly 16% less than men with moderate tumor movement within the consider, which included 190 men experiencing prostate surgery (54).

2.6 Aggressive prostate cancer vs. Vitamin D levels

After calculating age, PSA level, and abnormal rectal examinations, Murphy and his associates realized that a vitamin D level of less than 30 ng/mL of blood was associated with high-probability aggressive prostate cancer. (55).

2.7 The effect of age on the synthesis of vitamin D

In the skin of elderly people is less efficient in production Vitamin D shows up after absorbing ultraviolet rays, because their skin is thinner and has less cells for synthesizing vitamin D. At the same time, in elderly people, exposure to sunlight is less, kidneys have less ability to convert this vitamin into its active form. (Reduction of liver function and tissue blood flow) Two-thirds of the elderly with hip fracture show a deficiency of this vitamin. Sun exposure of the arms, legs, hands, and face for 10-15 minutes 2-3 times a week seems to provide adequate vitamin D even in winter, except in those who are unable or unwilling. They want to go out of the house. (55).

3. MATERIALS AND METHODS

Prostate cancer (PC) is the most prevalent cancer among men globally and its pathogenesis is complicated. Prostate cancer is affected by environmental and familial factors. Vitamin D can counteract both of these factors. In some cancerous tumors, a decrease in serum concentration has been announced. although, the findings about prostate cancer are uncoordinated with each other. (56). Polymorphism of the VDR gene may also be implicated in the expansion of this cancer. The Hardy-Weinberg Equilibrium (HWE) – $p > 0.05$ was in all studied polymorphisms. deflection from the HWE was not found. There was no distinction between the study group and the control group. No distinction was found when the groups were compared in consideration of age or the Gleason score. (57).

3.1. Sample Selection and Definition

In this research, patients recognized with prostate cancer (n=69) and the control group (n=63) were examined.

3.1.1 Patient group: The patient group included individuals obtained from Yeditepe University Hospital. This group consisted of individuals aged between 45-75 who were suffering from prostate cancer.

3.1.2 Control group: The control group contained individuals aged between 45 to 75 who were not diagnosed with prostate cancer by clinical studies.

3.2. Materials and Devices Used in the Experoment

3.2.1. Materials Used in DNA Isolation

DNA isolation was carried out from peripheral venous blood samples. Those samples were stored in tubed at +4^{o}C till the experiment started. EDTA contained tubes that were used in order to prevent blood clotting. For the DNA isolation, DNA Isolation Robot (Invitrogen and the Thermo Fischer Scientific Inc, Iprep pure link) system was applied.

3.2.2. The Equipment Used in the Experiment

In this study, DNA Isolation Robot (Iprep pure link, Invitrogen and the Thermo Fischer Scientific Inc), Nanodrop 2000 (Thermo Fisher Scientific Inc), Real-Time PCR (Applied Biosystems, Fast Real Time 7500), plate Centrifuge (Hettich), 7500 Fast Real-Time PCR Instrument, Centrifuge (Centrifuge 22R-Bechman Coulter),+4^{o}C Refrigerator (Haier), -20^{o}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. (58).

3.3. Methods

3.3.1. Genomic DNA Isolation from Blood

All venous blood of control and patient 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^{o}C A bot of iPrep DNA extraction (Invitrigen) and from the genomic DNA isolation Kit with iPrep was used for DNA isolation. By using this system, DNA can be isolated from 350 yL of peripheral blood, therefore this system allows to run 13 blood samples simultaneously. (59). For each sample one cartridge is used, and these cartridges are shaken for a while to ensure that the magnetic beads bind effectively with the DNA before placing the sample in their cartridge. (60)

iPrep robot operates according to ChargeSwitch^{R} (CST^{R}) technology, acting as a calibrated extraction procedure. This precedure Provides an advantage to isolate high amount of genomic DNA from the samples. In thid procedure, elevated amounts of pure genomic DNA may be obtained from samples by utilizing paramagnetic particles. (61).

3.3.2. Real Time PCR Procedure

The reagents used in Real-Time PCR and reaction mixture have been indicated in Table.6. The total volume for each sample was detected according to Real-Time PCR protocol. The reaction mixtures have been represented in table below for Real Time PCR protocol. (62).

Table 6. The mixtures of Real-Time PCR reaction

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

Real Time PCR steps were organized; After a waiting time of 10 minutes at 95ºC, the denaturation process was continued for 15 seconds per cycle at 92ºC and finally elongation to 60 seconds for each cycle at 60ºC. As can be seen in Table 7 denaturation and elongation / connecting processes were applied as 40 cycles. The conditions required for Real-Time PCR are comprehensively shown below.

Table 7. Real-Time PCR Conditions

40 cycle	Temperature ºC	Duration / Period of Time
Hold	95 ºC	10 minutess
Denaturation	92 ºC	15 seconds
Connection / Elongation	60 ºC	60 seconds

3.4. Statistical Analysis

To obtain the numeric values, the student's t-test was applied in this thesis. After all information was obtained by genotyping, this information was assessed in SPSS 26.0 with Fischer's Exact Tests and Chi-square tests to complete the statistical analysis. Fischer's Exact and Chi-square Tests were applied to comment the genotypes dispersion and alleled in groups. If P values could be lower than 0,05, it shows statistical significance.

4. RESULTS

4.1. Demographic Characteristics Results of Study Patients

To Analyse and indicate the single nucleotide polymorphism within the (rs2228570) gene, Extensive demographic results of 69 individuals diagnosed with prostate cancer and 63 healthy individuals as control have been represented in Table 8. Both the patient and control groups consist of male participants.

Table 8: Demographic Characteristics of Individuals

Parameter	Prostate cancer (n=69)	Control (n=63)	p Value
Age	67.61 ± 7.349	67.50 ± 7.813	0.960
BMI	27.01 ± 3.715	27.78 ± 3.817	0.486
Smoking	30.56 ± 18.696	27.64 ± 17.682	0.636
PSA	48.3391 ± 103.37796	3.2929 ± 2.298186	0.00001
Family history of cancer (%)			
Yes	37(44.6%)	-	-
No	46(55.4%)	-	-
Gleason score, mean ± SD	7.74 ± 0.885	-	-
Pathological T-stage, n (%)			
T_2a	7 % 5.6	-	-
T_2b	10 % 8.1	-	-
T_2c	24 % 19.4	-	-
T_3a	11 % 8.9	-	-
T_3b	9 % 7.3	-	-
Clinical T-stage n (%)			
CT_1c	25 % 20.2	-	-
CT_2	31 % 25.0	-	-
CT_3	5 % 4	-	-

Mann-Whitney U-test and Kruskal-Wallis test. Chi-square and Fisher's Exact Tests were applied to statistically analyzethe genotype distribution and alles in the groups.

The chi-squared test was used for cross tables. P values less than 0.05 were considered significant.

4.2. Statistical Assessment of Real-Time PCR Findings

Expression and readings of fluorescent radiation with the contribution of dyes in the probes. However, some samples could not be distinguished and interpreted. In this study, two different dyes corresponding to the FAM color blue and the VIC color green were used. ROX corresponds to nvestigate and interpret the glow curves, we placed a reference color to compare the FAM and VIC dyes. And we evaluated Allelic discrimination.

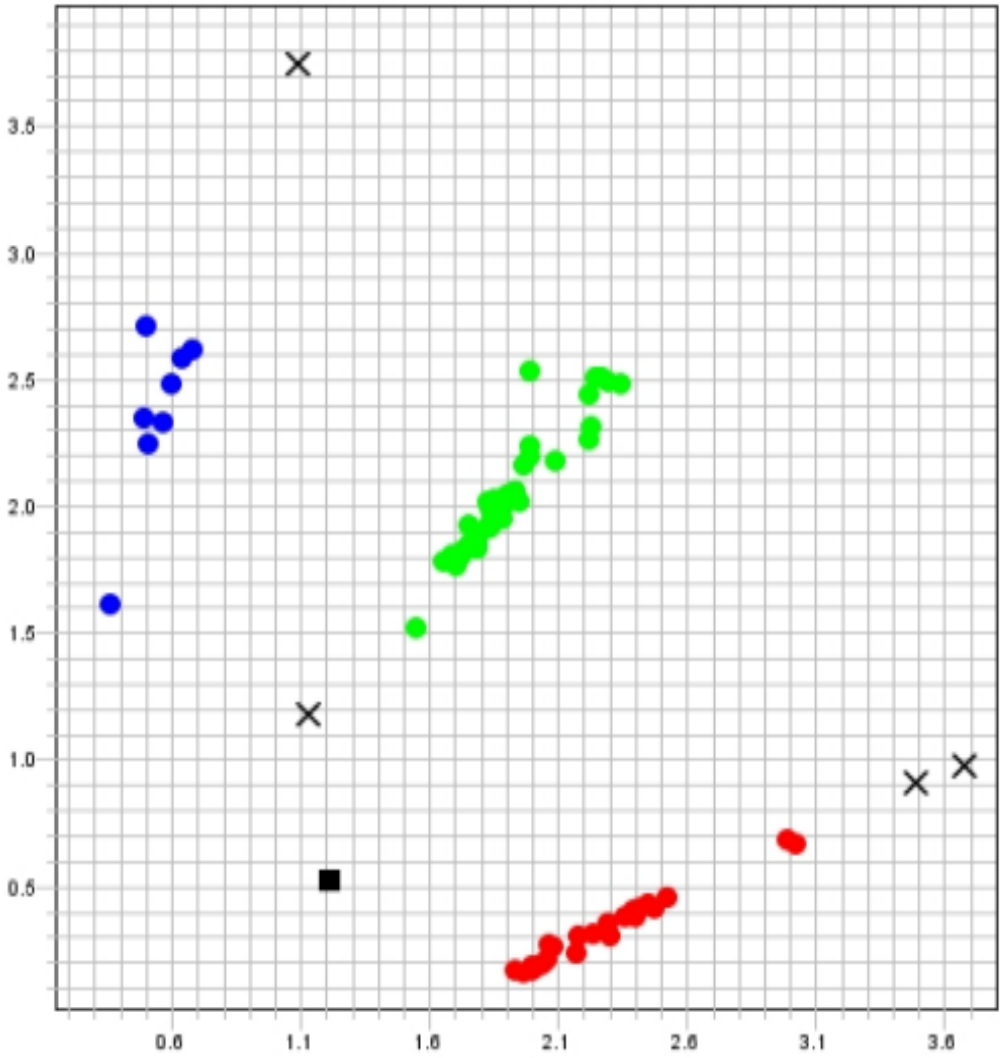

Figure 7. Allelic Discrimination plot of (rs2228570) genotype

According to (Figure.7), there is allelic discrimination shown. AA, which is seen as red dots is a homozygous wild type, AG which is seen as green dots is heterozygous, and GG which is seen as blue dots is a mutant homozygous type.

In Figure 8, the plot represents amplification plots of A and G allele. The yellow line is the threshold value as 0.03066.

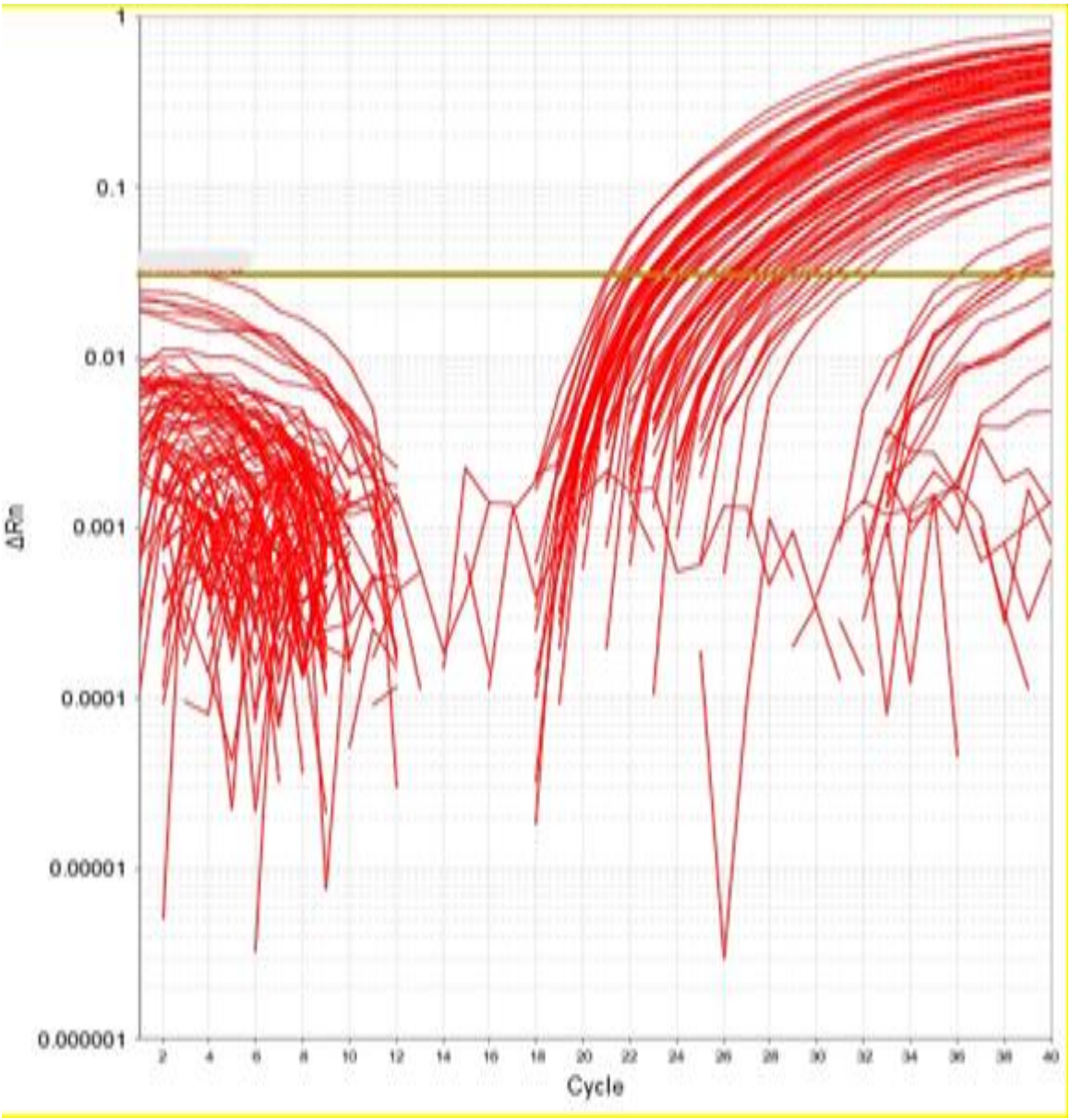

Figure 8. Allele A and G discrimination plot

4.3 Genotyping Analysis of Study Groups

In this study, VDR gene (rs2228570) polymorphism was analyzed in patients with PC and control groups. The relationship between these two groups is shown in Table 9. There was no significant difference obtained from this study regarding genotype analysis (p=0.475).

In the patient group, the genotype frequency of homozygous wild type (AA) was 4.6%, heterozygous genotype (AG) frequency was 41.7%, and homozygous mutant genotype (GG) was 53.9%. In the control group, the genotype frequency of homozygous wild type (AA) was 8.3%, heterozygous genotype (AG) frequency was 40%, and homozygous mutant genotype (GG) was 51.7%.

Table 9. Genotypes and alleles distribution of working groups.

VDR Genotype	PC Patient (n=69)	Control Group (n=63)	p Value	Odd Ratio (OR)	Confidence Interval (%)
AA	3 4.6%	5 8.3%	0.479 (NS)	0.532	0.122-2.330
AG	27 41.5%	24 40%	0.861 (NS)	0.861	0.532-2.177
GG	35 53.9%	31 51.7%	0.807 (NS)	1.091	0.532-2.204

**(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 strdent t-test was used to determine the differences between twp groups.

According to Table 9, VDR Genotypes were compared, and we found that homozygous mutant AA frequency was 8.3% in control groups and 4.6% in cases, heterozygous mutant AG was 40% in control and 41.5% in cases, and homozygous wild GG was 51.7% in controls and 53.9% in cases. Non-significant difference was shown between both groups for genotypes, homozygous mutant, homozygous wild, and heterozygous mutant.

As is shown in Table 10, there was no significant difference in allelic frequencies, too. When the control and patient groups were compared for the A allele, there was no significant difference between the groups (p=0.807). However, there was no significant difference

between the patient and control groups regarding the G allele (p=0.479), G allele frequency was higher in-patient group (74.7%) than in the control group (71.7%).

Among the cases group, one had Allele A, and 97 had Allele G. In controls groups, 34 had Allele A and 86 had Allele G, according to these results the VDR gene (rs2228570) Alleles distributionise a bit similar, and no significant difference was found between groups which p values of Allele A are 0.807 and Allele G is 0.479.

Table 10. VDR (rs2228570) genotype and alleles frequency comparison.

VDR Genotype	PC Patient (n=69)	Control Group (n=63)	p Value	Odd Ratio (OR)	Confidence Interval (%)
Allelic Distribution					
A Allele	33 25.3%	34 28.3%	0.807	0.916	0.454
G Allele	97 74.7%	86 71.7%	0.479	1.879	0.429

**(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 strdent t-test was used to determine the differences between twp groups.

Although there was no significant difference between the PC and healthy control groups regarding the VDR gene, the first and only study was the research association between the VDR gene (rs2228570) polymorphism and prostate cancer susceptibility. By the inside on this subject, the polymorphism in VDR (rs2228570) gene and prostate cancer should be investigated in different populations and bigger sample sizes, then the mechanism will be fully understood.

5. DISCUSSION AND CONCLUSION

In the last decades, Cancer is not one disease, it can be said to be a series of related diseases that can occur in almost any part of the body. At its most basic, cancer is a disease of the genes in the cells of our body. Genes control the way our cells work. all diseases to human life. Also, cancer leads to uncontrollable cell growth and abnormal expansion of the cells which are recognized as metastasis. So, cancer can be very risky and fatal. If the control mechanism of these cells is not controlled, cells become divided continuously and cancer can become visible (63).

The formation of cancer, which is known as carcinogenesis, is a very complicated biological mechanism. There are three stages of cancer formation: initiation, promotion, and progression, respectively. Also, smoking tobacco, excess alcohol consumption, and ionizing radiation can lead to the generation of cancer. Moreover, some hormones, tumor promoters, and some biological factors can affect cancer (19, 64-65).

Based on the types of cells, cancer is divided into two types malignant and benign cancers. Metastasis is the process through which malignant tumors spread across the body. Benign tumors can't infiltrate and spread like malignant ones (66). Malignant cancers include the lung, prostate, liver, and cervix. Cancer cells' symptoms vary depending on tumor location, growth, and size. Cancer diagnostics and treatments vary. Malignancies are often diagnosed using biomarkers, imaging, MRI, and PET. Early-stage cancer patients have a greater chance of surviving (67).

Cleaning the cancerous area with surgery, radiotherapy and chemotherapy is the most common treatment method. In addition, it can be supported by cancer-specific drug therapy and immunotherapy (68).

Prostate cancer is categorized as one of the most riskous and aggressive types of cancer all around the world. Although it is very common; it ranks fifth in cancer related death in the world (69,70).

Among VDR polymorphism including ApaI(rs7975232), FokI(rs2228570), Cdx2(rs11568820), TaqI(rs731236), and BsmI(rs1544410) in a meta-analysis the risk for women reproduction cancer like cervical, uterine, vaginal, endometrial, ovarian, and breast cancer, found an increased risk of developing breast and ovarian with FokI polymorphism and decrease risk with BsmI polymorphism (71). A meta-analysis for the connection between VDR polymorphism (ApaI, Cdx2, and TaqI) and the risk of basal cell cancer, head and neck cancer, EAC, hepatocellular cancer, colorectal cancer, oral non-Hodgkin lymphoma, squamous cell cancer, renal cell cancer, squamous cell cancer, and thyroid cancer found Cdx2 gg versus GG to be linked with a significantly rising risk for all cancer types and TaqI was related to significantly rising risk for colorectal cancer (72). The study concluded that significant associations exist between VDR polymorphisms and prostate. Significant variations in frequency also exist between diverse ethnic groups. VDR genotypes may have more significance when grouped with specific haplotypes when linked to additional gene polymorphisms involved in the vitamin D pathway, or when stratified by patient backgrounds or different tumor types. In many symptoms for proper performance, a correct connection is established between the ability of VDR. This part has a great impact on VDR polymorphism in the gastrointestinal tract. Exactly where vitamin D plays a special role. (73)

There are differences in PC frequency from one country to another. Incidence, mortality, and survival rates in PC 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 a combination of these factors. (74).

Moreover, patients with PC suffer from urination and nocturia as symptoms, Prostate specific antigen (PSA) level is one of the most prevalent ways to detect PC (72).

Additionally, many risk factors increase the generation of PC such as age, androgens, ethnicity, familial history and genetic tendency, life standards and habits, infection, and inflammation (73).

Numerous studies have investigated that along with external factors, some internal factors (as one of the growth factors) can also be in charge of PC progression. The cancer

risk was for the first time investigated in the VDR gene (rs2228570) polymorphism in the Turkish population with a case-control study. (74).

The first stage of PC diagnosis depends on the evaluation of prostate tissue under the microscope. By using transrectal ultrasound, prostate biopsy is conducted, and this method is used in order to achieve some samples in a pattern. After examining these tissue samples, the primary Gleason grade is applied for the highest pattern (75).

Results of several case-control studies have indicated the alterations in VDR gene (rs2228570) polymorphism may have a Useful treatment to prevent PC, the association among those alterations of vitamin D receptor and PC in Turkish population was not still fully understood. (76).

The polymorphism of VDR gene (rs2228570) in patients with PC has not been clearly studied before. According to the findings obtained from this study, it was determined that this polymorphism Reduces the risk of cancer. Depending on the evaluated results, having the AG heterozygous type can be considered as an advantage for the patients. The G allele can be counted as protective by itself, and its p value is not significant. However, having AG in patients was considered an advantage and it was observed that this type can contribute to decrease the risk of PC. (76).

Consequently, the polymorphism of the VDR gene (rs2228570) in Turkish patients with prostate cancer has not been studied before. In this study, we could not be able to display significant differences between the PC and healthy control groups. We think that the limited sample size is the biggest limitation of this thesis. The new studies with larger sample size should be conducted with the VDR polymorphism to show the effect of VDR on PC susceptibility.

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7. APPENDICES

7.1. Raw data

Crosstabs

Notes

Output Created		11-MAY-2023 20:43:08
Comments		
Input	Data	C:\Users\Work\Desktop\spss\vdr-pc-khademi.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	133
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=VDR_RS2228570 VDR_AA VDR_AG VDR_GG A_ALLEL G_ALLEL BY group /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.07
	Dimensions Requested	2
	Cells Available	524245

[DataSet2] C:\Users\Work\Desktop\spss\vdr-pc-khademi.sav

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
VDR_RS2228570 * group	125	94.0%	8	6.0%	133	100.0%
VDR_AA * group	125	94.0%	8	6.0%	133	100.0%
VDR_AG * group	125	94.0%	8	6.0%	133	100.0%
VDR_GG * group	125	94.0%	8	6.0%	133	100.0%
A_ALLEL * group	125	94.0%	8	6.0%	133	100.0%
G_ALLEL * group	125	94.0%	8	6.0%	133	100.0%

VDR_RS2228570 * group

Crosstab

			group		
			control	patient	Total
VDR_RS2228570	AA	Count	5	3	8
		% within VDR_RS2228570	62.5%	37.5%	100.0%
		% within group	8.3%	4.6%	6.4%
		% of Total	4.0%	2.4%	6.4%
	AG	Count	24	27	51
		% within VDR_RS2228570	47.1%	52.9%	100.0%
		% within group	40.0%	41.5%	40.8%
		% of Total	19.2%	21.6%	40.8%
	GG	Count	31	35	66
		% within VDR_RS2228570	47.0%	53.0%	100.0%
		% within group	51.7%	53.9%	52.9%
		% of Total	24.7%	28.2%	52.9%
Total		Count	60	65	125
		% within VDR_RS2228570	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.720[a]	2	.698
Likelihood Ratio	.724	2	.696
Linear-by-Linear Association	.286	1	.593
N of Valid Cases	125		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 3.84.

Symmetric Measures[d]

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Nominal by Nominal	Pearson's R	-,090	,097	-,918	,371[c]
Ordinal by Ordinal	Spearman Correlation	-,099	,097	-1,025	,318[c]
N of Valid Cases		125			

a. Not assuming the null hypothesis.

b. Correlation statistics are available for numeric data only.

c. Based on normal approximation.

Risk Estimate

	Value
Odds Ratio for VDR_RS2228570 (AA / AG)	[a]

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

VDR_AA * group

Crosstab

			group		
			control	patient	Total
VDR_AA	NO	Count	55	62	117
		% within VDR_AA	47.0%	53.0%	100.0%
		% within group	91.7%	95.4%	93.6%
		% of Total	44.0%	49.6%	93.6%
	YES	Count	5	3	8
		% within VDR_AA	62.5%	37.5%	100.0%
		% within group	8.3%	4.6%	6.4%
		% of Total	4.0%	2.4%	6.4%
Total		Count	60	65	125
		% within VDR_AA	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.720[a]	1	.396		
Continuity Correction[b]	.233	1	.629		
Likelihood Ratio	.724	1	.395		
Fisher's Exact Test				.479	.315
Linear-by-Linear Association	.714	1	.398		
N of Valid Cases	125				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 3.84.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for VDR_AA (NO/YES)	.532	.122	2.330
For group = control	.752	.425	1.330
For group = patient	1.413	.568	3.513
N of Valid Cases	125		

VDR_AG * group

Crosstab

			group		
			control	patient	Total
VDR_AG	NO	Count	36	38	74
		% within VDR_AG	48.6%	51.4%	100.0%
		% within group	60.0%	58.5%	59.2%
		% of Total	28.8%	30.4%	59.2%
	YES	Count	24	27	51
		% within VDR_AG	47.1%	52.9%	100.0%
		% within group	40.0%	41.5%	40.8%
		% of Total	19.2%	21.6%	40.8%
Total		Count	60	65	125
		% within VDR_AG	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.031[a]	1	.861		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.031	1	.861		
Fisher's Exact Test				1.000	.503
Linear-by-Linear Association	.030	1	.862		
N of Valid Cases	125				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 24.48.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	95% Confidence Interval Upper
Odds Ratio for VDR_AG (NO / YES)	1.066	.522	2.177
For group = control	1.034	.712	1.502
For group = patient	.970	.690	1.364
N of Valid Cases	125		

VDR_GG * group

Crosstab

			group		
			control	patient	Total
VDR_GG	NO	Count	29	30	59
		% within VDR_GG	49.2%	50.8%	100.0%
		% within group	48.3%	46.2%	47.2%
		% of Total	23.2%	24.0%	47.2%
	YES	Count	31	35	66
		% within VDR_GG	47.0%	53.0%	100.0%
		% within group	51.7%	53.9%	52.9%
		% of Total	24.7%	28.2%	52.9%
Total		Count	60	65	125
		% within VDR_GG	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.059[a]	1	.807		
Continuity Correction[b]	.004	1	.949		
Likelihood Ratio	.059	1	.807		
Fisher's Exact Test				.859	.474
Linear-by-Linear Association	.059	1	.808		
N of Valid Cases	125				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 28.32.

b. Computed only for a 2x2 table

Symmetric Measures[d]

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Nominal by Nominal	Pearson's R	-,036	,097	-,325	, 758[c]
Ordinal by Ordinal	Spearman Correlation	-,036	,097	-,325	, 758[c]
N of Valid Cases		125			

a. Not assuming the null hypothesis.

b. Correlation statistics are available for numeric data only.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for VDR_GG (NO / YES)	1.091	.540	2.204
For group = control	1.046	.727	1.507
For group = patient	.959	.684	1.345
N of Valid Cases	125		

A_ALLEL * group

Crosstab

			group		
			control	patient	Total
A_ALLEL	NO	Count	31	35	66
		% within A_ALLEL	47.0%	53.0%	100.0%
		% within group	51.7%	53.8%	52.8%
		% of Total	24.8%	28.0%	52.8%
	YES	Count	29	30	59
		% within A_ALLEL	49.2%	50.8%	100.0%
		% within group	48.3%	46.2%	47.2%
		% of Total	23.2%	24.0%	47.2%
Total		Count	60	65	125
		% within A_ALLEL	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.059[a]	1	.807		
Continuity Correction[b]	.004	1	.949		
Likelihood Ratio	.059	1	.807		
Fisher's Exact Test				.859	.474
Linear-by-Linear Association	.059	1	.808		
N of Valid Cases	125				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 28.32.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for A_ALLEL (NO / YES)	.916	.454	1.851
For group = control	.956	.664	1.376
For group = patient	1.043	.744	1.463
N of Valid Cases	125		

G_ALLEL * group

Crosstab

			group		
			control	patient	Total
G_ALLEL	NO	Count	5	3	8
		% within G_ALLEL	62.5%	37.5%	100.0%
		% within group	8.3%	4.6%	6.4%
		% of Total	4.0%	2.4%	6.4%
	YES	Count	55	62	117
		% within G_ALLEL	47.0%	53.0%	100.0%
		% within group	91.7%	95.4%	93.6%
		% of Total	44.0%	49.6%	93.6%
Total		Count	60	65	125
		% within G_ALLEL	48.0%	52.0%	100.0%
		% within group	100.0%	100.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.720[a]	1	.396		
Continuity Correction[b]	.233	1	.629		
Likelihood Ratio	.724	1	.395		
Fisher's Exact Test				.479	.315
Linear-by-Linear Association	.714	1	.398		
N of Valid Cases	125				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 3.84.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	95% Confidence Interval Upper
Odds Ratio for G_ALLEL (NO / YES)	1.879	.429	8.226
For group = control	1.330	.752	2.351
For group = patient	.708	.285	1.759
N of Valid Cases	125		

7.2. Ethical Committee Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2418 **06/10/2022**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Parinaz Khademi)

Yeditepe Üniversitesi Tıp Fakültesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu Üroloji AD. Prof. Dr. Faruk Yencilek'in sorumlu araştırmacı olduğu **"Vitamin D Reseptör Polimorfizmi ve Prostat Kanseri Arasındaki İlişkinin Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2482)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **05.10.2022** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No: 1660)**

Prof. Dr. Turgay ÇELİK

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

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

7.3. Case report form

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU OLGU RAPOR FORMU	TARİH: ..... VERSİYON: .....

ÇALIŞMANI ADI: D vitamini reseptörü Geni (rs2228570) polimorfizmi ile prostat kanseri arasındaki ilişkinin Arastirilmasi

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Gruplan Icin:

- Gönüllü Olma
- Prostat Kanseri Olma
- 40-70 Yaş Araliginda Olma
- Yukanda Belirtilenler Haricimde Bir Hastaliga Sahip Olmama

Kontrol Grubuicin:

- Gönüllü Olma
- Saglikli Olma (Yukanda Belirtilenlerde Dahil Olmak Üzere Hicbir Hastaliga Sahip Olmama)
- 40-70 Yaş Araliginda Olma

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü Olma
- 40-70 Yaş Araligi Disinda Olma
- Yukanda Belirtilenler Disinda Bir Hastaliga Sahip Olma

Sorumlu Araştırmacı

7.4. Curriculum Vitae

Personal Informations

Name	Parinaz	**Surname**	Khademi

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate			
Master			
University	Genetic	**Islamic Azad university of Varamin Branch**	2017
High school	Scince	Erfan	2014

Languages	Grades (#)
English	60

All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs),

Computer Skills

Program	Level
Spss	Average

***Excellent, good, average or basic**