



T.C.

BİRÜNİ UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

**DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS
MOLECULAR AND MEDICAL GENETICS MASTERS PROGRAM**

**DETERMINATION OF THE EXPRESSION LEVELS OF
THE KALLIKREIN (KLK) GENE FAMILY IN PROSTATE
CANCER**

FATMA BÜŞRA BÖYÜKÖZER

ADVISOR

ASST. PROF. DR. Elif Sibel Aslan

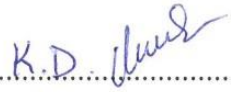
İSTANBUL

2018

ONAY SAYFASI

Biruni Üniversitesi Sağlık Bilimleri Enstitüsü Moleküler Biyoloji ve Genetik Anabilim Dalında Fatma Büşra BÖYÜKÖZER tarafından hazırlanan “Determination Of The Expression Levels Of The Kallikrein (KLK) Gene Family In Prostate Cancer” adlı tez çalışması aşağıdaki jüri tarafından YÜKSEK LİSANS tezi olarak kabul edilmiştir.

Tez Savunma Tarihi:08.08.2018

Jüri Üyesi; (Danışman)	Dr. Öğr. Üyesi Elif Sibel ASLAN Biruni Üniversitesi, Mühendislik ve Doğa Bilimleri Fakültesi, Moleküler Biyoloji ve Genetik Bölümü	
Jüri Üyesi;	Prof. Dr. M. Yusuf ÇELİK Biruni Üniversitesi, Tıp Fakültesi, Biyoistatistik ve Tıp Bilişimi Bölümü	
Jüri Üyesi;	Doç. Dr. Korkut ULUCAN Marmara Üniversitesi, Diş Hekimliği Fakültesi, Diş Hekimliği Temel Tıp Bilimleri Bölümü	

Tez hakkında alınan jüri kararı, Biruni Üniversitesi Sağlık Bilimleri Enstitüsü Yönetim Kurulu tarafından onaylanmıştır.



Doç. Dr. Leman ŞENTURAN
Sağlık Bilimleri Enstitü Müdürü

I. DECLARATION

I hereby declare that the study entitled ‘Determination of the expression levels of the Kallikrein (KLK) gene family in Prostate Cancer’ is my original work and fully and specifically acknowledged wherever adapted from other sources. It has not been published or submitted for any degree, diploma or other similar titles elsewhere. This information is purely of academic interest.

Fatma B şra B y k zer



II. ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my advisor Assistant Professor Dr. Elif Sibel Aslan, for her great help on my master's thesis.

Also, I thank Dr. Esra Güzel for her help in experiments and technical support, and Michael Ittmann for samples for our experiment.

I also thank to my lovely husband Mehmet Z., my supportive mother, my dearest father and my children Zehra, Emir Yahya and Reyhan for their positive motivation.



III. TABLE OF CONTENTS

Index	Page number
İç Kapak	-
Thesis Approval	-
I.Declaration	iii
II.Acknowledgement	iv
III.Table of Contents	v
IV.Symbols and Abbreviations	vi
V.List of Tables	vii
VI.List of Figures	viii
1.Özet ve anahtar kelimeler	1
2.Abstract	2
3.Introduction and Purpose	3
4.General Information	5
5.Methods and Materials	22
6.Results	29
7.Discussion	44
8.Conclusion	49
9.Resources	50
10.Appendix	
Ek 1.Gönüllü Olur Formu	59
Ek 2. Etik Kurul Onayı	61
Intihal raporu	63
11.Resume	64

IV. SYMBOLS / ABBREVIATIONS

AR : Androgen Receptor

cDNA : Complementary Deoxyribonucleic Acid

DHT : Dihydrotestosterone

DNA : Deoxyribonucleic Acid

DMSO : Dimethyl Sulfoxide

dH₂O : Distilled Water

M : Metastasis

N: Lymph Node Count

PIN : Prostatic Intraepithelial Neoplasia

PCa : Prostate Cancer

PSA : Prostate Specific Antigen

qRT-PCR : Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA : Ribonucleic Acid

RP : Radical Prostatectomy

5 α R : 5 Alfa Reductase

T : Tumor Size

μ l : Microliter

μ m : Micrometer

V. LIST OF TABLES

Table No	Table Name	Page
Table 1	TNM Stage	14
Table 2	Cancer Stage	15
Table 3	KLK Gene Family	18
Table 4	PCR conditions required for cDNA	24
Table 5	cDNA-synthesis mix for target gene quantification	25
Table 6	Real Time PCR conditions	25
Table 7	KLK primer pairs	27
Table 8	Sample RNA concentrations and purity of RNA	28
Table 9	Gleason scores of the tumor samples	30

VI. LIST OF FIGURES

Figure No	Figure Name	Page
Figure 1	Prostate gland	6
Figure 2	Global incidence rates for prostate cancer	7
Figure 3	Global mortality rates for prostate cancer	9
Figure 4	Percent of Deaths by Age Group: Prostate Cancer	10
Figure 5	Percent of Cases by Stage	12
Figure 6	KLK1 gene expression level	33
Figure 7	KLK1 gene expression levels in normal and tumor tissues	34
Figure 8	KLK2 gene expression level	35
Figure 9	KLK2 gene expression levels in normal and tumor tissues	36
Figure 10	KLK3 gene expression level	37
Figure 11	KLK3 gene expression levels in normal and tumor tissues	37
Figure 12	KLK4 gene expression level	38
Figure 13	KLK4 gene expression levels in normal and tumor tissues	39
Figure 14	KLK8 gene expression level	40
Figure 15	KLK8 gene expression levels in normal and tumor tissues	40
Figure 16	KLK9 gene expression level	41
Figure 17	KLK9 gene expression levels in normal and tumor tissues	42

1. ÖZET

Böyüközer, F.B. (2018). Prostat Kanserinde Kallikrein (KLK) Gen Ailesinin Ekspresyon Seviyelerinin Belirlenmesi. Biruni Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Biyoloji ve Genetik ABD. Yüksek Lisans Tezi. İstanbul.

Prostat kanseri günümüzde erkek toplumunu ilgilendiren ve ölümle sonuçlanan en ciddi kanser türlerindendir. Genellikle 50 yaşından sonra görülmeye başlar ve ileri yaşlarda görülme sıklığı artar. Prostat kanserinin erken evrede teşhisi mortalite ve morbiditeyi azaltılması açısından önem taşımaktadır. Ancak prostat kanseri başlangıç tedavisini takiben kanserin büyümesi yavaş olabilir ve özellikle yüksek riskli prostat kanseri olan erkeklerin % 30-90'ında nüks oluşabilmektedir. Prostat kanseri tanısında en yaygın kullanılan belirteç olan prostat spesifik antijen (PSA)'in özgüllüğü ve pozitif öngörü değeri düşük olup kanser tanısı için mutlak alt sınır değeri henüz tanımlanamamıştır. Radikal prostatektomi sonrasında takipte iki defa ölçülen PSA değerinin >0.2 ng/mL üzerinde ve radyoterapi sonrasında ise nadir PSA seviyesinin ≥ 2 ng/mL üzerindeki artışı biyokimyasal nüks olarak tanımlanmaktadır.

Kallikrein (KLK) gen ailesi hem DNA hem de amino asit seviyesinde önemli homolojileri paylaşan salgılanmış serin proteazları kodlayan 15 gen içerir. İnsan kallikrein gen ailesinin iki üyesi, prostat spesifik antijen ve insan kallikrein 2, prostat kanseri biyobelirteçleri olarak klinik uygulamada kullanılmaktadır. Çalışmamız kapsamında 34 adet tekrarlayan 36 adet tekrarlamayan prostat kanseri ve 19 normal doku örneklerinde 15 adet KLK gen ekspresyon düzeylerinde değişimleri incelenmiştir. Bu noninvaziv yöntemi PKa tanısında güçlü bir aday yapmaktadır. Sonuç olarak, tekrarlayan PKa'da KLK gen ailesinin ekspresyon seviyelerindeki farklılık literatürde ilk kez tarafımızdan gösterilmiştir. PSA'nın rutin muayenede kolay uygulanabilir olması ve prostat kanserinin diğer KLK'lar ile olan ilişkisi incelenmiştir. Sonuçlarımız, PKa'da farklı eksprese olan KLK'ların nüks belirteci olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler : Prostat Kanseri, KLK gen ailesi, gen ekspresyonu

2. ABSTRACT

Böyüközer, F.B. (2018). Biruni University, Institute of Health Sciences, Molecular Biology and Genetics. Masters Thesis. İstanbul.

Prostate cancer is one of the deadliest cancer types affecting the male population that results with loss of life. Usually begin to appear after the age of 50, and the incidence increases with advanced age. Diagnosis of prostate cancer at the early stage is important in reducing mortality and morbidity. However, after initial treatment, cancer growth may be slow and recur in 30-90% of male patients with increased prostate cancer risk. The specificity and positive predictive value of prostate specific antigen (PSA), the most widely used biomarker for prostate cancer, is low and the absolute lower limit value for cancer diagnosis has not yet been identified. Biochemical recurrence is defined as an increase in PSA value >0.2 ng/mL, measured twice after radical prostatectomy and nadir PSA value ≥ 2 ng/mL, after radiotherapy.

The Kallikrein (KLK) gene family consist of 15 genes encoding secreted serine proteases that have relevant homologies at both the DNA and amino acid level. Two members of the human kallikrein gene family, prostate specific antigen (PSA) and human kallikrein 2 (hK2), are used clinically as prostate cancer biomarkers. In our study, changes in 15 KLK gene expression levels in 34 recurring 36 non-recurring prostate cancers and 19 normal tissue specimens were investigated. This non-invasive method makes a strong candidate for PCa diagnosis. In conclusion, the difference in expression levels of the KLK gene family in recurrent PCa has been shown for the first time by us in the literature. Easy application of PSA in routine examination and the relationship between prostate cancer and other KLKs have been examined. Our results have shown that KLKs, which are differently expressed in PCa, can be used as recurrence markers.

Key words: Prostate Cancer, KLK gene family, gene expression

3. INTRODUCTION AND PURPOSE

Prostate cancer is the most typical cancer in males and can lead to death (Coffey 1993). Serum test for prostate-specific antigen, and procedures developed for surgical intervention and radiation therapy substantially reduce cancer mortality with early diagnosis of prostate cancer (Pezaro, *et al.* 2014). Clinical cancer staging of prostate cancer; PSA, digital rectal checkup, prostate biopsy findings and imaging methods are applied. In this view, it is possible to select the most appropriate treatment by determining the extensiveness of the disease using the pre-treatment clinical parameters and estimating the prognosis of the disease. When staging is done in prostate cancer, the risk assessment of the disease and the possibility of cancer recurrence are taken into consideration. The recurrence of prostate cancer depends on the treatment previously received by a patient and tumor characteristics. Most recurrent cancer patients have an increase in PSA levels, while some have evidence of recurrent cancer with x-rays or screening (Hanna and Jones 2015; Woodrum, *et al.* 2015). However, today, there is still no effective cure for advanced prostate cancer patients. For this reason, research continues for prognostic determinants that can differentiate prostate cancer from aggressive forms.

In the treatment planning of prostate cancer; patient age, tumor stage, accompanying health problems and risk groups are taken into consideration. Radical prostatectomy (RP) is the process of excising prostate tissue together with prostatic urethra that is between external sphincter and bladder neck and seminal vesicles so that negative surgical margins are obtained. Follow-up is of great importance in patients who have undergone RP surgery at an early age and who are in the moderate or high risk group for recurrence (Sternberg, *et al.* 2014). In patients with moderate or high risk (PSA ≥ 10 and/or Gleason score ≥ 7 and/or clinical stage $\geq T2b$), follow-ups are required once every 3 months for first 2 years, semiannually for next 2 years and every year for the following years (Mithal, *et al.* 2015).

The Kallikrein (KLK) gene family is a subgroup of serine proteases and is located on the same chromosomal locus 19q13.4. The KLK genes share significant homogeneity at nucleotide and protein levels. These genes are encoded for supposed serine proteases and majority of these genes are regulated by steroid hormones

(Yousef and Diamandis 2001). In recent studies, it is known that at least a few of the kallikrein genes play a role in malignancy formation that includes gene and protein structure, enzymatic activities, tissue expression, hormonal regulation and alternative splicing.

It has been shown that KLK genes are expressed at various levels in a broad range of tissues showing a functional involvement of human kallikrein proteases at various degrees of physiological processes. For example, it is known that certain kallikrein genes (NES1, protease M, PSA) have reduced expressions in prostate, breast and other cancers. NES1 has been shown to be a potent angiogenesis inhibitor of a novel breast cancer tumor suppressor protein and PSA (Diamandis, *et al.* 2000).

This study aimed to explore the potential biological effects of the KLK gene family on recurrence of prostate cancer since PSA has failed to predict prostate cancer. PCa mechanism in the direction of the obtained data will be better understood and lead to new projects for treatment.

4. GENERAL INFORMATION

4.1. Prostate

The prostate is the organ of the reproductive system, located just at the exit of the bladder. In males, the prostate is a small gland about the size of a chestnut that produces seminal fluid that nourishes and transports sperm it is approximately 4 cm in diameter, 2 cm in thickness, and 20 grams in weight (Wilson 2014). The prostate secretes the fluid that feeds and protects the semen. During ejaculation, the prostate compresses this fluid into the urethra and excretes it out as semen. Prostate secretions allow the male reproductive cell sperm to survive on the female reproductive tract (Lee, *et al.* 2011).

4.2. Prostate Cancer

Prostate cancer is one of the most common types of cancer in men developing in the prostate, the second most common cause of death after lung cancer. Usually the prostate cancer grows gradually and is initially limited to the prostate gland, which does not cause serious damage. However, some types of PCa grow slowly while other types are aggressive and can spread rapidly (Schrecengost and Knudsen 2013). Every year around the world, 899,000 new prostate cancers are detected and 258,000 people lose their lives due to prostate cancer. By 2030, it is predicted that 1.7 million new prostate cancer cases will be seen annually and 499,000 patients will lose their lives due to prostate cancer (Bellier, *et al.* 2018; Peisch, *et al.* 2017).

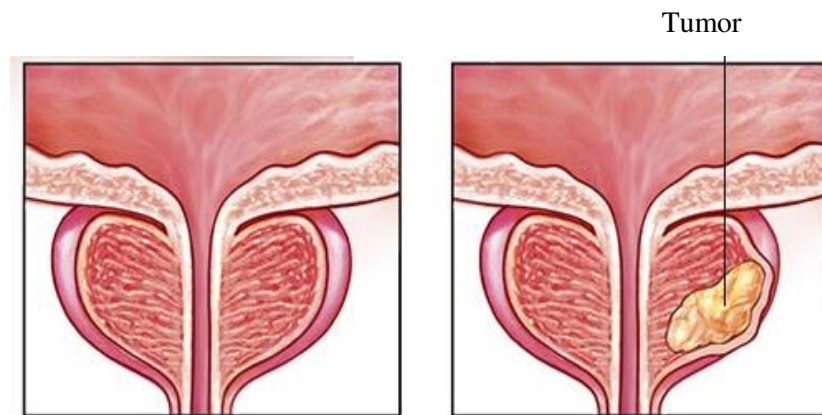


Figure 1. Prostate gland

Prostate cancer occurs in the prostate gland, which is located below a male's bladder. A normal prostate gland and a prostate with a tumor are shown in this figure.

(<https://www.mayoclinic.org/diseases-conditions/prostate-cancer/symptoms-causes/syc-20353087>,
Date of access: 21.06.2018)

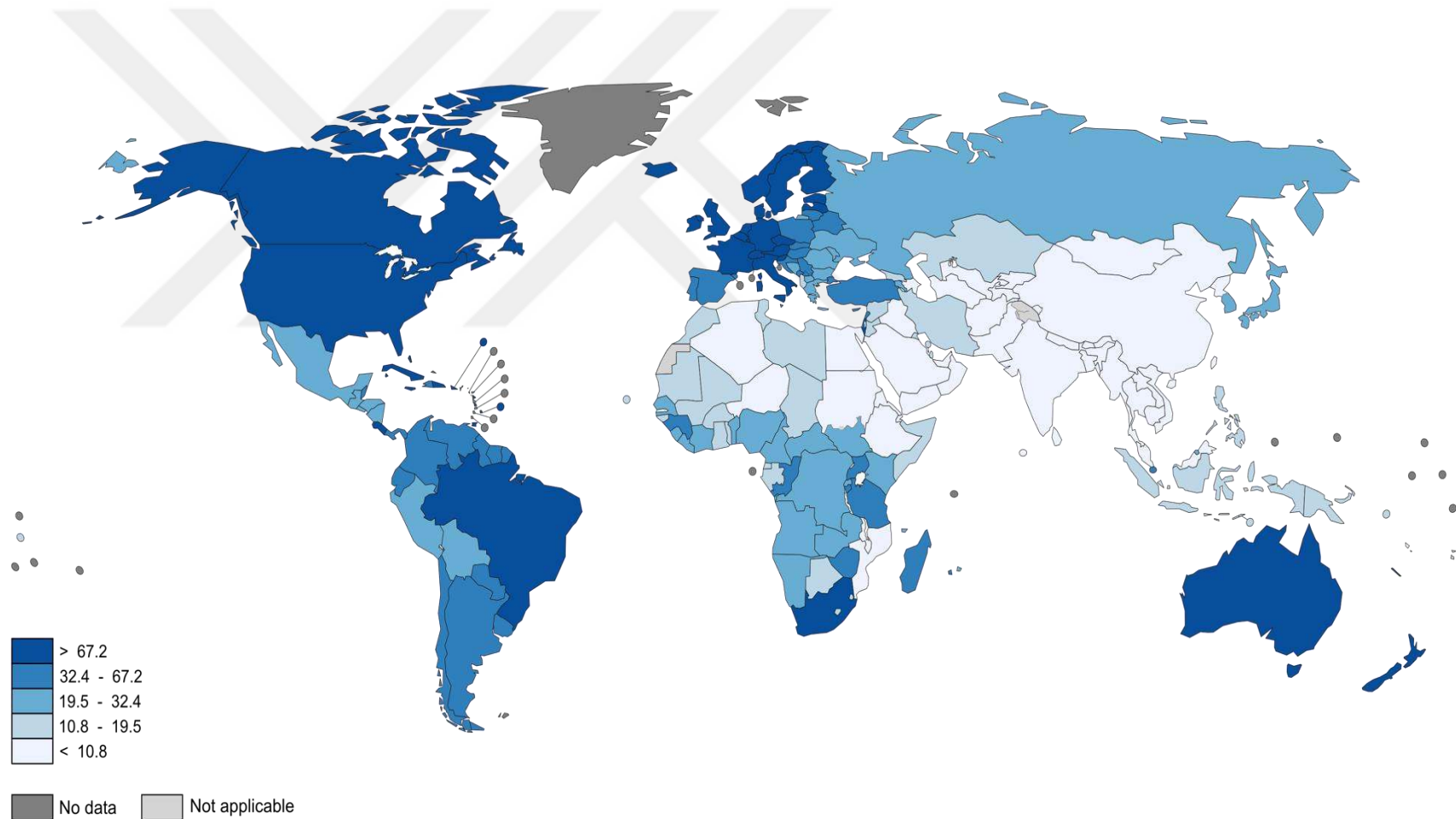


Figure 1. Global incidence rates for prostate cancer Prostate cancer incidence rates are higher at North America, Australia, Northern and Western Europe. The rates are also high at less developed regions such as South America and South Africa.

(<http://globocan.iarc.fr/old/FactSheets/cancers/prostate-map-i1.png>, Date of access: 23.06.2018)

The global incidence rates for prostate cancer varies more than 25 times in the world. The difficulty to diagnose prostate cancer at early stage and access to PSA (prostate specific antigen) test in different regions causes the variety. The prostate cancer usually arises from the outer part of the gland; it does not cause any urination symptoms, makes the diagnosis of prostate cancer at onset very difficult. The cancer may or may not show any symptoms. More advanced prostate cancer may have symptoms such as difficulty in urination, decreased urine flow, erectile dysfunction, blood in the semen, discomfort in the pelvic area and bone pain. However, access to PSA (prostate specific antigen) test makes early diagnosis of prostate cancer possible. The PSA (prostate specific antigen) test is prominently being used in the regions where incidence discovery rate is the highest such as Australia/New Zealand and North America (ASR 111.6 and 97.2 per 100,000, respectively) and Western and Northern Europe. Incidence rates are also relatively high in less developed regions such as Caribbeans (ASR 79.8 per 100,000), South Africa (ASR 61.8 per 100,000) and South America (ASR 60.1 per 100,000). PSA test has a greater effect on incidence than mortality thus worldwide mortality rate changes (ten times between approximately 3 and 30 in 100,000) are less likely to be observed than changes in incidence and, rates are much less compared to developed regions (165,000 and 142,000, respectively). Mortality rates are generally found to be very low in black populations (Caribbeans, 100,000 and sub-Saharan Africa 29) and Asia (for example 2.9 in 100,000 in South-Central Asia), modestly high in America and Oceania (Khazaei, *et al.* 2016) (Figure 2-3). According to GLOBOCAN data, prostate cancer with approximately 307,000 deaths in 2012 and 6.6% of total men deaths was reported to be the fifth leading cause of cancer death in men (Hassanipour-Azgomi, *et al.* 2016; Khazaei, *et al.* 2016). It is reported that prostate cancer is the 6th most common cancer in our country and it is present in rates of 6.1%. (Haydaroglu A 2007). In addition, lifetime risk of developing prostate cancer is 30%, risk of developing clinical prostate cancer is 10%, and risk of prostate cancer death is 3%. Among other factors, age is the most important risk factor, the incidence of cancer increases starting from the age of 50. The mortality rate for age less than 44 years age and ages over 44 is 0.01% to 99.99% respectively. In addition to age, genetics, black race, high-fat diets play a role in the development of cancer (Grossman, *et al.* 2018). It is predicted that in 2018, new prostate cancer cases will constitute 9.5% of all new cancer cases with 164,690, and %4.8 of all cancer deaths with the estimated deaths of 29,430. The age rates of prostate cancer in all men between 2011 and 2015 are as shown in Figure 4 (<https://seer.cancer.gov/statfacts/html/prost.html>, Date of access : 16.05.2018).

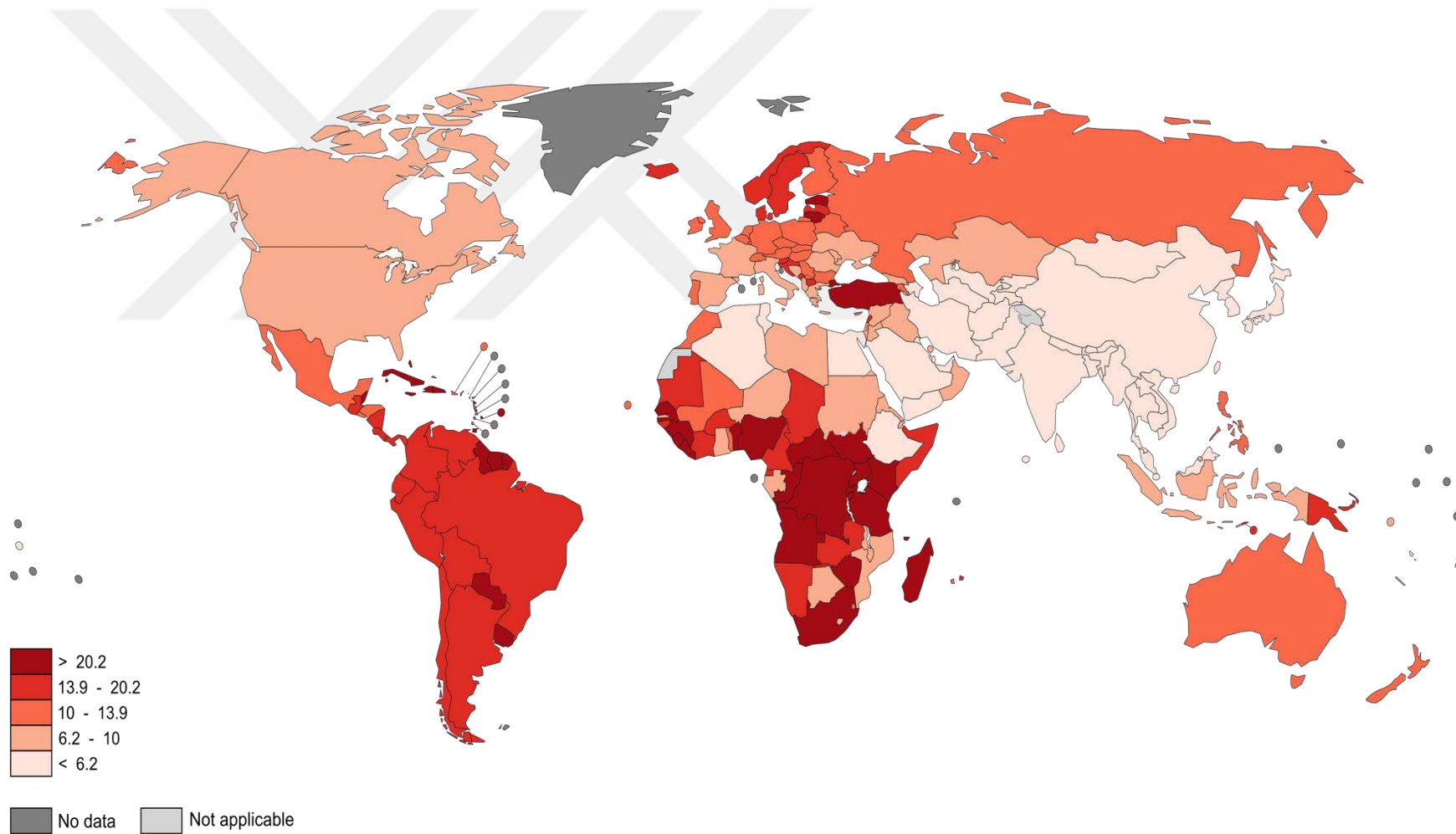


Figure 2. Global mortality rates for prostate cancer Prostate cancer mortality rates are higher at less developed regions such as South Africa and South America. Diversely, mortality rates are lower at South Asia and sub-Saharan Africa.

(<http://globocan.iarc.fr/old/FactSheets/cancers/prostate-map-m1.png>, Date of access: 23.06.2018)

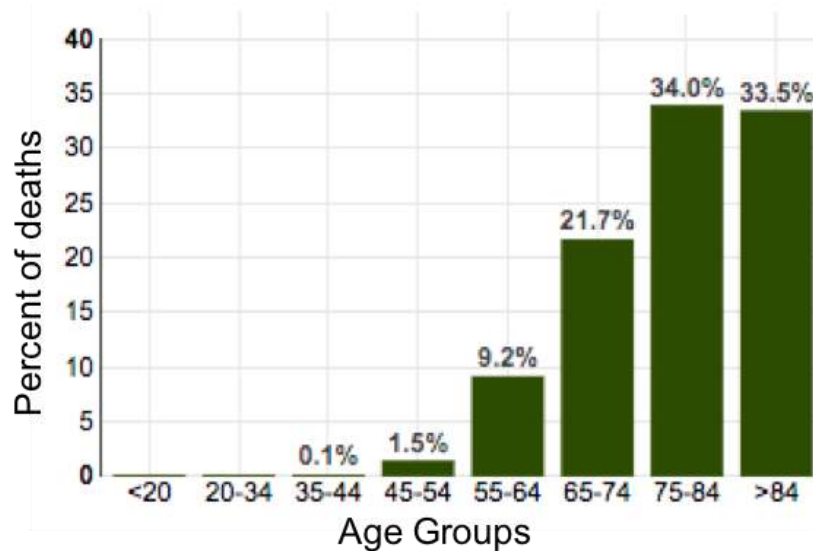


Figure 3. Percent of Deaths by Age Group: Prostate Cancer (2011-2015). The percent of prostate cancer deaths is highest among men aged 75-84.

(<https://peterattiamd.com/wp-content/uploads/2018/02/prostate-survival-1.png>, Date of access: 29.06.2018)

4.2.1. Diagnosis of Prostate Cancer

4.2.1.1. Prostate Specific Antigen

Prostate Specific Antigen (PSA) is an enzyme released from the prostate gland in men providing sperm liquidation. Essentially, it is secreted from the cells surrounding and forming the inner part of the ducts of the prostate (Kornberg, *et al.* 2018). PSA is a mostly prostate-specific enzyme that is also secreted by the pancreas and salivary glands in very small quantities. The concentration of PSA in the sperm is much higher than in blood, and only a fraction of the produced PSA circulate in the blood. In healthy individuals, serum levels of PSA may vary depending on prostate volume, age, and race (Blanc-Lapierre, *et al.* 2017; McVary and Rademaker 1990). Increased levels of PSA in the blood is important for prostate cancer, however it is not specific to prostate cancer alone. PSA levels may increase in ejaculation, transurethral catheterization, transrectal ultrasonography, trauma and benign growth of the prostate, prostate infections as well as diseases such as PCa, BPH, cystoscopy and prostatitis (Caster, *et al.* 2015). PSA level of 4.0 ng/mL is considered normal. Therefore, if a man has a PSA level above 4.0 ng/mL, digital prostate examination, the other important modality in the early diagnosis

of prostate cancer, should be done and evaluated together with the PSA level. Although 25% of patients diagnosed with prostate cancer have PSA levels within normal limits, the diagnosis is made with the stiffness and irregularity determined by digital examination. When there is a suspicion on the digital examination or in the PSA, ultrasonography guided needle biopsy is performed and prostate tissue samples are obtained (Hayes and Barry 2014).

Additional tests such as computed tomography, bone scintigraphy, and MRI may be required depending on the disease stage. Cancers limited to the organ respond very well to surgical treatment. In recent years, the success of surgeries has been reported to increase with developments of laparoscopic and robotic methods. In advanced stages; hormone-therapy, radiotherapy, chemotherapy and surgery are among the treatment methods (Fiz, *et al.* 2018).

4.2.2. Prostate Cancer Formation

There are many studies about the familial and genetic side of prostate cancer. Known risk factors for prostate cancer are age, family history and race. Age is the most important risk factor known in prostate cancer. In older ages, the incidence of prostate cancer is increasing (Gann 2002).

The earlier the age of the person who has been diagnosed with prostate cancer in the family, the higher the number of persons who have acquired prostate cancer in the family and the degree of relativity, the greater the risk. For example, a man with a father with prostate cancer is twice as likely to have prostate cancer, while with a brother with prostate cancer is 3 times as likely compared to normal risk. If both brothers and father have prostate cancer within the same family, the risk is 5 times higher. Generally, prostate cancer, which is a disease of men aged 50 years or older, is due to both genetic and environmental factors (Bostwick, *et al.* 2004; Leitzmann and Rohrmann 2012).

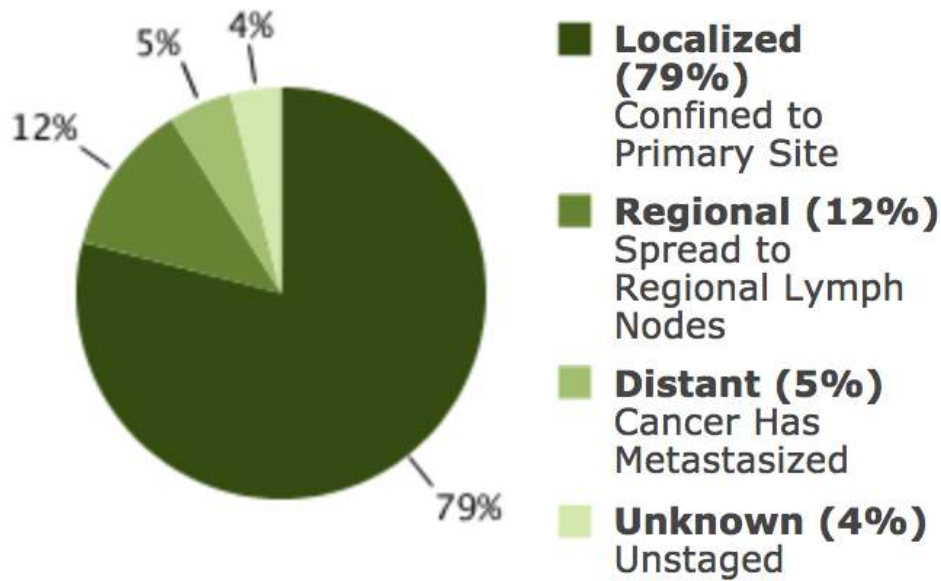


Figure 5. Percent of Cases by Stage. (2007-2013) The percent of the prostate cancer cases varies by stage. Prostate cancer is mostly localized to primary site. However, it could be spread to other parts of the male's body.

(<https://peterattiamd.com/wp-content/uploads/2018/02/prostate-survival-1.png>, Date of access: 29.06.2018)

4.2.2.1. Age

Age is among the causes of prostate cancer, in fact it is considered to be the most important risk factor for cancer formation. Although pre-neoplastic lesions known as prostatic intraepithelial neoplasia (PIN) are seen at an early ages, the incidence is high in males aged 50 years (Sakr, *et al.* 1993), and it is much more common in individuals aged between 60-70 years. It was recorded that the incidence of PCa in our country was 8.3% in individuals in their 50s and increased up to 33.3% in individuals in their 80s and above. The appearance of precancerous lesions in male elderly individuals is significantly more common than the incidence of prostate carcinoma. For this reason, cancer-related morphological changes occur early in life and invasive carcinoma formation is more common as a consequence of aging (Mahran, *et al.* 2018).

4.2.2.2. Environmental factors

The incidence of prostate cancer in the United States is significantly higher than that of Asian countries, with the presence of histologic pre-neoplastic lesions (Dhom 1983). For this reason, it is assumed that diet and environmental factors play a crucial role in prostate carcinogenesis, as in other epithelial cancers (Steinberg, *et al.* 1990).

4.2.2.3. Familial inheritance

The ratio of familial inheritance is a small percentage (10%) of prostate cancers and most reports reflect association with early stage disease (Carter, *et al.* 1992; Nelson, *et al.* 2013). Although the candidate genes responsible for the formation of cancer have not yet been identified, studies have shown the presence of 2 loci (Ostrander and Stanford 2000) in predisposing families located in X chromosome and chromosome 1q region. In addition, statistical relationship between breast and prostate cancer has been reported in many studies however the molecular basis of such a link is still unclear (Anderson and Badzioch 1993; Bennett, *et al.* 2002).

4.2.2.4. The role of steroid hormones

In prostate carcinogenesis, steroid hormone receptors play a significant role in all phases of cancer formation. In particular, it has been reported that androgens have a substantial age-related decrease in men compared to estrogens and androgens have been reported to be a contributing factor in the onset of prostate cancer (Prehn 1999). The steroid binding protein in the prostate gland is increased by the effects of androgens. Dihydrotestosterone (DHT) is essential for the differentiation, development and secretion functions of the prostate. Testosterone-derived DHT produced with 5 alpha-reductase (5 α R) causes the secretion of keratinocyte and fibroblast growth factors in prostate mesenchymal cells. These growth factors cause hypertrophy and hyperplasia in the prostate gland (Bhargava 2014).

4.2.2.5. Heterogeneity and multifocality

The heterogeneous and multifocal character of prostate cancer lesions creates significant challenges in the diagnosis and treatment of the disease. Regarding heterogeneity, a histological examination of the prostate cancer gland typically reveals

benign glands, PIN foci and neoplastic foci at varying degrees of severity are side by side. For taking this heterogeneity into account, Gleason proposed a rating system, a common system used by pathologists, and is used to develop appropriate treatment methods. In this system, a higher Gleason grade related to the two most common neoplastic foci indicates the presence of a more advanced carcinoma (Gleason 1992).

4.2.3. TNM Staging in Prostate Cancer

The treatment of prostate cancer is planned based on the cancer staging and risk assessment. It is done by assessment of limitation of cancer to an organ, spreading to regional lymph nodes, distant metastases (Cheng, *et al.* 2012). Radiological imaging (computed tomography [CT], MRI) or bone scintigraphy are methods used for staging. In the TNM system, prostate cancer limitation to the organ is defined as T-staging; lymph node involvement as N-staging and distant metastasis is defined as M-staging (Mottet, *et al.* 2017).

Table 1. TNM Stage

Tumor (T)	
T0	No evidence of primary tumor
T1	Non-palpable tumor that is not evident from radiographic imaging
T2	Palpable tumor confined to the prostate
T3	Palpable tumor extending beyond the prostate
T4	Palpable tumor that is fixed or that invades adjacent structures
Lymph Nodes (N)	
N0	No lymph node metastases
N1	Metastases in one regional lymph node that is ≤ 2 cm wide
N2	Metastases in one or more regional lymph nodes, each ≤ 5 cm wide
N3	Metastases in at least one regional lymph node each > 5 cm wide
Distant metastases (M)	
M0	No evidence of distant metastases
M1	At least one distant metastasis

The staging of prostate cancer reveals how much cancer is present in the body. It is used to determine the best method of treatment as well as the course and condition of cancer. Cancer stage is based on tests performed in prostate cancer, including blood PSA level, and biopsy results (Mottet, *et al.* 2011).

Table 2. Cancer Stages

Stage I	the cancer is small and only in the prostate
Stage II	the cancer is larger and may be in both lobes of the prostate but is still confined to the prostate
Stage III	the cancer has spread beyond the prostate to close by lymph glands or seminal vesicles
Stage IV	the cancer has spread to other organs such as the bone and is referred to as metastatic vesicles

(www.cancer.gov, Date of access: 30.06.2018)

4.2.4. Gleason Scoring

It should be considered together with the stage, and shows "how important cancer is" in the cancerous tissue detected with biopsy. The grading system used to determine is called the Gleason Score. Cancer cells are graded by giving a score of 1 to 5 (1: closest to normal, best; 5: unusually different, worst), which are different from the normal structural changes shown in microscopic images of cancer cells. The Gleason score is a value between 2 and 10 because it is a total value. The pathologist determines the two most frequent differences in tissues obtained by biopsy and gives a total "Gleason score", in which the most common becomes first (Chen and Zhou 2016; Stark, *et al.* 2009).

If there is no prostate tissue in the biopsy tissues, it should be evaluated as inadequate for diagnosis and a biopsy should be performed again. Intraductal carcinoma, lymphovascular invasion and presence of cancer exceeding the prostate,

presence of cancer in each biopsy piece, length and ratio should be indicated. Because this data is very important in determining the cancer volume and therefore the treatment to be performed. A biopsy piece with >5 mm or >50% cancer presence indicates an important cancer presence that needs to be treated immediately. Also, if only one of the received biopsies has cancer in a small focus, the cancer grade, length and percentage should be indicated. This is because it may require a re-biopsy before deciding on treatment. As the Gleason score increases, cancer proliferation, progression, exceeding of prostate and spreading (aggressiveness) increases. Also, the most common degree level is also important. For example, a Gleason score of 4+3=7 is more aggressive than 3+4=7, even though it has the same score (Epstein, *et al.* 2016; Wright, *et al.* 2009).

4.2.5. Recurrent Prostate Cancer

The PSA test is used in the follow-up of patients to see if cancer has recurred after treatment. Elevation in PSA level after prostate cancer treatment may be the first indication of recurrence. Such a "biochemical relapse" occurs much earlier than other clinical signs and symptoms of prostate cancer recurrence (Marks and Bostwick 2008). The factors predicting prostate cancer in recurrent biopsies cannot be determined precisely. For this reason, the optimal biopsy strategy is still undetermined. In the literature, many factors are studied which suggest the possibility of recurrence of prostate cancer in cases with negative biopsies and to prevent unnecessary biopsies with PSA, PSA velocity, free/total PSA ratio, prostate cancer antigen gene 3 (PCA-3), high grade intraepithelial neoplasia, atypical small acinar proliferation, prostate volume and radiological methods (Ploussard and de la Taille 2018).

4.2.6. Treatment

The treatment of prostate cancer is performed with the use of surgical (radical prostatectomy, RP), radiotherapy (RT) and hormonal therapy (HT) approaches alone or in combination with risk groups. Determination of treatment in prostate cancer is one of the controversial areas (Logothetis, *et al.* 2013). The main reasons for this are: varying treatment depending on the patient's life expectancy and general health status, presence of controversial issues in the areas of expertise, difficulties in projecting the guidelines for clinical practice, and clinical trials in the literature that form basis for treatment and guidelines to be depending on mostly retrospective data and their value of evidence are limited. As a result, there are different treatment options for prostate cancer treatment that can be applied instead of each other in similar clinical situations according to the choice of the physician and the patient (Incrocci 2015; Klotz and Emberton 2014).

4.3. Kallikrein Gene Family

Kallikreins are a subset of serine proteases and enzymes capable of cleaving peptide bonds in proteins. In humans, while plasma kallikrein has a known paralogue, peptidases (KLKs) associated with tissue kallikrein encode a family of fifteen related serine proteases. These genes are localized on the 19q13 chromosome, which constitutes the largest contiguous cluster of proteases in the human genome. Kallikreins are responsible for the coordination of various physiological functions such as blood pressure, semen liquefaction and skin shedding (Yousef and Diamandis 2001). Features table with general information about KLKs is given in Table 3.

Table 3. KLK gene family. KLK gene family has 15 members which encodes different types of proteins, belongs to tissue kallikreins. KLK gen family members are located contiguously on the 19q13 chromosome.

Approved Symbol	Approved Name	Other protein names/symbols	Chromosome
<i>KLK1</i>	kallikrein 1	Pancreatic/renal kallikrein, hPRK	
<i>KLK2</i>	kallikrein related peptidase 2	Human glandular kallikrein 1, hGK-1	19q13.33
<i>KLK3</i>	kallikrein related peptidase 3	Prostate-specific antigen, PSA	19q13.33
<i>KLK4</i>	kallikrein related peptidase 4	Prostase, KLK-L1 protein, EMSP1	19q13.41
<i>KLK5</i>	kallikrein related peptidase 5	KLK-L2 protein; HSCTE	19q13.41
<i>KLK6</i>	kallikrein related peptidase 6	Zyme, protease M, neurosin	19q13.3
<i>KLK7</i>	kallikrein related peptidase 7	HSCCE	19q13.33
<i>KLK8</i>	kallikrein related peptidase 8	Neuropsin; ovasin; TADG-14	19q13
<i>KLK9</i>	kallikrein related peptidase 9	KLK-L3 protein	19q13.33
<i>KLK10</i>	kallikrein related peptidase 10	NES1 protein	19q13.41
<i>KLK11</i>	kallikrein related peptidase 11	TLSP/hippostasin	19q13.41
<i>KLK12</i>	kallikrein related peptidase 12	KLK-L5 protein	19q13.41
<i>KLK13</i>	kallikrein related peptidase 13	KLK-L4 protein	19q13.41
<i>KLK14</i>	kallikrein related peptidase 14	KLK-L6 protein	19q13.41
<i>KLK15</i>	kallikrein related peptidase 15		19q13.33

Kallikrein enzymes are generally divided into two main categories: plasma kallikrein and tissue kallikreins. These two categories differ significantly in terms of molecular weight, substrate specificity, immunological properties, gene structure and type of quinine released. Plasma kallikrein or Fletcher factor (official symbol KLKB1) is encoded by a single gene located on human chromosome 4q35 (Björkqvist, *et al.* 2013; Diamandis, *et al.* 2000) The gene consists of 15 exons and encodes an enzyme that secretes bioactive peptide bradykinin from a high molecular weight precursor molecule (high mol wt kininogen) produced by the liver. Plasma kallikrein is expressed only by the liver cells. The function of plasma kallikrein plays a role in the regulation of

vascular tone and inflammatory reactions through the involvement of blood clotting and fibrinolysis process and release of bradykinin (Biyashev, *et al.* 2006).

Tissue kallikreins are members of a large multigene family and exhibit significant similarities at the gene and protein level and at the same time in the tertiary structure. The term "kallikrein" is generally used to describe an enzyme that acts on a precursor molecule (kininogen) for the release of a bioactive peptide (kinin) (Charest-Morin, *et al.* 2015; Yayama, *et al.* 2003). Another term is often used to define these enzymes is "kininogenases". The term "kininase"; is used to describe other enzymes that can inactivate kinin. Between known human and animal tissue kallikreins, only one enzyme has the ability to efficiently release a bioactive kinin from kininogen (Biyashev, *et al.* 2006).

Similarities between the members of the new human kallikrein gene family

1. All genes belonging to the family are located in the same chromosomal region (19q13.4).
2. All genes encode with a conserved catalytic triad structure (histidine, aspartic acid and serine in appropriate positions) for serine protease variants.
3. All genes belonging to the family have five coding exons (some members contain one or more 5' untranslated exons).
4. The encoding exon sizes are similar or identical.
5. The intron phases are completely protected between the members of the gene families of the 15 human and rodent kallikreins.
6. All genes belonging to the family have significant sequence homology at DNA and amino acid levels (40-80%).
7. Most genes belonging to the gene family are regulated by steroid hormones.

The intron phase indicates the position of the intron in the codon: the intron phase I, intron, occurs after the first nucleotide of the codon; II, intron occurs after the second nucleotide; 0, intron occurs between the codons (Pavlopoulou, *et al.* 2010).

4.3.1. Gene Organization

All members of the new human kallikrein multigene family are coded for serine proteases. The organization of all genes is very similar, with the first encoding exon having a short 5'-untranslated regions, the second exon containing the catalytic triple amino acid histidine. Towards the end of the exon, at the beginning of middle catalytic triple aspartic acid and exon, the third exon contains the fifth exon, which contains the catalytic triple serine. Beyond the stop codon, there is a 3'-untranslated region of variable length (Murray, *et al.* 1990).

While making sure that the classical kallikreins do not have 5' untranslated exons, many other members of this multigene family have one or two 5' untranslated exons. The gene family also contains 5' untranslated exons that have not yet been identified. In addition, most of the 3p-untranslated regions of these genes sometimes vary, leading to variants with different mRNA lengths, however they encode for the same protein. Although the intron lengths of these genes vary considerably, the exon lengths are quite similar or identical (Digby, *et al.* 1989).

5. MATERIALS AND METHOD

5.2. Materials

5.2.1. Samples

All samples were obtained in accordance with approved ethical standards of the responsible committee of Baylor College of Medicine. 19 normal and 70 Prostate Cancer tissue samples (which are grouped as recurrent and non-recurrent) were obtained from Baylor College of Medicine, Texas, USA. The environmental conditions for storing the tissues are in a freezer with temperature set in -80 °C.

The specimens were prepared to be more than 70% tumor tissue from radical prostatectomy materials. According to this method, punch biopsies were taken from fresh tissue and the percentage of cancer in this area was determined complementarily by observing the tumor around the punch biopsy cavity during a routine histopathological examination of the material of radical prostatectomy. The validity and consistency of this method have been pointed out in various studies. For example, it has been shown that cancer tissues prepared by this method have expressed higher prostate cancer specific markers AMACR and PSGR, compared to prostate tissues without tumor and included prostate cancer specific gene fusions.

5.2.2. Equipments and Devices

5.2.2.1. Equipments

Hybridization Oven (Shel Lab, Oregon, USA)

Mini Centrifuge (Thermo Scientific, Germany)

Centrifuge (Hettich Retina 420R, Germany)

Refrigerator (4 ° C) (Vestel, Turkey)

Freezer (-20° C) (Vestel, Turkey)

Freezer (-80° C) (Wisd, DAIHAN Scientific, Korea)

Nanodrop spectrophotometer (Thermo Scientific-ND 8000, Germany)

Hot Plate (Thermo Scientific, Germany)

Laminar air-flow (Class II Safety Cabinet) (Metisafe, Turkey)

Vortex (Wise Mix-VM10, Korea)

Micropipette (Thermo Scientific, Germany)

Multi channel pipette (Thermo Scientific, Germany)

Thermal cycler (PCR) (Biorad, USA)

Roche LightCycler 480 (Basel, Switzerland)

5.2.2.2. Solutions

cDNA Synthesis Kit (Transcriptor High Fidelity cDNA Synthesis Kit, Basel Stadt, Switzerland)

TRIzol (Ambion, USA)

Isopropanol (Sigma-Aldrich, USA)

Ethanol (Merck, Darmstadt, Germany)

First-Strand cDNA Synthesis Kit for Real-Time PCR (Applied Biosystems, Foster City, CA, USA)

Syber Green PCR Master miX (Applied Biosystems, Foster City, CA, USA)

Primers (Sentegen, Turkey)

5.2.3.Computer Software

SPSS 21 (Istanbul University, Software License Server © 01-2013)

5.3. Method

5.3.1. Processing of tissues with nitrogen

The specimens taken from the patient tissues were treated with liquid nitrogen and divided into small pieces.

5.3.2. RNA isolation with Trizol

1 mL of Trizol solution was added to the cells for RNA isolation from the tissues separated into the small particles and stored at -80° C until isolation. Pipetting for fragmentation of the homogenized cells and 5 minutes of incubation for complete dissociation of the proteins bound to the nucleic acids were carried out. Each sample was pipetted for 5 minutes after adding 0.2 mL of chloroform. The samples were centrifuged at 12000 x g for 25 minutes at +4° C. After the centrifuge, three phases were observed in the tubes. The lower phase includes extracellular membranes and polysaccharides, middle phase includes high 22 molecular weight DNA and top phase includes RNA. The RNA included clear phase was transferred to the new micro-tube. 0.5 mL isopropyl alcohol was added to each sample. The samples were incubated in ice for 30 min and then centrifuged at +4° C for 30 min at 12000 x g. After this step, RNA was observed as a whitish structure. After removal of the supernatant, 0.5 mL of 75% ethanol was added and centrifuged at 7500 x g for 10 min at +4° C. At the last stage, ethanol was removed from the RNA pellets by drying. The RNA pellets were then suspended in 30 µl of nuclease free water. Concentration measurement was obtained by taking 3 µl from each of the RNA samples.

5.3.3. Determination of RNA concentration and purity

RNA concentration and purity quality were measured spectrophotometrically with optical density (OD) measurement. The concentrations and purity degrees of the RNA samples were measured on the NanoDrop ND-2000c spectrophotometer device with absorbance values of 260 nm and 280 nm wavelengths.

Purity measurements of the samples were compared with the ideal value for RNA 2 (OD_{260 nm}/OD_{280 nm} > ~ 2.0), determined by the absorbance ratio at a wavelength of 260/280 nm. RNA integrity was checked by agarose gel electrophoresis and the tubes were put to -80° C to hold for further steps.

5.3.4. cDNA synthesis

The cDNAs were synthesized with Transcriptor HighFidelity Reverse Transcription kit from Roche (Switzerland) using the same amount of samples from the isolated RNAs.

5.3.4.1. Qualitative RT-PCR

Reverse transcriptase PCR protocol was used with Oligo (dt) 18 primers for single chain cDNA synthesis.

Table 4. PCR conditions required for cDNA

Phase	Temperature(°C)	Time	Cycle
Incubation	50	2 min	1
Enzyme Activation	95	10 min	1
Denaturation	95	15 sec	40
Binding / Extension	60	1 min	

The isolated RNA of each sample is incubated in ice for 10 min at +65° C with primer addition. Then, the buffer, protector, dNTPs, DTT and reverse transcriptase enzyme are added to medium and respectively incubated at optimal conditions (10-30 min at 45-55° C, 5 min at 85° C) and then PCR procedure is carried out.

Table 5. cDNA-synthesis mix for target gene quantification

Components	Volume (µL) per rxn
Total RNA (500ng)	depends on sample
Random hexamer primer	2.0
Nuclease-free water	Up to 9.4
5 X RT-reaction buffer	4.0
Protector RNase inhibitor	0.5
dNTP mix	2.0
DTT	1.0
RT – enzyme	1.1
Total Volume	20.0

5.3.5. Quantitative RT-PCR

Quantitative PCR analysis was performed by SYBR Green Master Mix of Roche (Switzerland). Quantitative gene expression assay for microRNAs and microRNA specific primers (KLK primers) were purchased from Exiqon Inc. (Denmark).

Table 6. Real Time PCR conditions

Program	Temperature	Time	Cycle
Pre-incubation	95 °C	10 min	1
Amplification	95 °C	15 sec	40
	60 °C	40 sec	
	72 °C	1 sec (reading)	
Cooling	95 °C	30 sec	1

The mix is optimized for SYBR Green reactions and contains SYBR Green I Dye, AmpliTaq Gold® DNA Polymerase, dNTPs with dUTP, Passive Reference, and optimized buffer components. It was performed in LightCycler480-II qRT-PCR. β -actin was used as an internal control.

5.3.6. Statistical Analysis

For statistical evaluations of the determined expression variations, data were plotted as mean $\pm$ standard deviation and p value was calculated from Student's t -test. A p value of 0.05 or below was accepted statistically significant.



Table 7.KLK primer pairs. KLK primer pairs were designed by us in our laboratory at Biruni University.

KLK1-F	CAGACTTCATGCTGTGTGTCG
KLK1-R	TTCTCCGCTATGGTGTCTC
KLK2-F	AGCCTGCCAAGATCACAGAT
KLK2-R	CCTTCTCAGAGTAAGCTCTAGCACA
KLK3-F	CGTGACGTGGATTGGTGC
KLK3-R	GCCGCAGACTGCCCTG
KLK4-F	CTCGCTAACGACCTCATGCT
KLK4-R	TGCAGACCTCCTCAGACACC
KLK5-F	TCCTCTCATTGTCCCTCTGC
KLK5-R	CGCAGAACATGGTGTTCATCT
KLK6-F	GATGGTGGTGCTGAGTCTGA
KLK6-R	CCCACAGTGGATGGATAAGG
KLK7-F	CTGTCATCCATGGTGAAGAAAGT
KLK7-R	TTGACATCCACGCACATGA
KLK8-F	GTGGCAACTGGGTCCTTACA
KLK8-R	TGCTCTGGGCCATCTTTATT
KLK9-F	TCCACCTTACTCGGCTCTTC
KLK9-R	GCTGAGGTCCTTGTTGAAGC
KLK10-F	TCTCGCTCTTCAACGGCCT
KLK10-R	CCCTACTCGAGCCCACAGT
KLK11-F	GGCAACATCACAGACACCA
KLK11-R	CCCAGGAGATAATGCCTTGA
KLK12-F	TGTGTGTTCTTGGGCTCAGC
KLK12-R	CCCACCTGTGGTCAATAAGGAC
KLK13-F	GCACAAAAGAGGGTGGCAA
KLK13-R	CGGATCCACAGGACGTATCT
KLK14-F	GCCTATCCTAGAACCATCACG
KLK14-R	CTGGAGCTGTCCTCTGCA
KLK15-F	GGAAGGTGACGAGTGTGC
KLK15-R	TTGCGCAGGTTGTGCTCT

6. RESULTS

In accordance with purity measurements, RNA concentrations of the samples are given below. (Table 8)

Table.8 Sample RNA concentrations and purity of RNA

Sample No	Tissue Type	Tissue Status	Concentration (ng/mL)
NR1	Prostate	No Recurrence	1351
NR2	Prostate	No Recurrence	429
NR3	Prostate	No Recurrence	519
NR4	Prostate	No Recurrence	303
NR5	Prostate	No Recurrence	426
NR6	Prostate	No Recurrence	1072
NR7	Prostate	No Recurrence	644
NR8	Prostate	No Recurrence	595
NR9	Prostate	No Recurrence	709
NR10	Prostate	No Recurrence	641
NR11	Prostate	No Recurrence	350
NR12	Prostate	No Recurrence	622
NR13	Prostate	No Recurrence	411
NR14	Prostate	No Recurrence	646
NR15	Prostate	No Recurrence	971
NR16	Prostate	No Recurrence	561
NR17	Prostate	No Recurrence	347
NR18	Prostate	No Recurrence	370
NR19	Prostate	No Recurrence	743
NR20	Prostate	No Recurrence	559
NR21	Prostate	No Recurrence	454
NR22	Prostate	No Recurrence	856
NR23	Prostate	No Recurrence	453
NR24	Prostate	No Recurrence	541
NR25	Prostate	No Recurrence	675
NR26	Prostate	No Recurrence	489
NR27	Prostate	No Recurrence	765
NR28	Prostate	No Recurrence	959
NR29	Prostate	No Recurrence	456

NR30	Prostate	No Recurrence	796
NR31	Prostate	No Recurrence	547
NR32	Prostate	No Recurrence	567
NR33	Prostate	No Recurrence	863
NR34	Prostate	No Recurrence	645
NR35	Prostate	No Recurrence	1154
NR36	Prostate	No Recurrence	596
R1	Prostate	Recurrence	520
R2	Prostate	Recurrence	473
R3	Prostate	Recurrence	842
R4	Prostate	Recurrence	806
R5	Prostate	Recurrence	377
R6	Prostate	Recurrence	789
R7	Prostate	Recurrence	989
R8	Prostate	Recurrence	760
R9	Prostate	Recurrence	832
R10	Prostate	Recurrence	1411
R11	Prostate	Recurrence	435
R12	Prostate	Recurrence	551
R13	Prostate	Recurrence	964
R14	Prostate	Recurrence	1118
R15	Prostate	Recurrence	1165
R16	Prostate	Recurrence	1287
R17	Prostate	Recurrence	269
R18	Prostate	Recurrence	913
R19	Prostate	Recurrence	740
R20	Prostate	Recurrence	311
R21	Prostate	Recurrence	857
R22	Prostate	Recurrence	496
R23	Prostate	Recurrence	1024
R24	Prostate	Recurrence	527
R25	Prostate	Recurrence	634
R26	Prostate	Recurrence	837
R27	Prostate	Recurrence	486
R28	Prostate	Recurrence	493
R29	Prostate	Recurrence	572

R30	Prostate	Recurrence	448
R31	Prostate	Recurrence	589
R32	Prostate	Recurrence	324
R33	Prostate	Recurrence	567
R34	Prostate	Recurrence	765

Gleason scores of tumor samples are given in Table 9.

Table 9. Gleason scores of the tumor samples

Sample No	Tissue Type	Tissue Status	Gleason of Sample
NR1	Prostate	No Recurrence	3+4
NR2	Prostate	No Recurrence	3+3
NR3	Prostate	No Recurrence	4+3
NR3	Prostate	No Recurrence	3+4
NR4	Prostate	No Recurrence	2+3
NR5	Prostate	No Recurrence	3+4
NR6	Prostate	No Recurrence	3+4
NR7	Prostate	No Recurrence	3+4
NR8	Prostate	No Recurrence	4+4
NR9	Prostate	No Recurrence	3+3
NR10	Prostate	No Recurrence	3+3
NR11	Prostate	No Recurrence	3+3
NR12	Prostate	No Recurrence	5+5
NR13	Prostate	No Recurrence	3+3
NR14	Prostate	No Recurrence	3+3
NR15	Prostate	No Recurrence	3+3
NR16	Prostate	No Recurrence	3+4
NR17	Prostate	No Recurrence	3+4
NR18	Prostate	No Recurrence	3+3
NR19	Prostate	No Recurrence	4+3
NR20	Prostate	No Recurrence	3+3
NR21	Prostate	No Recurrence	3+4

NR22	Prostate	No Recurrence	3+3
NR23	Prostate	No Recurrence	3+3
NR24	Prostate	No Recurrence	3+3
NR25	Prostate	No Recurrence	3+4
NR26	Prostate	No Recurrence	3+4
NR27	Prostate	No Recurrence	3+3
NR28	Prostate	No Recurrence	4+3
NR29	Prostate	No Recurrence	3+3
NR30	Prostate	No Recurrence	5+4
NR31	Prostate	No Recurrence	3+3
NR32	Prostate	No Recurrence	3+3
NR33	Prostate	No Recurrence	4+3
NR34	Prostate	No Recurrence	3+4
NR35	Prostate	No Recurrence	4+4
NR36	Prostate	No Recurrence	3+3
R1	Prostate	Early Recurrence	3+4
R2	Prostate	Early Recurrence	3+4
R3	Prostate	Early Recurrence	3+4
R4	Prostate	Early Recurrence	2+4
R5	Prostate	Early Recurrence	3+3
R6	Prostate	Early Recurrence	3+4
R7	Prostate	Early Recurrence	4+3
R8	Prostate	Early Recurrence	3+4
R9	Prostate	Early Recurrence	3+4
R10	Prostate	Early Recurrence	4+4
R11	Prostate	Early Recurrence	3+4
R12	Prostate	Early Recurrence	3+3
R13	Prostate	Early Recurrence	4+3
R14	Prostate	Early Recurrence	4+5
R15	Prostate	Early Recurrence	2+4
R16	Prostate	Early Recurrence	4+4
R17	Prostate	Early Recurrence	3+4
R18	Prostate	Early Recurrence	4+3
R19	Prostate	Early Recurrence	4+3
R20	Prostate	Recurrence	4+3
R21	Prostate	Recurrence	3+4

R22	Prostate	Recurrence	3+4
R23	Prostate	Recurrence	3+4
R24	Prostate	Recurrence	4+4
R25	Prostate	Recurrence	3+4
R26	Prostate	Recurrence	4+3
R27	Prostate	Recurrence	4+3
R28	Prostate	Recurrence	4+3
R29	Prostate	Recurrence	3+4
R30	Prostate	Recurrence	4+4
R31	Prostate	Recurrence	5+4
R32	Prostate	Recurrence	3+4
R33	Prostate	Recurrence	4+3
R34	Prostate	Recurrence	4+3

6.1. KLK Gene Expression Levels in Tissue Samples

Expression levels of the KLK gene family were shown by qRT-PCR in prostate samples (recurrent, non-recurrent and normal tissues). Recurrent, non-recurrent, and normal tissue specimens are evaluated with expression level comparison.

When the recurrent prostate cancer tissues and normal tissues for KLK1 gene were compared, it was determined that the level of expression in recurrent cancer tissues decreased and the p value was 0.03. In normal and non-recurrent cases, expression level in non-recurrent cancer tissue was decreased and p value was 0.03. In comparison with normal tissue and tumor (R + NR) tissue, decreased expression level in tumor tissues was observed and p value was 0.02. (Figure 6-7)

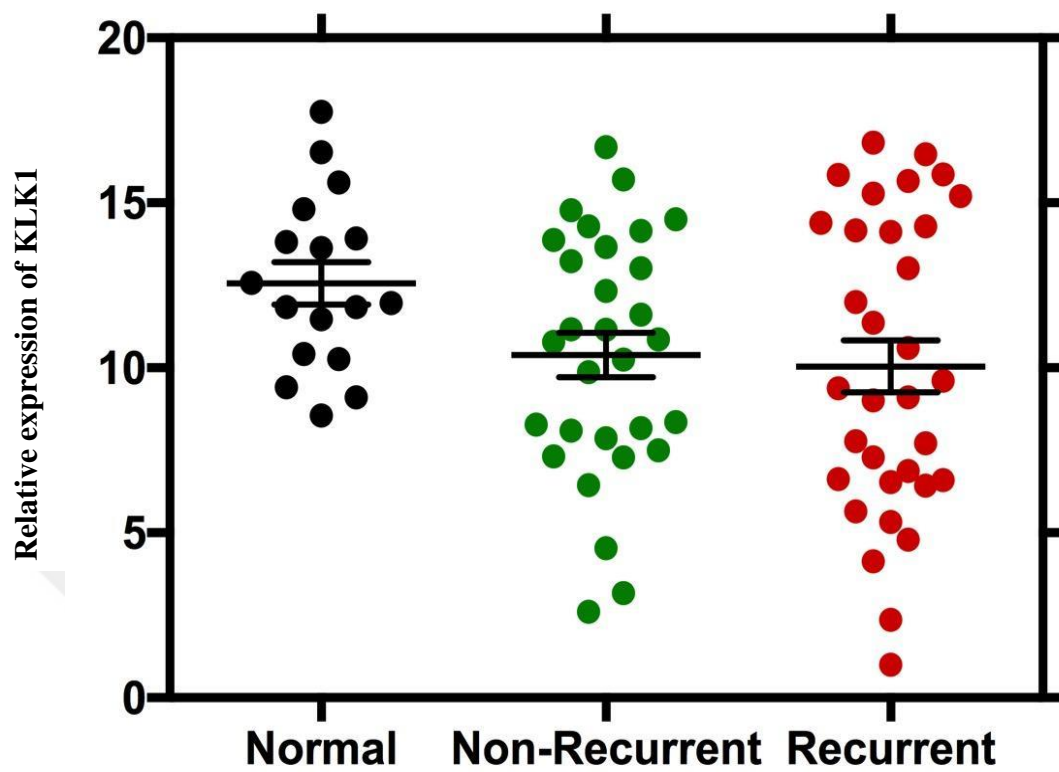


Figure 6. KLK1 gene expression levels. The expression levels of KLK1 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. Non-recurrent and recurrent cancer patients have similar expression levels.

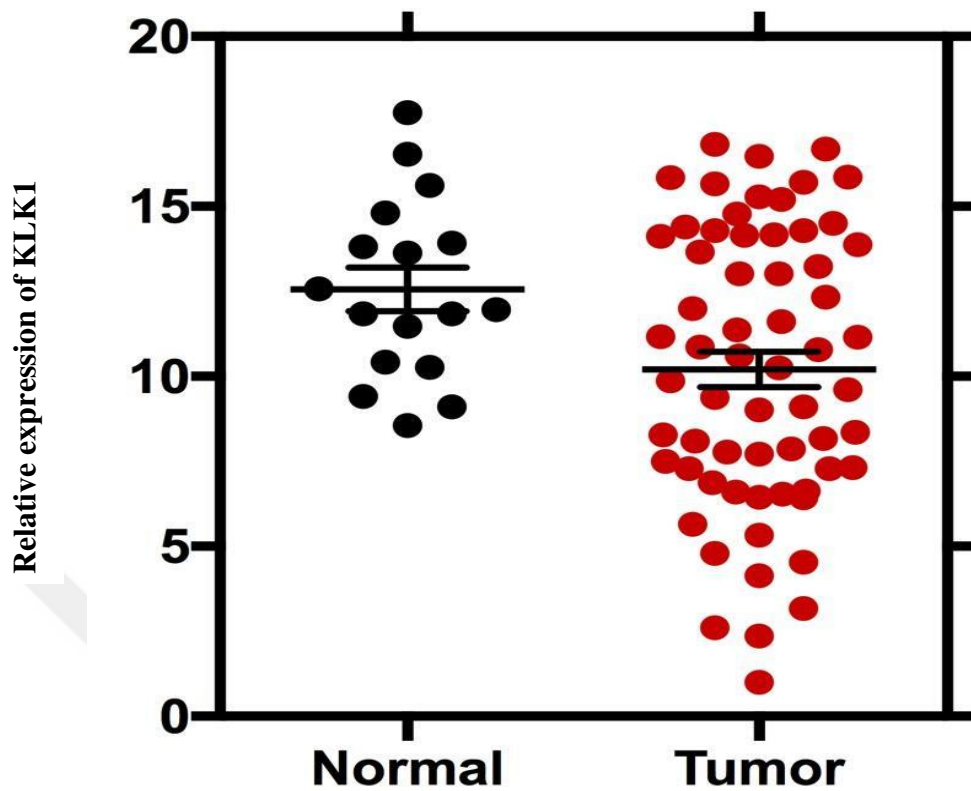


Figure 7. KLK1 gene expression levels in normal and tumor tissues. KLK1 Gene expression levels of prostate cancer is relatively lower than in normal tissues.

In comparison to recurrent prostate cancer tissues and normal tissues, the KLK2 expression level of the recurrent tumor tissues was down-regulated and the p value was 0.02. There was no significant difference in expression levels between normal and non-recurrent tissues. Also, we could not determine a remarkable difference in normal tissues and tumor (R + NR) tissues gene expression levels.

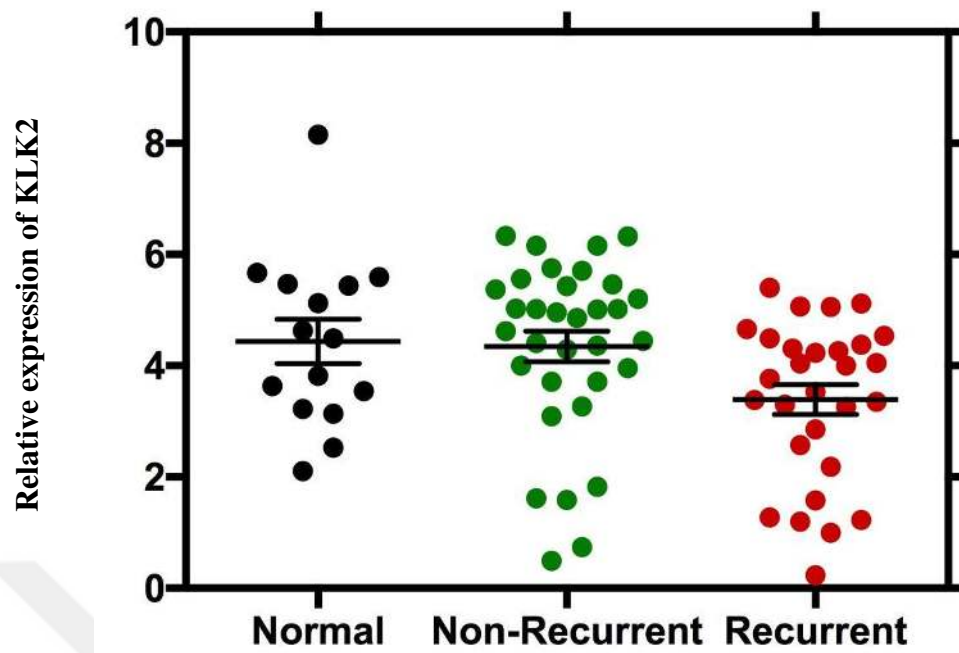


Figure 8. KLK2 gene expression levels. The expression levels of KLK2 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. While normal and non-recurrent cancer patients have similar expression levels, recurrent cancer patients have lower expression levels.

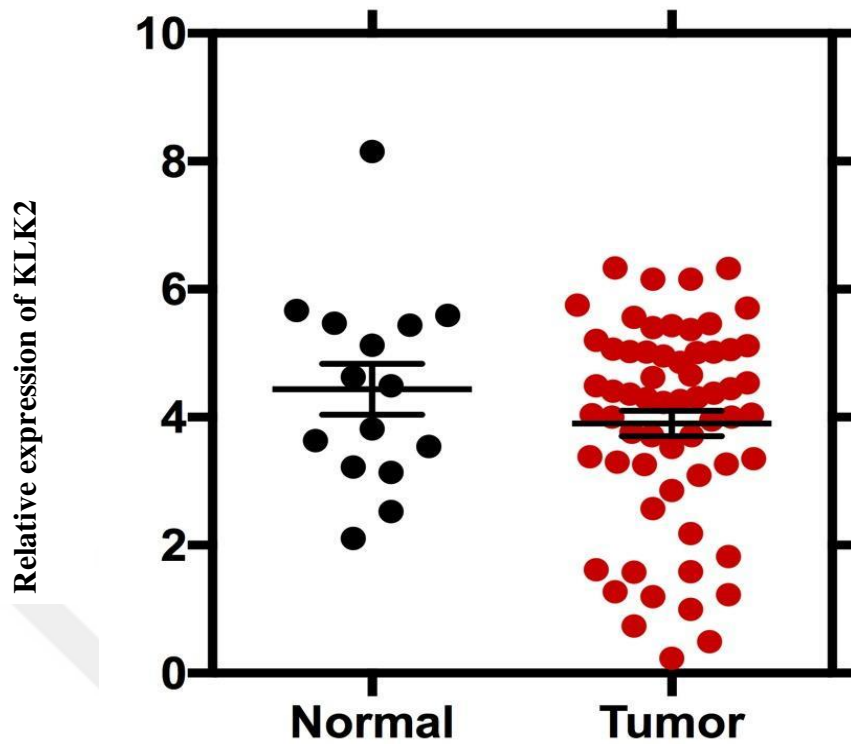


Figure 9. KLK2 gene expression levels in normal and tumor tissues. KLK2 Gene expression levels of prostate cancer is relatively lower than in normal tissues.

It was determined that comparing the recurrent prostate cancer tissues and normal tissues for the KLK3 gene, the level of gene expression in the recurrent tissues increased and the p value was 0.01. In comparison to non-recurrent prostate cancer tissues and normal tissues, KLK3 is overexpressed in non-recurrent cancer tissues and p value was determined to be 0.02. When compared with normal tissues and tumor (R + NR) tissues, increased expression level was observed in tumor tissues with a p value of 0.01.

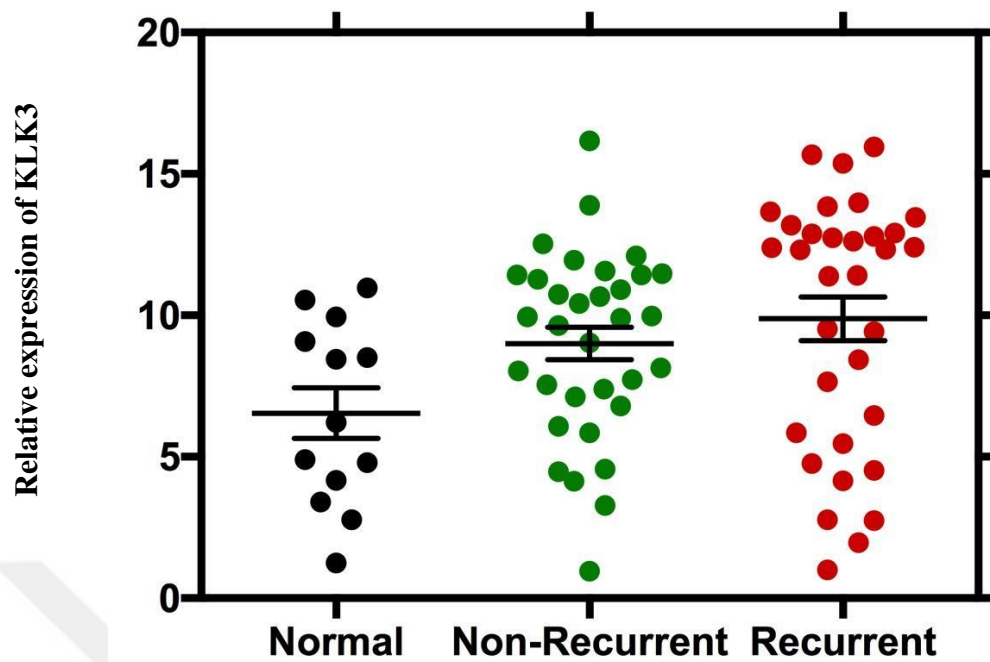


Figure 10. KLK3 gene expression levels. The expression levels of KLK3 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. Recurrent cancer patients have higher expression levels than in non-recurrent and normal tissues, respectively.

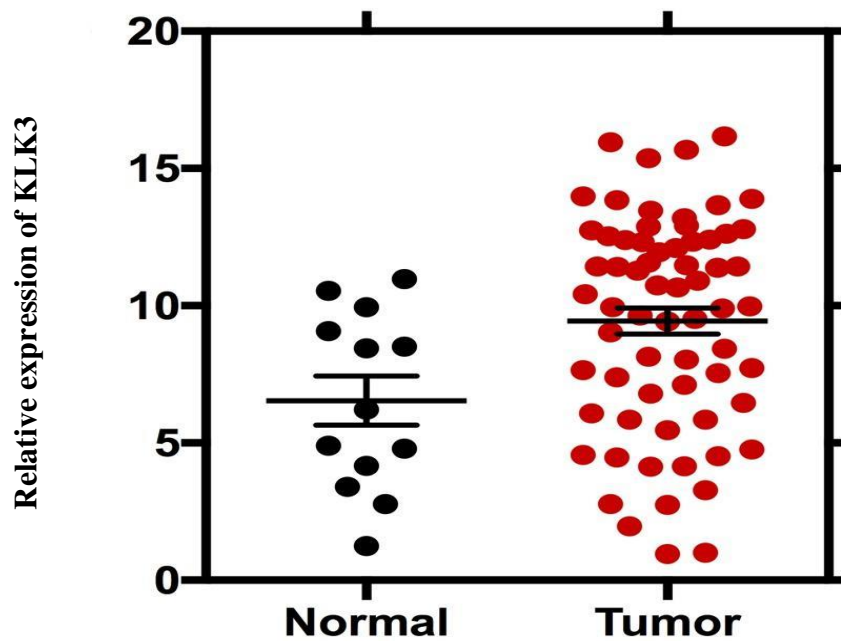


Figure 11. KLK3 gene expression levels in normal and tumor tissues. KLK3 gene expression levels of prostate cancer is higher than in normal tissues.

When the recurrent prostate cancer tissues and normal tissues for the KLK-4 gene were compared, the level of expression increased in recurrent tissues and the p value was 0.003. In comparison to normal and non-recurrent cancer tissues, the KLK4 was overexpressed in non-recurrent tissues and p value was determined to be 0.02. Up-regulated expression levels were observed in tumor tissues (R + NR) compared to normal tissues, and the p value was 0.004.

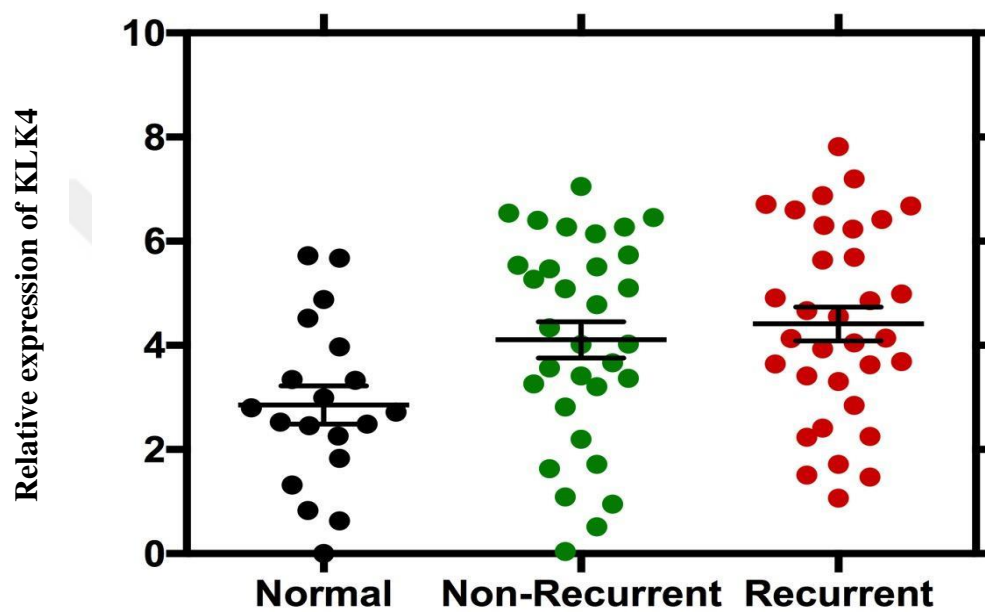


Figure 12. KLK4 gene expression levels. The expression levels of KLK4 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. Recurrent cancer patients have higher expression levels than in non-recurrent and normal tissues, respectively.

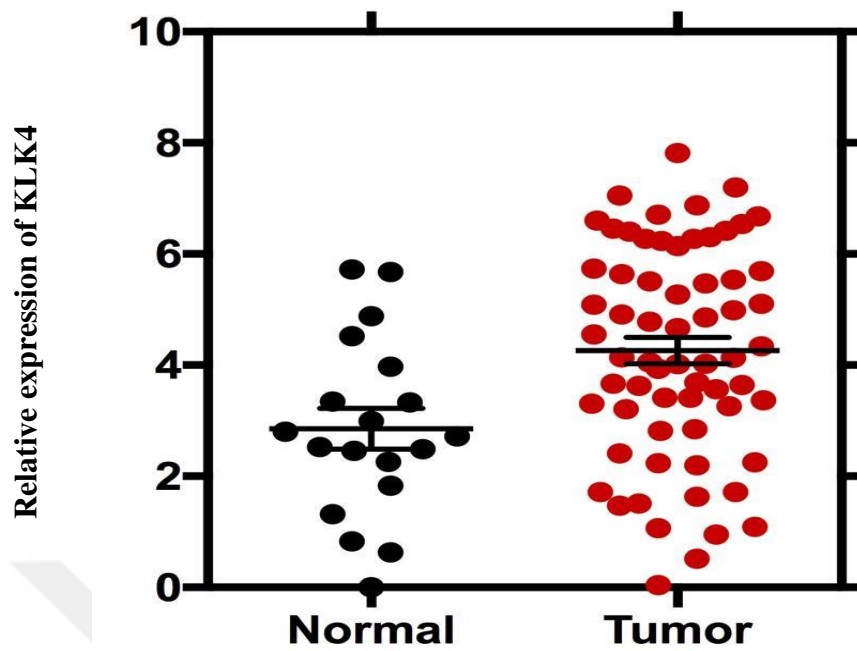


Figure 13. KLK4 gene expression levels in normal and tumor tissues. KLK4 gene expression levels of prostate cancer is higher than in normal tissues.

There was no significant change in KLK8 gene expression level in recurrent tissues compared to normal prostate cancer tissues, and the p value was 0.2. In comparison to normal and non-recurrent cancer tissues, the level of expression in non-recurrent cancer tissues increased and the p value was found 0.008. In tumor tissues (R+NR), KLK8 was overexpressed compared to normal tissues, and the p value was 0.02.

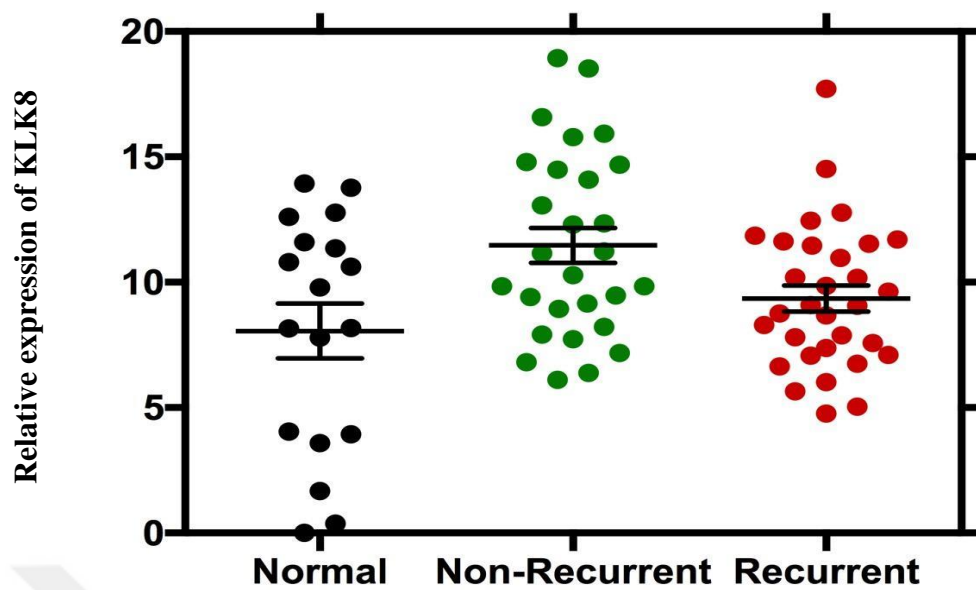


Figure 14. KLK8 gene expression levels. The expression levels of KLK8 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. Non-recurrent cancer patients have the highest expression levels compared to recurrent cancer patients.

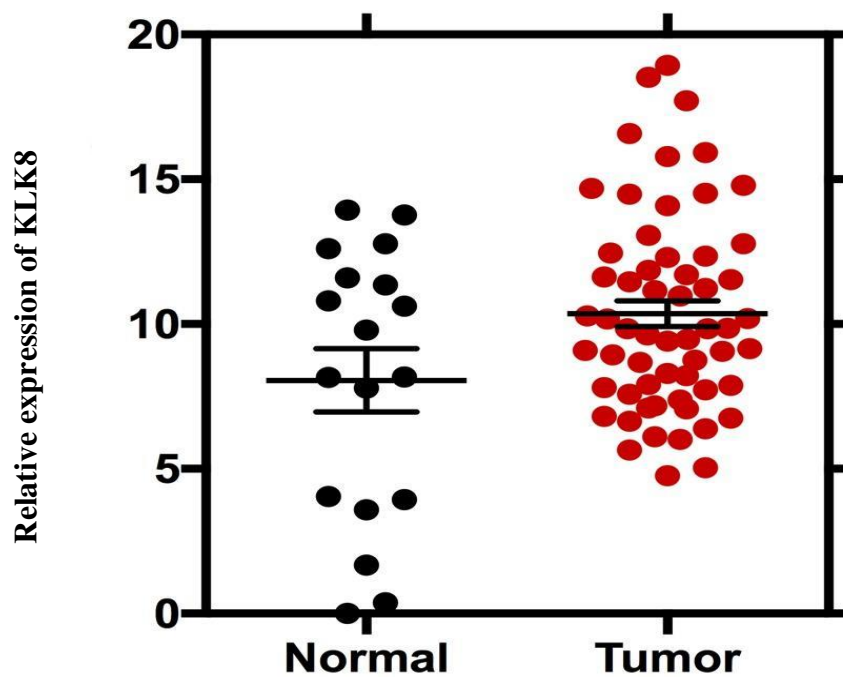


Figure 15. KLK8 gene expression levels in normal and tumor tissues. KLK8 gene expression levels of prostate cancer are higher than in normal tissues.

It was determined that the level of KLK9 gene was overexpressed in recurrent cancer tissues comparing to normal tissues, and the value of p was 0.006. When compared with normal and non-recurrent tissues, the level of expression in non-recurrent tissues increased and p value was found to be 0.02. There was a substantial increase in expression level in tumor tissues (R + NR) compared with normal tissues, and p value was 0.0002.

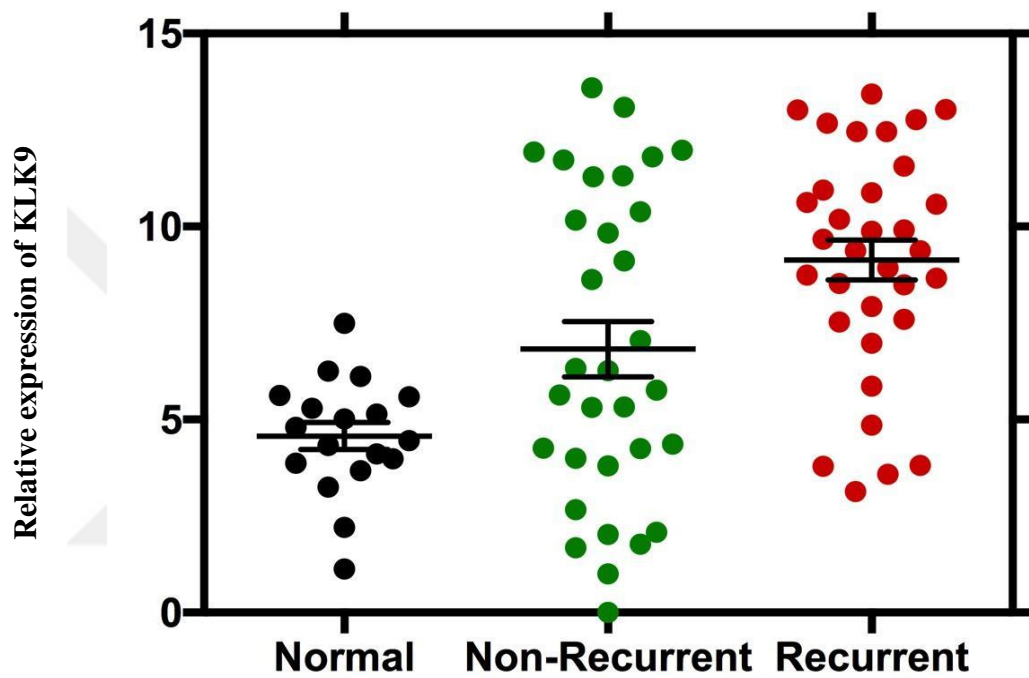


Figure 16. KLK9 gene expression levels. . The expression levels of KLK9 gene are shown in normal, non-recurrent and recurrent prostate cancer patients. Recurrent cancer patients have higher expression levels than in non-recurrent and normal tissues, respectively.

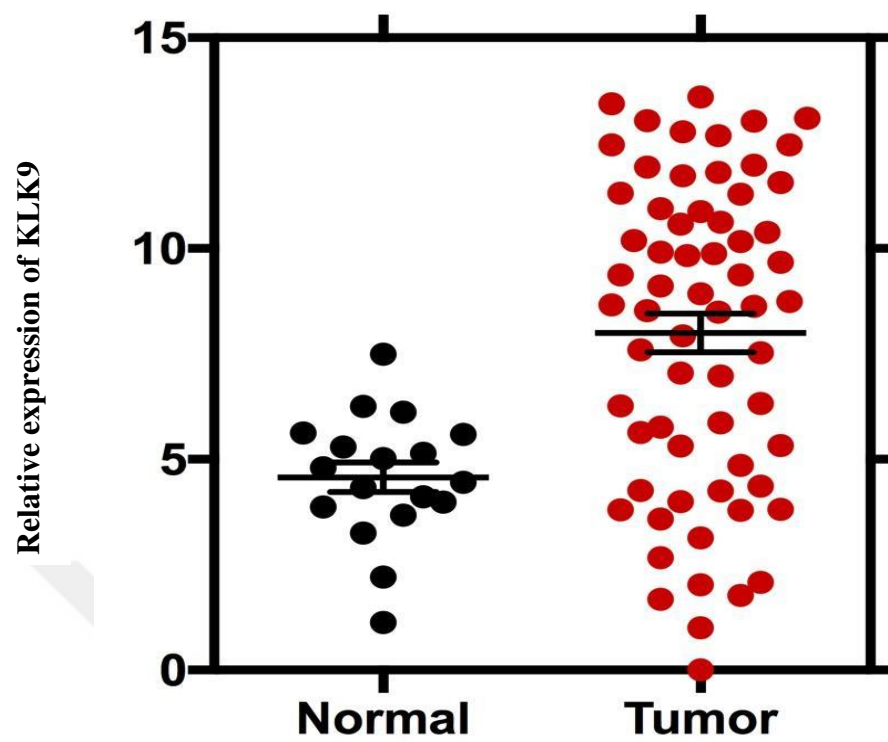


Figure 17. KLK9 gene expression levels in normal and tumor tissues. KLK9 gene expression levels of prostate cancer is significantly higher than in normal tissues.

7. DISCUSSION

The samples we used in our study were taken from a wide range of patients and the effects of the Kallikrein gene family on prostate cancer were evaluated by determining their expression levels. Prostate cancer is the most frequently encountered cancer in men after lung cancer (Rees, *et al.* 2014). It often progresses very gradually and may not cause serious harm. However more aggressive types can spread quickly without treatment. Additionally, there is a risk of recurrence of the prostate cancer after chemotherapy, radiotherapy or surgical intervention. The genetic changes that causes to the development and clinical progression of prostate cancer are poorly identified. It is also limited to research genetic alterations on the onset stage of cancer tissues due to the small number of tumor cells (Wallis and Nam 2015).

Prostate-specific antigen, or PSA, is a protein produced by normal, as well as malignant, cells of the prostate gland. The PSA level in blood is often elevated in men with prostate cancer. However, a number of benign conditions can also cause a man's PSA level to rise. The most frequent benign prostate conditions that cause an elevation in PSA level are prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate). The PSA test can also miss prostate cancer (Gaudreau, *et al.* 2016).

It has recently been reported that to improve cancer prediction in biopsies, using the Prostate Cancer Gene 3 (PCA3) parameter yields significant results (Aubin, *et al.* 2010). This parameter is more valuable than free PSA (Haese, *et al.* 2008). Cancer can be detected in 50% of cases with a PCA3 score of 100 (Marks, *et al.* 2007) and PCA3 may reduce unnecessary biopsies by 44% (Aubin, *et al.* 2010). In another study, it was stated that unnecessary biopsy was reduced by 67%, whereas 9-21% could miss high-grade tumors. In a related study, it was reported that the PCA3 rise significantly increased the rates of re-biopsy cancer detection, especially if HGPIN was present (Remzi, *et al.* 2010).

Among the serine proteases, KLKs form a family of 15 trypsin- and chymotrypsin-like proteases (Borgoño and Diamandis 2004; Lawrence, *et al.* 2010). Protease activity is controlled by several mechanisms, including regulation of gene expression, activation of their inactive pro-forms (zymogens) either autocatalytically or

by other proteases, inhibition of their activity by endogenous protease inhibitors, and phosphorylation (Turk, *et al.* 2012). Thus, kallikrein-related peptidase (KLK) family members are prospective targets for treatment of prostate cancer (Sotiropoulou and Pampalakis 2012).

In humans, the kallikrein gene family was known to include only three members: the gene encoding pancreatic/renal kallikrein (KLK1), the gene encoding kallikrein 2 (KLK2) and the gene encoding prostate-specific antigen (PSA; KLK3) (Riegman, *et al.* 1992). It is reported that all 15 KLKs are expressed in the prostate at the mRNA level (Shaw and Diamandis 2007). However, the PSA and some of the KLK gene family members may enhance the growth of prostate cancer by stimulating cell proliferation (Williams, *et al.* 2011).

In our study, the expression profile of 6 genes (KLK1, KLK2, KLK3, KLK4, KLK8, KLK9) that related to prostate cancer tissues were characterized.

KLK1 is underexpressed in recurrent and non-recurrent prostate cancer tissues compare to normal prostate tissues. This is in contrast to findings by Mingxin (Zuo, *et al.* 2016) of KLK1 expression in the GBC (gallbladder cancer), especially in female GBC patients.

There are potent indications that the KLK2, KLK3 and KLK4 genes are expressed in various tissues, such as breast and prostate (Yousef and Diamandis 1999).

The KLK2 protein product, human kallikrein 2 (hK2) is emerging as an additional prostatic tumor marker. hK2 is responsible for the activation of PSA by cleaving its pro-form to the enzymatically active mature form (Kumar, *et al.* 1997). The cancer risk is also correlated with lower hK2 and higher %fPSA, both of which are associated with lower tPSA (Klein, *et al.* 2010). In related to prior study, KLK2 is underexpressed in recurrent prostate cancer tissues in comparison to normal tissues in our case.

PSA (KLK3) is one of the family members of human tissue kallikreins that play vital role in both normal biology and tumor development and progression (Yousef and Diamandis 2001).

KLK3 is overexpressed in recurrent and non-recurrent prostatic cancer tissues in our experiments.

In our study, KLK4 is found to be overexpressed in recurrent and non-recurrent prostate cancer tissues. Similarly, in another experiment, KLK4 was found to be expressed significantly in a subset of ovarian cancer tissue extracts in the ratio of 55%. Severe positive association was found between KLK4 expression and tumor grade ($P = 0.02$) and clinical stage ($P < 0.001$) (Obiezu, *et al.* 2001).

KLK5 is overexpressed in ovarian cancer tissues (Diamandis and Yousef 2001). In another study a significant decrease is found in KLK5 expression levels in prostate cancer tissues, compared to their normal counterparts (Yousef, *et al.* 2002)

KLK6 is previously shown as a biomarker for ovarian carcinoma (Diamandis, *et al.* 2003) and also shown to be overexpressed in colorectal (Ogawa, *et al.* 2005) and gastric cancers (Nagahara, *et al.* 2005).

KLK7 mRNA was significantly lowered in either stage I or stage II breast cancer patients and higher level of KLK7 mRNA was found to be associated with better prognosis (Holzscheiter, *et al.* 2006; Talieri, *et al.* 2004). KLK7 (stratum corneum chymotryptic enzyme, HSCCE) has been shown to be expressed at abnormally high levels in ovarian cancer (Ogawa, *et al.* 2005).

KLK8 is upregulated in both recurrent and non-recurrent prostate cancer tissues. Interestingly, KLK8 is expressed lower in recurrent prostate cancer compared to non-recurrent cancer. The patients who have recurrent prostate cancer, might have a loss of KLK8 gene sequences or are affected by other factors.

In ovarian tumors, the expression of the KLK8 gene and its spliced variants indicated that the new variants were expressed very frequently. This full-length KLK8 expression is an independent and favorable prognostic marker for ovarian cancer (Magklara, *et al.* 2001).

KLK9 is upregulated in recurrent and non-recurrent prostate cancer tissues in our case. Furthermore, KLK9 expression is significantly higher in breast cancer patients with early stages compared with advanced stages ($p = 0.039$) and in patients with tumor size < 2 cm compared with larger tumors ($p = 0.028$) (Yousef, *et al.* 2003). It is also reported for the first time that higher KLK9 expression has favorable prognostic value in ovarian cancer (Yousef, *et al.* 2001).

KLK11 is expressed in several human tissues. The highest levels of KLK11 were found in the prostate, followed by stomach, trachea, skin, and colon (Diamandis, *et al.* 2002).

It is provided that the expression of KLK13, recently identified family members of kallikreins, is significantly up-regulated in metastatic lung adenocarcinoma. While overexpression of KLK13 resulted in an increase in malignant cell behavior, knockdown of its endogenous gene expression caused a significant decrease in cell migratory and invasive properties. Functional studies further demonstrated that KLK13 is activated via demethylation of its upstream region (Chou, *et al.* 2011).

7 genes (KLK5-8, KLK10, KLK11, and KLK14) were analyzed in another study and they were found to be up-regulated in ovarian cancer tissues and cell lines, compared with normal ovary (Yousef, *et al.* 2003).

KLK12, KLK13 and KLK14 genes are downregulated in breast cancer (Diamandis and Yousef 2001).

Although KLK15 was found to be overexpressed in more aggressive forms of prostate cancer tissues in other studies, we did not determine a significant result in our case (Stephan, *et al.* 2003).

While, KLK1 and KLK2 genes (only in recurrent prostate cancer tissues) are down-regulated in prostatic cancer tissues, other genes (KLK3, KLK4, KLK8, KLK9) are found to be overexpressed in both recurrent and non-recurrent prostate cancer tissues in our experiment.

We could not determine a significant expression results in other KLK genes, such as KLK5, KLK6, KLK7, KLK10, KLK11, KLK12, KLK13, KLK14, KLK15.

Gene fusion and chromosomal rearrangements were previously thought to be primarily the oncogenic mechanism of hematological malignancies and sarcomas.

We suggest that the functions of KLKs include either promoting or inhibiting tumor growth and/or metastasis by regulated gene expression. In recent studies, majority of prostate cancers have affected by recurrent gene fusions which have important clinical and biological implications in the study of common epithelial tumors (Kumar-Sinha, *et al.* 2008). Polymorphisms, gene fusions and mutations could affect

the higher or lower expression levels on both non-recurrent and recurrent prostate cancers.



8. CONCLUSION

In conclusion, the difference in expression levels of the KLK gene family in recurrent prostate cancer has been shown for the first time by us in the literature.

For the further studies, these genes have the potential to be the prognostic marker genes for progression of the prostate cancer. It should be aimed to identify novel therapies to affect the expression levels of KLK genes.

New immunoassays are developed for new kallikrein proteins to diagnose other cancers (ovarian) and other diseases (Alzheimer's) (Obiezu, *et al.* 2001). It should also be aimed to diagnose prostate cancer at early stages by detecting kallikrein proteins with newly developed immunoassay kits.

9. REFERENCES

Anderson DE, Badzioch MD (1993). Familial effects of prostate and other cancers on lifetime breast cancer risk. *Breast Cancer Res Treat* 28:107-113

Aubin SM, Reid J, Sarno MJ, Blase A, Aussie J, Rittenhouse H, Rittmaster R, Andriole GL, Groskopf J (2010). PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial. *J Urol* 184:1947-1952

Bellier A, Colonna M, Delafosse P, Seigneurin A (2018). Incidence of prostate cancer and net survival by grade in a geriatric population: A population-based study in a French administrative entity from 1991 to 2013. *Cancer Epidemiol* 56:60-66

Bennett KE, Howell A, Evans DG, Birch JM (2002). A follow-up study of breast and other cancers in families of an unselected series of breast cancer patients. *Br J Cancer* 86:718-722

Bhargava S (2014). Increased DHT levels in androgenic alopecia have been selected for to protect men from prostate cancer. *Med Hypotheses* 82:428-432

Biyashev D, Tan F, Chen Z, Zhang K, Deddish PA, Erdös EG, Hecquet C (2006). Kallikrein activates bradykinin B2 receptors in absence of kininogen. *Am J Physiol Heart Circ Physiol* 290:H1244-1250

Björkqvist J, Jämsä A, Renné T (2013). Plasma kallikrein: the bradykinin-producing enzyme. *Thromb Haemost* 110:399-407

Blanc-Lapierre A, Rousseau MC, Parent ME (2017). Perceived Workplace Stress Is Associated with an Increased Risk of Prostate Cancer before Age 65. *Front Oncol* 7:269

Borgoño CA, Diamandis EP (2004). The emerging roles of human tissue kallikreins in cancer. *Nat Rev Cancer* 4:876-890

Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, Morrison H, Sonawane B, Shifflett T, Waters DJ, Timms B (2004). Human prostate cancer risk factors. *Cancer* 101:2371-2490

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC (1992). Mendelian inheritance of familial prostate cancer. *Proc Natl Acad Sci U S A* 89:3367-3371

Caster JM, Falchook AD, Hendrix LH, Chen RC (2015). Risk of Pathologic Upgrading or Locally Advanced Disease in Early Prostate Cancer Patients Based on Biopsy Gleason Score and PSA: A Population-Based Study of Modern Patients. *Int J Radiat Oncol Biol Phys* 92:244-251

Charest-Morin X, Raghavan A, Charles ML, Kolodka T, Bouthillier J, Jean M, Robbins MS, Marceau F (2015). Pharmacological effects of recombinant human tissue kallikrein on bradykinin B2 receptors. *Pharmacol Res Perspect* 3:e00119

Chen N, Zhou Q (2016). The evolving Gleason grading system. *Chin J Cancer Res* 28:58-64

Cheng L, Montironi R, Bostwick DG, Lopez-Beltran A, Berney DM (2012). Staging of prostate cancer. *Histopathology* 60:87-117

Chou RH, Lin SC, Wen HC, Wu CW, Chang WS (2011). Epigenetic activation of human kallikrein 13 enhances malignancy of lung adenocarcinoma by promoting N-cadherin expression and laminin degradation. *Biochem Biophys Res Commun* 409:442-447

Coffey DS (1993). Prostate cancer. An overview of an increasing dilemma. *Cancer* 71:880-886

Dhom G (1983). Epidemiologic aspects of latent and clinically manifest carcinoma of the prostate. *J Cancer Res Clin Oncol* 106:210-218

Diamandis EP, Okui A, Mitsui S, Luo LY, Soosaipillai A, Grass L, Nakamura T, Howarth DJ, Yamaguchi N (2002). Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. *Cancer Res* 62:295-300

Diamandis EP, Scrilas A, Fracchioli S, Van Gramberen M, De Bruijn H, Henrik A, Soosaipillai A, Grass L, Yousef GM, Stenman UH, Massobrio M, Van Der Zee AG, Vergote I, Katsaros D (2003). Human kallikrein 6 (hK6): a new potential serum

biomarker for diagnosis and prognosis of ovarian carcinoma. *J Clin Oncol* 21:1035-1043

Diamandis EP, Yousef GM (2001). Human tissue kallikrein gene family: a rich source of novel disease biomarkers. *Expert Rev Mol Diagn* 1:182-190

Diamandis EP, Yousef GM, Luo LY, Magklara A, Obiezu CV (2000). The new human kallikrein gene family: implications in carcinogenesis. *Trends Endocrinol Metab* 11:54-60

Digby M, Zhang XY, Richards RI (1989). Human prostate specific antigen (PSA) gene: structure and linkage to the kallikrein-like gene, hGK-1. *Nucleic Acids Res* 17:2137

Epstein JI, Zelefsky MJ, Sjoberg DD, Nelson JB, Egevad L, Magi-Galluzzi C, Vickers AJ, Parwani AV, Reuter VE, Fine SW, Eastham JA, Wiklund P, Han M, Reddy CA, Ciezki JP, Nyberg T, Klein EA (2016). A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Eur Urol* 69:428-435

Fiz F, Dittman H, Campi C, Morbelli S, Marini C, Brignone M, Bauckneht M, Piva R, Massone AM, Piana M, Sambuceti G, la Fougère C (2018). Assessment of Skeletal Tumor Load in Metastasized Castration-Resistant Prostate Cancer Patients: A Review of Available Methods and an Overview on Future Perspectives. *Bioengineering* (Basel) 5(3):58

Gann PH (2002). Risk factors for prostate cancer. *Rev Urol* 4 Suppl 5:S3-S10

Gaudreau PO, Stagg J, Soulières D, Saad F (2016). The Present and Future of Biomarkers in Prostate Cancer: Proteomics, Genomics, and Immunology Advancements. *Biomark Cancer* 8:15-33

Gleason DF (1992). Histologic grading of prostate cancer: a perspective. *Hum Pathol* 23:273-279

Grossman DC, Curry SJ, Owens DK, Bibbins-Domingo K, Caughey AB, Davidson KW, Doubeni CA, Ebell M, Epling JW, Kemper AR, Krist AH, Kubik M, Landefeld CS, Mangione CM, Silverstein M, Simon MA, Siu AL, Tseng CW, Force UPST (2018).

Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 319:1901-1913

Haese A, de la Taille A, van Poppel H, Marberger M, Stenzl A, Mulders PF, Huland H, Abbou CC, Remzi M, Tinzi M, Feyerabend S, Stillebroer AB, van Gils MP, Schalken JA (2008). Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol* 54:1081-1088

Hanna C, Jones RJ (2015). Emerging treatments for recurrent prostate cancer. *Future Oncol* 11:2873-2880

Hassanipour-Azgomi S, Mohammadian-Hafshejani A, Ghoncheh M, Towhidi F, Jamehshorani S, Salehiniya H (2016). Incidence and mortality of prostate cancer and their relationship with the Human Development Index worldwide. *Prostate Int* 4:118-124

Haydaroglu A BY, Özşaran Z. (2007). Cancer registry analyzes in Ege University:

Evaluation of 34134 cases. *Turkish Journal of Oncology* . 22(1):22-8

Hayes JH, Barry MJ (2014). Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence. *JAMA* 311:1143-1149

Holzschelter L, Biermann JC, Kotzsch M, Prezas P, Farthmann J, Baretton G, Luther T, Tjan-Heijnen VC, Talieri M, Schmitt M, Sweep FC, Span PN, Magdolen V (2006). Quantitative reverse transcription-PCR assay for detection of mRNA encoding full-length human tissue kallikrein 7: prognostic relevance of KLK7 mRNA expression in breast cancer. *Clin Chem* 52:1070-1079

Incrocci L (2015). Radiotherapy for prostate cancer and sexual health. *Transl Androl Urol* 4:124-130

Khazaei S, Rezaeian S, Ayubi E, Gholamalinee B, Pishkuhi MA, Mansori K, Nematollahi S, Sani M, Hanis SM (2016). Global Prostate Cancer Incidence and Mortality Rates According to the Human Development Index. *Asian Pac J Cancer Prev* 17:3793-3796

- Klein RJ, Halldén C, Cronin AM, Ploner A, Wiklund F, Bjartell AS, Stattin P, Xu J, Scardino PT, Offit K, Vickers AJ, Grönberg H, Lilja H (2010). Blood biomarker levels to aid discovery of cancer-related single-nucleotide polymorphisms: kallikreins and prostate cancer. *Cancer Prev Res (Phila)* 3:611-619
- Klotz L, Emberton M (2014). Management of low risk prostate cancer: active surveillance and focal therapy. *Curr Opin Urol* 24:270-279
- Kornberg Z, Cooperberg MR, Spratt DE, Feng FY (2018). Genomic biomarkers in prostate cancer. *Transl Androl Urol* 7:459-471
- Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS (1997). Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. *Cancer Res* 57:3111-3114
- Kumar-Sinha C, Tomlins SA, Chinnaiyan AM (2008). Recurrent gene fusions in prostate cancer. *Nat Rev Cancer* 8:497-511
- Lawrence MG, Lai J, Clements JA (2010). Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. *Endocr Rev* 31:407-446
- Lee CH, Akin-Olugbade O, Kirschenbaum A (2011). Overview of prostate anatomy, histology, and pathology. *Endocrinol Metab Clin North Am* 40:565-575, viii-ix
- Leitzmann MF, Rohrmann S (2012). Risk factors for the onset of prostatic cancer: age, location, and behavioral correlates. *Clin Epidemiol* 4:1-11
- Logothetis CJ, Gallick GE, Maity SN, Kim J, Aparicio A, Efstathiou E, Lin SH (2013). Molecular classification of prostate cancer progression: foundation for marker-driven treatment of prostate cancer. *Cancer Discov* 3:849-861
- Magklara A, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Danese S, Diamandis EP (2001). The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. *Clin Cancer Res* 7:806-811

Mahran A, Turk A, Buzzy C, Wang M, Yang J, Neudecker M, Jaeger I, Ponsky LE (2018). Younger Men with Prostate Cancer have Lower Risk of Upgrading while on Active Surveillance: A Meta-analysis and Systematic Review of the Literature. *Urology*

Marks LS, Bostwick DG (2008). Prostate Cancer Specificity of PCA3 Gene Testing: Examples from Clinical Practice. *Rev Urol* 10:175-181

Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SM, Cancio AT, Desaulniers M, Ellis WJ, Rittenhouse H, Groskopf J (2007). PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* 69:532-535

McVary KT, Rademaker AW (1990). Re: Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. *J Urol* 144:1485-1486

Mithal P, Howard LE, Aronson WJ, Kane CJ, Cooperberg MR, Terris MK, Amling CL, Freedland SJ (2015). Prostate-specific antigen level, stage or Gleason score: which is best for predicting outcomes after radical prostatectomy, and does it vary by the outcome being measured? Results from Shared Equal Access Regional Cancer Hospital database. *Int J Urol* 22:362-366

Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, Fossati N, Gross T, Henry AM, Joniau S, Lam TB, Mason MD, Matveev VB, Moldovan PC, van den Bergh RCN, Van den Broeck T, van der Poel HG, van der Kwast TH, Rouvière O, Schoots IG, Wiegel T, Cornford P (2017). EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol* 71:618-629

Mottet N, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V, Schmid HP, Van der Kwast T, Wiegel T, Zattoni F, Heidenreich A (2011). EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. *Eur Urol* 59:572-583

Murray SR, Chao J, Lin FK, Chao L (1990). Kallikrein multigene families and the regulation of their expression. *J Cardiovasc Pharmacol* 15 Suppl 6:S7-16

Nagahara H, Mimori K, Utsunomiya T, Barnard GF, Ohira M, Hirakawa K, Mori M (2005). Clinicopathologic and biological significance of kallikrein 6 overexpression in human gastric cancer. *Clin Cancer Res* 11:6800-6806

Nelson Q, Agarwal N, Stephenson R, Cannon-Albright LA (2013). A population-based analysis of clustering identifies a strong genetic contribution to lethal prostate cancer. *Front Genet* 4:152

Obiezu CV, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Rigault de la Longrais IA, Arisio R, Diamandis EP (2001). Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. *Clin Cancer Res* 7:2380-2386

Ogawa K, Utsunomiya T, Mimori K, Tanaka F, Inoue H, Nagahara H, Murayama S, Mori M (2005). Clinical significance of human kallikrein gene 6 messenger RNA expression in colorectal cancer. *Clin Cancer Res* 11:2889-2893

Ostrander EA, Stanford JL (2000). Genetics of prostate cancer: too many loci, too few genes. *Am J Hum Genet* 67:1367-1375

Pavlopoulou A, Pampalakis G, Michalopoulos I, Sotiropoulou G (2010). Evolutionary history of tissue kallikreins. *PLoS One* 5:e13781

Peisch SF, Van Blarigan EL, Chan JM, Stampfer MJ, Kenfield SA (2017). Prostate cancer progression and mortality: a review of diet and lifestyle factors. *World J Urol* 35:867-874

Pezaro C, Woo HH, Davis ID (2014). Prostate cancer: measuring PSA. *Intern Med J* 44:433-440

Ploussard G, de la Taille A (2018). The role of prostate cancer antigen 3 (PCA3) in prostate cancer detection. *Expert Rev Anticancer Ther* 1-8

Prehn RT (1999). On the prevention and therapy of prostate cancer by androgen administration. *Cancer Res* 59:4161-4164

Rees JR, Zens MS, Gui J, Celaya MO, Riddle BL, Karagas MR (2014). Non melanoma skin cancer and subsequent cancer risk. *PLoS One* 9:e99674

Remzi M, Haese A, Van Poppel H, De La Taille A, Stenzl A, Hennenlotter J, Marberger M (2010). Follow-up of men with an elevated PCA3 score and a negative biopsy: does an elevated PCA3 score indeed predict the presence of prostate cancer? *BJU Int* 106:1138-1142

Riegman PH, Vlietstra RJ, Suurmeijer L, Cleutjens CB, Trapman J (1992). Characterization of the human kallikrein locus. *Genomics* 14:6-11

Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD (1993). The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. *J Urol* 150:379-385

Schrecengost R, Knudsen KE (2013). Molecular pathogenesis and progression of prostate cancer. *Semin Oncol* 40:244-258

Shaw JL, Diamandis EP (2007). Distribution of 15 human kallikreins in tissues and biological fluids. *Clin Chem* 53:1423-1432

Sotiropoulou G, Pampalakis G (2012). Targeting the kallikrein-related peptidases for drug development. *Trends Pharmacol Sci* 33:623-634

Stark JR, Perner S, Stampfer MJ, Sinnott JA, Finn S, Eisenstein AS, Ma J, Fiorentino M, Kurth T, Loda M, Giovannucci EL, Rubin MA, Mucci LA (2009). Gleason score and lethal prostate cancer: does $3 + 4 = 4 + 3$? *J Clin Oncol* 27:3459-3464

Steinberg GD, Carter BS, Beaty TH, Childs B, Walsh PC (1990). Family history and the risk of prostate cancer. *Prostate* 17:337-347

Stephan C, Yousef GM, Scorilas A, Jung K, Jung M, Kristiansen G, Hauptmann S, Bharaj BS, Nakamura T, Loening SA, Diamandis E (2003). Quantitative analysis of kallikrein 15 gene expression in prostate tissue. *J Urol* 169:361-364

Sternberg CN, Petrylak DP, Madan RA, Parker C (2014). Progress in the treatment of advanced prostate cancer. *Am Soc Clin Oncol Educ Book* 117-131

- Talieri M, Diamandis EP, Gourgiotis D, Mathioudaki K, Scorilas A (2004). Expression analysis of the human kallikrein 7 (KLK7) in breast tumors: a new potential biomarker for prognosis of breast carcinoma. *Thromb Haemost* 91:180-186
- Turk B, Turk D, Turk V (2012). Protease signalling: the cutting edge. *EMBO J* 31:1630-1643
- Wallis CJ, Nam RK (2015). Prostate Cancer Genetics: A Review. *EJIFCC* 26:79-91
- Williams SA, Jelinek CA, Litvinov I, Cotter RJ, Isaacs JT, Denmeade SR (2011). Enzymatically active prostate-specific antigen promotes growth of human prostate cancers. *Prostate* 71:1595-1607
- Wilson AH (2014). The prostate gland: a review of its anatomy, pathology, and treatment. *JAMA* 312:562
- Woodrum DA, Kawashima A, Gorny KR, Mynderse LA (2015). Magnetic Resonance-Guided Thermal Therapy for Localized and Recurrent Prostate Cancer. *Magn Reson Imaging Clin N Am* 23:607-619
- Wright JL, Salinas CA, Lin DW, Kolb S, Koopmeiners J, Feng Z, Stanford JL (2009). Prostate cancer specific mortality and Gleason 7 disease differences in prostate cancer outcomes between cases with Gleason 4 + 3 and Gleason 3 + 4 tumors in a population based cohort. *J Urol* 182:2702-2707
- Yayama K, Kunimatsu N, Teranishi Y, Takano M, Okamoto H (2003). Tissue kallikrein is synthesized and secreted by human vascular endothelial cells. *Biochim Biophys Acta* 1593:231-238
- Yousef GM, Diamandis EP (1999). The new kallikrein-like gene, KLK-L2. Molecular characterization, mapping, tissue expression, and hormonal regulation. *J Biol Chem* 274:37511-37516
- Yousef GM, Diamandis EP (2001). The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr Rev* 22:184-204

Yousef GM, Kyriakopoulou LG, Scorilas A, Fracchioli S, Ghiringhello B, Zarghooni M, Chang A, Diamandis M, Giardina G, Hartwick WJ, Richiardi G, Massobrio M, Diamandis EP, Katsaros D (2001). Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. *Cancer Res* 61:7811-7818

Yousef GM, Polymeris ME, Yacoub GM, Scorilas A, Soosaipillai A, Popalis C, Fracchioli S, Katsaros D, Diamandis EP (2003). Parallel overexpression of seven kallikrein genes in ovarian cancer. *Cancer Res* 63:2223-2227

Yousef GM, Scorilas A, Chang A, Rendl L, Diamandis M, Jung K, Diamandis EP (2002). Down-regulation of the human kallikrein gene 5 (KLK5) in prostate cancer tissues. *Prostate* 51:126-132

Yousef GM, Scorilas A, Nakamura T, Ellatif MA, Ponzzone R, Biglia N, Maggiorotto F, Roagna R, Sismondi P, Diamandis EP (2003). The prognostic value of the human kallikrein gene 9 (KLK9) in breast cancer. *Breast Cancer Res Treat* 78:149-158

Zuo M, Rashid A, Wang Y, Jain A, Li D, Behari A, Kapoor VK, Koay EJ, Chang P, Vauthey JN, Li Y, Espinoza JA, Roa JC, Javle M (2016). RNA sequencing-based analysis of gallbladder cancer reveals the importance of the liver X receptor and lipid metabolism in gallbladder cancer. *Oncotarget* 7:35302-35312

(Date of access: 21.06.2018), <https://www.mayoclinic.org/diseases-conditions/prostate-cancer/symptoms-causes/syc-20353087>

(Date of access: 23.06.2018), <http://globocan.iarc.fr/old/FactSheets/cancers/prostate-map-il.png>

(Date of access : 16.05.2018), <https://seer.cancer.gov/statfacts/html/prost.html>

(Date of access: 23.06.2018), <http://globocan.iarc.fr/old/FactSheets/cancers/prostate-map-m1.png>

(Date of access: 29.06.2018), <https://peterattiamd.com/wpcontent/uploads/2018/02/prostate-survival-1.png>

(Date of access: 30.06.2018), www.cancer.gov

GÖNÜLLÜ OLUR FORMU

“Prostat Kanserinde Kallikrein (KLK) Gen Ailesinin Ekspresyon Seviyelerinin Belirlenmesi” başlıklı çalışmada prostat kanserinin nüks etmesinde bu genlerin rolleri anlaşılarak kanser prognozundaki etkilerinin aydınlatılması hedeflenmektedir. Tez çalışması kapsamında tekrar eden ve tekrar etmeyen prostat kanserli hastalar ile kontrol gruplarından elde edilen prostat dokularında KLK gen ailesine ait 15 genin ekspresyon düzeyinin karşılaştırılması amaçlanmaktadır.

19 Normal, 36 tekrar etmeyen ve 34 tekrar eden prostat kanseri doku örneklerinden RNA izolasyonu gerçekleştirilecektir. Konsantrasyonları ve saflık oranları spektrofotometre ile ölçülecektir.

Bu çalışma ile prostat kanserinin biyolojisi daha iyi anlaşılabilecek ve yeni bulgular ile tedavi için yeni yöntemler geliştirmek mümkün olacaktır. Prostat kanseri alanında elde edilecek bu bulgular ışığında prostat kanseri mekanizması daha iyi anlaşılabilir ve tedaviye yönelik yeni projelere öncülük edilebilecektir.

Araştırmaya katılmayı red etme hakkına sahipsiniz. İstediğiniz anda araştırmacıya haber vererek çalışmadan çekilebilir ya da araştırmacı tarafından gerek görüldüğünde araştırma dışı bırakılabilirsiniz. Araştırmayı kabul etmemeniz durumunda veya herhangi bir nedenle çalışma programından çıkmanız halinde, hastalığı ile ilgili tedavisinde bir aksama olmayacaktır.

Kabul etmeniz halinde katılacağınız bu çalışma bir araştırmadır. Araştırmanın amacı hastalığınız ile ilişkisi olduğu düşünülen gen düzeylerini incelemektir. Gönüllü olarak sizin araştırma üzerinde herhangi bir sorumluluğunuz bulunmamaktadır. Araştırma boyunca sizden bir maddi katkı talebi olmayacaktır ayrıca size de bir ödeme yapılmayacaktır. Sizin için herhangi bir rahatsızlık veya risk oluşturmayacak bu çalışma, kısa dönemde size bir fayda da sağlamayacaktır. Ancak uzun dönemde hastalığın gelişiminin daha iyi anlaşılabilmesini ve alternatif tedavi geliştirebilmek için çalışma önemlidir.

Alınacak veriler yalnızca adı geçen çalışmada kullanılacaktır. Araştırmada yer almak tamamen sizin isteğinize bağlı olup araştırmada yer almayı reddedebilirsiniz. Reddetmeniz halinde yararınıza engel ya da cezai bir durum ortaya çıkmayacaktır. İzleyiciler, yoklama yapanlar, etik kurullar ve resmi makamlar size ait tıbbi bilgilere

ulařılabilecek ancak bu bilgiler ve kimlik bilgileriniz gizli tutulacaktır.

Sizi ‘***Prostat Kanserinde Kallikrein (KLK) Gen Ailesinin Ekspresyon Seviyelerinin Belirlenmesi***’ bařlıklı bir **arařtırmaya** davet ediyoruz. Bu arařtırmaya katılıp katılmama kararını vermeden önce, arařtırmanın neden ve nasıl yapılacağını bilmeniz gerekmektedir. Bu nedenle bu formun okunup anlaşılması büyük önem taşımaktadır. Ařağıdaki bilgileri dikkatlice okumak için zaman ayırınız. İsterseniz bu bilgileri aileniz ve/veya yakınlarınız ile tartıřınız. Eęer anlayamadığınız ve sizin için açık olmayan řeyler varsa, ya da daha fazla bilgi isterseniz bize sorunuz.

Bu alıřmaya katılmak tamamen **gönüllölük** esasına dayanmaktadır. alıřmaya katılmama hakkına sahiptir. Katılımcılardan elde edilecek bilgiler tamamen arařtırma amacı ile kullanılacaktır.

Katılımcının/Vasisinin/Velisinin Adı Soyadı :

İmza/Tarih :

Onama Tanıklık Eden Kiřinin Adı Soyadı

İmza/Tarih

Sorumlu Arařtırmacı:

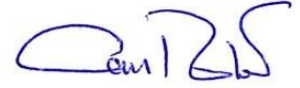
İmza:

Biruni Üniversitesi Girişimsel Olmayan Araştırmalar Etik Kurulu

25.09.2018

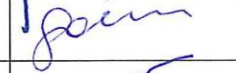
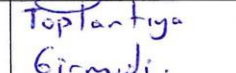
Sayın Dr.Öğr.Üyesi.Elif Sibel ASLAN

Biruni Üniversitesi Girişimsel Olmayan Araştırmalar Etik Kurulu yapılan inceleme sonucunda planladığı “ **Prostat Kanserinde Kallikrein (KLK) Gen Ailesinin Ekspresyon Seviyelerinin Belirlenmesi**” isimli araştırmanızın kurulumuzun **25.09.2018** tarihli toplantısında etik yönden uygun olduğuna karar verilmiştir.



Etik Kurul Başkanı
Prof.Dr.Can Polat EYİGÜN

T.C.
BİRÜNİ ÜNİVERSİTESİ
GİRİŞİMSEL OLMAYAN KLİNİK ARAŞTIRMALAR ETİK KURUL KARARI

	Karar No: 2018/20-14			
Tarih: 25.09.2018 Toplantı Sayısı:20	Dr.Öğr.Üyesi.Elif Sibel ASLAN'ın planladığı “ Prostat Kanserinde Kallikrein (KLK) Gen Ailesinin Ekspresyon Seviyelerinin Belirlenmesi ” konulu araştırma incelendi, yapılan inceleme sonucunda araştırmanın etik yönden uygun olduğuna karar verildi.			
ÜYELER				
Adı soyadı	Alanı	Bölümü	Katılım	İmza
Prof.Dr.Can Polat EYİĞÜN	Tıp Fakültesi	Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji A.D	Etik Kurul Başkanı	
Doç.Dr.Leman ŞENTURAN	Sağlık Bilimleri Fakültesi	Hemşirelik Bölümü	Etik Kurul Başkan Yardımcısı	
Prof.Dr.Fatma ÇELİK	Sağlık Bilimleri Fakültesi	Beslenme ve Diyetetik Bölümü	Üye	
Doç.Dr.Şölen HİMMETOĞLU	Tıp Fakültesi	Tıbbi Biyokimya A.D.	Raportör	
Doç.Dr.Burcu KARADUMAN	Diş Hekimliği Fakültesi	Periodontoloji A.D.	Üye	
Dr.Öğr.Üyesi.Ayşe Tuba CEYHUN	Eğitim Fakültesi	Zihin Engelliler Bölümü	Üye	
Dr.Öğr.Üyesi.Yonca ZENGİNLER	Sağlık Bilimleri Fakültesi	Fizyoterapi ve Rehabilitasyon Bölümü	Üye	 Toplantıya Girmeli.

DETERMINATION OF THE EXPRESSION LEVELS OF THE KALLIKREIN (KLK) GENE FAMILY IN PROSTATE CANCER

ORIJINALLIK RAPORU

%30

BENZERLIK ENDEKSI

%24

İNTERNET
KAYNAKLARI

%21

YAYINLAR

%9

ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.utoronto.ca İnternet Kaynağı	%6
2	www.genenames.org İnternet Kaynağı	%1
3	www.wildirismedical.com İnternet Kaynağı	%1
4	www.cancer.gov İnternet Kaynağı	%1
5	www.ifcc.org İnternet Kaynağı	%1
6	zerocancer.org İnternet Kaynağı	%1
7	Diamandis, E.P.. "The New Human Kallikrein Gene Family: Implications in Carcinogenesis", Trends in Endocrinology & Metabolism, 20000301 Yayın	%1

RESUME

Name and family name : Fatma Busra Boyukozer

Birth date and place : 11/11/1984 - Istanbul

E-mail: busraguzel@yahoo.com

Öğrenim Durumu:

Graduation	School and Department Name	Graduation year
Undergraduate	Istanbul University Molecular Biology and Genetics	2008