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ESTIMATE SOME BIOCHEMICAL TESTS AND TGF-B (+869 C/T) POLYMORPHISM IN PATIENTS WITH PROSTATE CANCER

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WISSAM JUMAAH AHMED AHMED

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By Wissam Jumaah Ahmed AHMED

August 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

ESTIMATE SOME BIOCHEMICAL TESTS AND TGF-B (+869 C/T) POLYMORPHISM IN PATIENTS WITH PROSTATE CANCER

Wissam Jumaah Ahmed AHMED

Master of Science in Biology

Advisor: Asst. Prof. Dr. Songül ŞAHİN

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August 2022

The current research sought to assess hepatic and renal functions as well as investigate the relationship between prostate cancer and the TGF-β (+869 C/T) polymorphism. A total of (80) males with prostate cancer were involved in this research. The patients were between the ages of 25 and 70. These patients were hospitalized to Kirkuk oncology center between January to March (2022). 50 healthy individuals served as the control group in this experiment. Electrophoresis is used identify DNA fragments after extraction or to detect PCR results if standard DNA is present. According to the study's findings, the patients' creatinine level (2.17±0.35) increased significantly (P≤0.05) when compared to the control group (0.93±0.28). the urea level in the patients group (52.91±4.57) increased significantly (P≤0.05) when compared to the control group (21.52±1.84). About the liver enzymes, ALT level in the patients (43.42±8.29) was significantly (P≤0.05) higher than that of the control group (16.03±1.64). AST level was seen a significant (P≤0.05) rise in the patient group (49.72±6.53) compared to the control group (20.42±2.54). Finally, this work identifies a mechanism for prostate cancer's constitutive stimulation of TGF-β signalling. The secondary structures of the mRNA, the signal peptide, and the resulting instability of the mRNA were also connected to the SNPs.

2022, 55 pages

Keywords: Transforming growth factor b, Prostate cancer, Liver functions, Renal functions

ÖZET

PROSTAT KANSERLİ HASTALARDA BAZI BİYOKİMYASAL TESTLERİN VE TGF-B (+869 C/T) POLİMORFİZMİNİN DEĞERLENDİRMESİ

Wissam Jumaah Ahmed AHMED
Biyoloji, Yüksek Lisans
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Eş Danışman: Dr. Öğr. Üyesi Ahmed Hamad SALEH
Ağustos 2022

Mevcut araştırma, karaciğer ve böbrek fonksiyonlarını değerlendirmenin yanı sıra prostat kanseri ile TGF-β (+869 C/T) polimorfizmi arasındaki ilişkiyi araştırmayı amaçladı. Bu araştırmaya prostat kanserli toplam (80) erkek dahil edildi. Hastalar 25-70 yaşları arasındaydı. Bu hastalar Ocak-Mart (2022) arasında Kerkük onkoloji merkezine yatırıldı. Bu deneyde 50 sağlıklı birey kontrol grubu olarak görev yaptı. Agaroz jeli üzerindeki PCR etkileşiminin sonuç bantlarının boyutunu belirlemek için, ekstraksiyon prosedüründen sonra DNA fragmanlarını belirlemek veya standart DNA mevcut olduğunda PCR etkileşiminin sonucunu saptamak için elektroforez kullanılmıştır. Çalışma bulgularına göre hastaların kreatinin düzeyi (2,17±0,35) kontrol grubuna (0,93±0,28) göre anlamlı olarak arttı (P≤0,05). Hasta grubunda (52,91±4,57) üre düzeyi, kontrol grubuna (21,52±1,84) göre anlamlı düzeyde (P≤0,05) yükseldi. Karaciğer enzimleri açısından hastalarda (43,42±8.29) ALT düzeyi kontrol grubuna (16,03±1,64) göre anlamlı (P≤0,05) yüksekti. Hasta grubunda (49,72±6,53) kontrol grubuna (20,42±2,54) göre AST düzeyinde anlamlı (P≤0,05) artış görüldü. Son olarak, bu çalışma, prostat kanserinin TGF-β sinyalinin yapısal uyarımı için bir mekanizma tanımlar.

2022, 55 sayfa

Anahtar Kelimeler: Dönüştürücü büyüme faktörü b, Prostat kanseri, Karaciğer fonksiyonları, Böbrek fonksiyonları

PREFACE AND ACKNOWLEDGEMENTS

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It is God's success

Wissam Jumaah Ahmed AHMED

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

°C	Degree celsius
dL	Deciliter
mg	Milligram
mL	Millilitre
µL	Microliter

LIST OF ABBREVIATIONS

ACS	American cancer society
AJCC	American joint committee on cancer
AMH	Anti-Mullerian hormone
BMP	Bone morphogenetic protein
BPH	Benign prostatic hyperplasia
CES	Equina syndrome
CRPC	Castrate-resistant prostate cancer
CT	Computerized tomography
DHT	Dihydrotestosterone
DRE	Digital rectal examination
EAU	European association of urology
ERSPC	European randomized study of screening for prostate cancer
ESTRO	European society for radiotherapy and oncology
ESUR	European society of urogenital radiology
GDF	Growth differentiation factor
ISUP	International society of urological pathology
KLK3	Termed kallikrein-3
LUTS	Lower urinary tract symptoms
PC	Prostate cancer
PCA3	Prostate cancer antigen 3
PSA	Prostate-specific antigen
SIOG	International society of geriatric oncology
SNPs	Single nucleotide polymorphisms
SPECT	Singlephoton emission CT
TGF-b	Transforming growth factor b
TME	Tumor microenvironment
Treg	Regulatory T
TRUS-PBx	Transrectal ultrasound-guided prostate biopsy
TURP	Transurethral resection of prostate
UICC	Union for international cancer control
USP	Ubiquitin specific peptidases
WHO	World health organization

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1 INTRODUCTION

The diagnosis of prostate cancer occurs in the vast majority of cases of noncutaneous cancer in males, more than a million new cases were reported in 2012, making up 15% of all male cancer diagnoses, 70% of which took place in industrialised areas. Prostate cancer, which affects over 1.4 million people in Europe and was the cause of 10% of all cancer-related deaths in 2012, is the type of cancer that affects men the most frequently. (Bray *et al.* 2013). According to American Cancer Society (ACS), there will be a total of 164.690 new cases of prostate cancer diagnosed in men in the United States in 2018. This accounts for 19% of all newly diagnosed cases of cancer. 29.430 men will die from the disease, accounting for 9% of all cancer deaths among males; and the number of new prostate cancer cases will account for 19% of all new cancer cases (Siegel *et al.* 2018).

Numerous research investigating various approaches to address this public health issue, including secondary prevention, were initiated as a result of the epidemiology of prostate cancer (i.e., screening). Before a patient is clinically aware of a condition, screening seeks to identify and treat it. A diagnosis of cancer is typically not made until symptoms are clinically obvious in the majority of individuals. On the other hand, There is a window of opportunity for screening in the case of prostate cancer since there is a long period of time between the stage of the illness at which it can be diagnosed and the stage at which it presents its symptoms. (which may never occur in low risk disease). The European Randomized Study of Screening for Prostate Cancer (ERSPC) was the largest study to examine the impact of repeated screening on mortality. It was conducted in eight European countries (Belgium, Finland, France, Italy, the Netherlands, Spain, and Sweden) and nearly 200.000 men (aged 50 to 74) were randomly assigned to either a screening or a control arm. (Schröder *et al.* 2014). Every two to four years, men in the screening group received prostate-specific antigen (PSA) testing, and those with increased PSA levels (i.e., PSA>3.0 ng/mL) got a thorough prostate biopsy (Schröder *et al.* 2014).

After 13 years of follow-up, the ERSPC results were recently updated, demonstrating that systematic screening decreases prostate cancer mortality by 21% when compared to no or restricted screening (Schröder *et al.* 2014). Additionally, with longer follow-up, there was a significant decline in the number of screenings (from 1.410 at nine years to 781 at 13 years) and treatments (from 48 at nine years to 27 at 13 years) required to prevent a single prostate cancer mortality. These figures are currently comparable to those from breast cancer studies (Roobol *et al.* 2010, Alberts *et al.* 2016).

When compared to no screening at all, screening can reduce disease-specific mortality for a man completely complying with the ERSPC procedure by 50% (Bokhorst *et al.* 2014). However, prostate cancer screening is debatable due to the possibility of overdiagnosis and overtreatment (Mottet *et al.* 2021). The term "overdiagnosis" refers to the finding of prostate tumours that would not have been discovered without screening (i.e., patients with cancer that would have remained asymptomatic) (Draisma *et al.* 2003). According to estimates, screening might incorrectly detect up to 50% of all cases of prostate cancer (Draisma *et al.* 2009). If these guys receive therapy, it would be called excessive (Draisma *et al.* 2003).

The prostate cancer guidelines of the EAU, ESTRO, ESUR, and SIOG recommend an individualised risk-adapted strategy for early detection for a well-informed man with a good performance status and a life expectancy of at least ten years (Elwyn *et al.* 2010, Loeb 2014).

1.1 Objectives of the Thesis

- Investigate if or not there is a link between the TGF-(+869 C/T) polymorphism and prostate cancer.
- Assess the capabilities of the liver.
- Assess the capabilities of the kidneys.

2 LITERATURE REVIEW

2.1 The Prostate Gland

2.1.1 Anatomy and histology of the prostate

2.1.1.1 Surface anatomy

It has the form of a pyramid, with the apex pointing in a downward direction and the base pointing in an upward direction so that it faces the bladder. The prostate sits in front of the rectum and beneath the urinary bladder in a normal human body. The prostate in young guys weighs about ~20 grammes, measures about ~3 centimetres in length, ~4 centimetres in width, and 2 centimetres in thickness (Figure 2.1). Anatomically, the prostate gland is divided into three distinct zones, which are as follows: The transition zone, which ranges in size from 5 to 10 percent of the prostate's total volume, surrounds the urethra on all sides; the peripheral zone, It houses the gland's lateral portions; and the center region, which makes up 25% of the volume of the prostate and lies in the middle of the transition and peripheral zones. The middle zone is traversed by the ejaculatory ducts (Partin *et al.* 2015).

The prostate is separated into the left and right lateral lobes by the prostatic urethra, which is a passageway that runs through the gland. During a digital rectal examination, it is possible to feel the median sulcus as well as the rear of the lateral lobes (DRE). After connecting to the prostatic urethra, The seminal colliculus is where the paired ejaculatory duct exits the prostate (verumontanum). Two puboprostatic ligaments connect the prostate to the pubic bone within a tight capsule. Both sides are wrapped in endopelvic fascia., covers the prostate ventrally, and encircles the levator ani muscle. It also extends to both sides of the pelvis. Between the rectum and the prostate is a fascia called the Denovilliers fascia, which acts as the dorsal barrier. Under the bladder, the prostate gland produces chemicals that aid in the production of semen. the prostatic urethra and ejaculatory duct (Partin *et al.* 2015, Stacey *et al.* 2017).

2.1.1.2 Histology

The pseudostratified columnar epithelium of the prostate, which is composed of tubuloalveolar glands. Granules for secretion are present in columnar cells. The foundational cells for epithelial regeneration are found between the columnar cells and are called basal cells. Seventy percent of the prostate's bulk, or the fibromuscular stroma, is made up of smooth muscle and connective tissue, and it surrounds each gland. The smooth muscles contract and expel glandular material during ejaculation (McNeal 1981).

2.1.1.3 Vascular, lymphatic and nerve supply

The internal iliac vessels are the source of the blood supply. The urethral branches of the inferior vesical artery reach thAt 4 and 8 o'clock, the basal prostate and bladder neck, respectively, feeding the transition zone. The branches also split into capsular and urethral branches. The capsular branches, which subsequently go to the pelvic floor and give rise to smaller arteries that pierce the capsule, laterally connect the cavernous nerves. The middle rectal, internal pudendal, and obturator arteries all assist to blood supply. The venous vessels enter the deep venous complex at the location known as the vesicoprostatic plexus. This is the point at which they reach the internal iliac veins. The deep penile vein is the source of blood that is sent to the vesicoprostatic plexus, which may be found underneath the puboprostatic ligaments and the pubic bone. The obturator and internal iliac nodes are the destinations of the lymphatic drainage that originates in the prostate. Additionally, lymphatic contact exists with the para-aortic, presacral, and external iliac lymph nodes. The lateral cavernous nerves carry the autonomic innervation to the prostate. The alpha-receptors on parasympathetic (S2-4) and sympathetic (L1-2) neurons govern the contraction of smooth muscle, respectively (Partin *et al.* 2015).

2.1.1.4 Structures

The seminal vesicles are paired, that are found underneath the bladder and contain ducts that emerge into the ductus deferens, are responsible for the formation of the ejaculatory

duct. The ductus deferens is located in the pelvic region. The seminal vesicles is made up of glandular tissue that resembles ducts and is 15 centimetres long. Their walls are comprised of muscles. The seminal vesicle is responsible for producing a gelatinous, alkaline (pH 7.4) secretion that contains fructose. This secretion accounts for approximately half of the semen's total volume. The testicles are responsible for producing sperm as well as testosterone, while the epididymis is responsible for storing mature sperm. The vas deferens is a tube that is present during ejaculation and is responsible for transporting sperm & epididymal to ejaculatory duct semen flow (Figure 2.1).

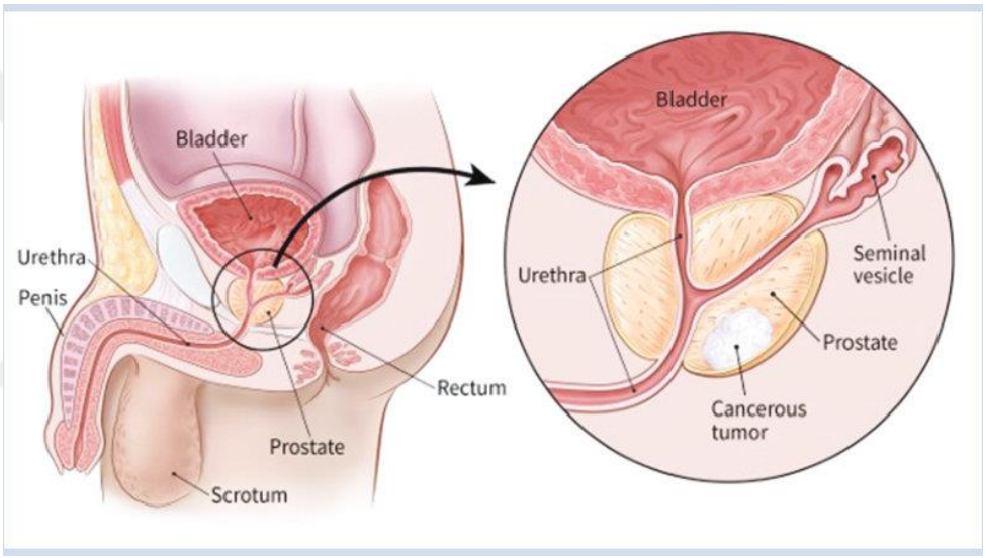

Figure 2.1 Anatomy of the prostate

2.1.2 Functions of the prostate

Prostate-specific antigen is a glycoprotein enzyme that is secreted by the prostate epithelial cells (PSA). It is also known as kallikrein-3 since it belongs to the kallikrein-related peptidase family (KLK3). In order to facilitate the transit of sperm and facilitate fertilisation, PSA is created to liquefy the semen in the ejaculate (Balk *et al.* 2003).

Twenty percent of the volume of semen is made up of fluid secreted by the prostate. Spermine, the thin, acidic (pH 6.4), and protease-rich prostatic fluid contains

immunoglobulins, phosphatases, and proteases. In addition to providing sustenance and the ideal environment for sperm to travel and fertilise the ovum, constituents aid in the liquefaction of semen. During ejaculation, the prostate acts as a valve to stop the passage of pee. The parasympathetic nervous system regulates urination, which causes the bladder neck to relax. The sympathetic nervous system regulates ejaculation, which is brought on by the smooth muscle stroma contracting. Androgens are necessary for the prostate to function properly. Dihydrotestosterone (DHT), a metabolite of testosterone that is primarily responsible for regulating the prostate, is generated mostly by the testicles (Partin *et al.* 2015).

Prostate cancer

In the United States, prostate cancer affects more men than any other non-cutaneous tumour, with 3.3 million survivors and an expected 161.000 new cases diagnosed in 2017 (Siegel *et al.* 2017). Although prostate cancer frequently has a sluggish growth rate, it is nonetheless the third most common cancer among men. Urinary retention, back pain, bone pain, & hematuria were the most typical presenting symptoms before prostate-specific antigen (PSA) testing was available. the majority of patients are asymptomatic when they are diagnosed (Koh *et al.* 2006, Caini *et al.* 2014, Eggener *et al.* 2015).

Sub-Saharan African origin, family history, certain genetic mutations (BRCA 1 or 2), and advanced age are all risk factors for prostate cancer. Prostate cancer screening is still debatable because of the slow progression of the condition and the morbidity linked to overtreatment. 3 a lot of improvements in identifying illness risk and broadening therapy choices have been developed in the last ten years (Eggener *et al.* 2015).

Signs and symptoms

The majority of PC are located in the prostate gland's periphery and may be palpable on DRE when the volume is more than >0.2 mL. Regardless of PSA level, DRE can identify prostate cancer in about 18% of cases. A PSA test and PBx are required in cases of

abnormal DRE results. Early PC is typically asymptomatic. Approximately 30% of males show no symptoms at all (Miller *et al.* 2003). When cancer affects the urethra and prevents the passage of urine, symptoms develop (Figure 2.2). Lower urinary tract symptoms (LUTS), such as dribbling at the end of the flow and trouble starting the flow, may be present (Kupelian *et al.* 2006).

The possibility of urine retention with or without kidney damage exists in cases of severe blockage. Patients with advanced or metastatic illness may have back discomfort (bony metastases), weariness, or sluggishness. The diagnosis and treatment of cauda equina syndrome (CES), which is caused by cancer that affects the spinal nerves, must take place as soon as possible and include steroid medication, magnetic resonance imaging (MRI), radiation therapy, and surgical decompression. Back discomfort, numbness and weakness in the lower limbs, perineal (saddle) anaesthesia, and urinary and faecal disturbances are all symptoms associated with CES (Dy *et al.* 2008).

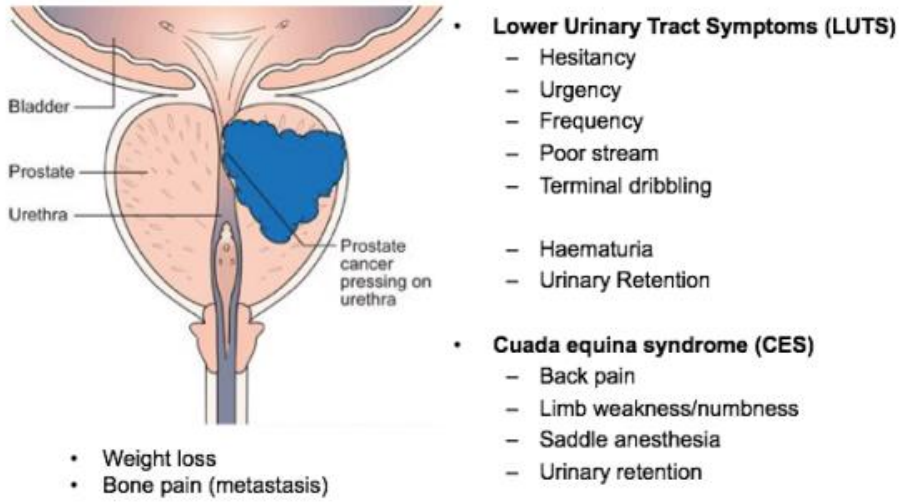

Figure 2.2 Signs and symptoms

2.1.3 Classification

Histopathological staging and clinical evaluation (DRE findings), as well as tissue biopsies and imaging, are used to classify and stage prostate cancer. WHO created a

categorization system for male genital organ and urinary system tumours in 2004. In 2016, this grading scheme was upgraded and changed (Humphrey *et al.* 2016). The categorization is intended to support risk management procedures and stratification.

2.1.3.1 Grading

Two grading scores are combined to get the Gleason grade. The most frequent cell type/pattern observed upon inspection of specimens is, respectively, the first (primary) and second (secondary) Gleason scores. An improved grading method from the ISUP 2005 was introduced at the PCa's 2014 Gleason grading conference by the International Society of Urological Pathology (ISUP) (Epstein *et al.* 1994). There is a scale from 1 to 5, with 1 representing well-differentiated and 5 representing poorly-differentiated.

2.1.3.2 Staging

PCa is staged using the popular TNM (tumour, node, metastasis) staging approach. T1-2 refers to the prostate capsule alone, whereas T3-4 signifies invasion of the prostate capsule to adjacent tissues, like the seminal vesicles. The T-stage defines the initial tumour and the amount of local disease invasion. T1 disease is a tumour discovered through TRUS-PBx or transurethral resection of the prostate (TURP), whereas T2 disease is a carcinoma palpable during DRE (Figure 2.3). The N-stage emphasises how PCa has progressed with respect to the neighbourhood lymph nodes that the prostate gland empties (N1) (Mottet *et al.* 2017, Maistro *et al.* 2019).

These include the pelvic nodes that are situated below the point at which the common iliac arteries branch off into two separate arteries. An MRI of the pelvis can examine the prostate as well as highlight any lymph nodes in the region that are abnormally enlarged or swollen. The M-stage is defined as any spread to distant tissues such as the bones (M1b), distant organs such as the hepatic structure, the brain structure, and the lung (M1c), and lymph nodes (M1a- non-pelvic nodes). The M-stage is the last stage of cancer staging (Figure 2.4). It is possible to evaluate non-regional lymph nodes and distant organ

metastases using an MRI that covers the whole body. In addition, single-photon emission CT, often known as SPECT, can be utilised to assess bone metastases. In conclusion, staging necessitates the use of a mix of diagnostic procedures, such as a DRE, PBx, imaging in the form of CT or MRI, and an isotope bone scan. The amount of prostatic invasion may be determined based on the tumour (T) stage. Nodal and distant organ involvement is reflected in the stages of node (N) & metastasis, respectively (M). The TNM staging system was created either by AJCC or the Union for International Cancer Control (UICC). Both organisations are a part of the American Cancer Society (adapted from the 2017 European Association of Urology guidelines) (Van Der Cruijsen-Koeter *et al.* 2005, Mottet *et al.* 2017).

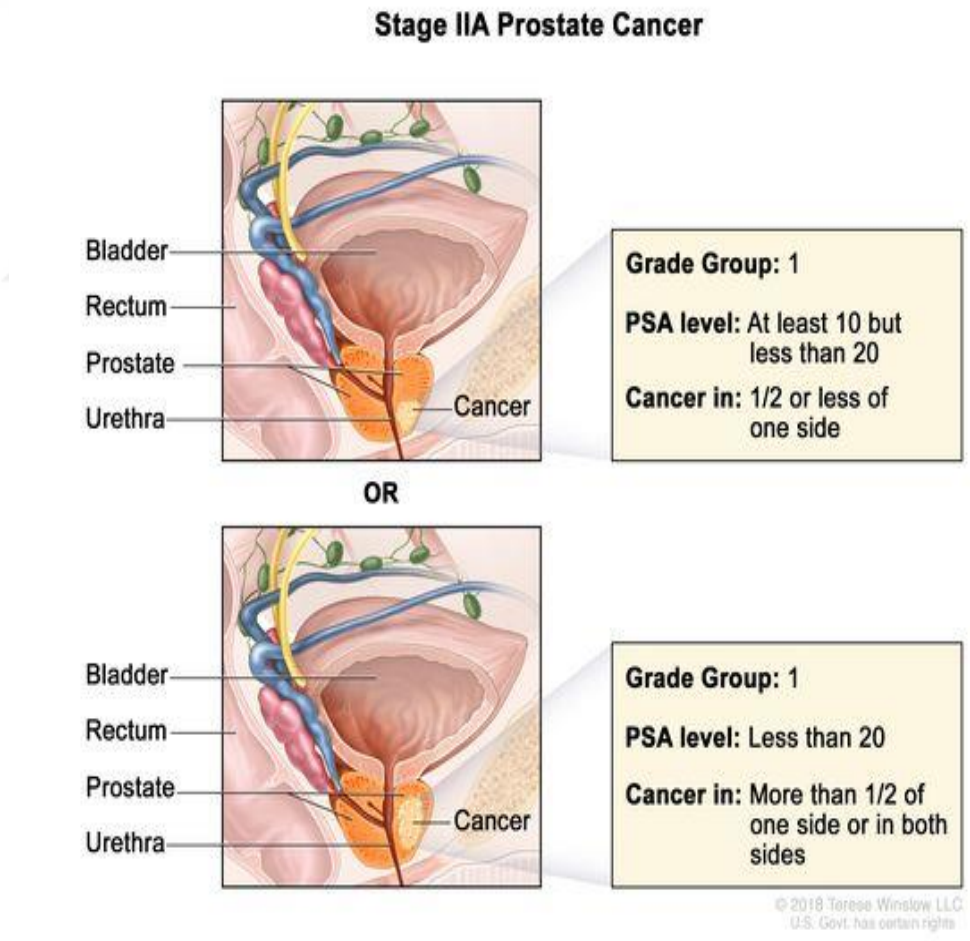

Figure 2.3 Stages of prostate cancer

Risk factors

The only two known risk factors for PC development are age and heredity, specifically grandparents, parents, siblings, or other close relatives with a history of breast, ovarian, or cervical cancers.

- Age. Approximately 36.3% of cases are diagnosed in the seventh decade, with 31.6% occurring between the ages of 70 and 79.
- Heredity. In 10% of instances, PC may be genetic, with a 2- to 3-fold increased risk; if more than one family is afflicted, the risk might be up to 5-fold higher. PC is a polygenic illness since several genes have been shown to be involved in its development. In order to predict the behaviour and aggressiveness of tumours, studies have shown the value of analysing polymorphisms in genes like ELAC2 (which plays a role in tubulin function), RNASEL (an endoribonuclease that functions as a tumour suppressor gene), and MSR1 (mutations in which confer a predisposition to chronic inflammatory conditions).
- Race. Every year, there are 27.000 fatalities from PC and 250.000 new cases in the United States of America (USA). African Americans account for more than half of these fatalities, followed by whites, Hispanic Americans, and less often Asians. PC has a 17 percent chance of developing, and the same condition has a 3 percent fatality rate.
- Inflammation. Chronic inflammatory processes that impact the prostate have been identified as a risk factor for PC. This idea, however, is debatable, and no causation has been shown.
- Hormones. It is generally known that androgens and estrogens played a role in the development of PC. Rarely may patients with congenital androgen deficits have Benign prostatic hyperplasia (BPH). However, these patients run the risk of developing PC or BPH if androgen ablation is done after puberty (Wein *et al.* 2011, Heidenreich *et al.* 2014).
- Metabolic disorder A increased risk of PC, recurrence, and/or advancement has been linked to the presence of two or more metabolic syndrome components (Castillejos-Molina *et al.* 2011).

- The preventive effects of vitamins E and D, selenium, calcium, and omega 3 and 6 fatty acids in the prevention of PC have been the subject of several research. However, no clear cause-and-effect connection has been discovered. A Mediterranean diet rich in antioxidants is associated with reduced PC frequency in those who consume it (Ma and Chapman 2009, Bristow *et al.* 2013, Lin *et al.* 2015). Comparatively to Asian groups that have stayed in their home countries, American Asian people have a greater frequency of PC. This may be because they have adopted new dietary habits that differ from those in their place of origin. This trend lends credence to the hypothesis that some dietary components may affect PC rates.
- Smoking. Smoking has been linked to an increased risk of PC because more circulating cadmium causes more cellular oxidation. The relationship between these two conditions hasn't been proven to be causative, though.
- Exercise. Exercise is strongly advised since it is thought to be a protective factor, mostly for quality of life with or without a PC (Mohamad *et al.* 2015).

Screening and diagnosis

The measurement of PSA, either with or without digital rectal examination, is the primary method for diagnosing prostate cancer in men who do not have any symptoms of the disease (DRE). Prostate epithelial cells are responsible for the production of the PSA glycoprotein. PSA levels grow in patients who have prostate cancer because of the increased synthesis of PSA and the breakdown of tissue that occurs between the prostate and the capillaries, both of which cause more PSA to be present in the blood (Eggener *et al.* 2015, Grossman *et al.* 2018).

PSA, on the other hand, is not exclusive to cancer despite the fact that it can only be found in prostate tissue. Prostate procedures (DRE, biopsy), prostatitis, benign prostatic hyperplasia (BPH), & ejaculation are some of the other conditions and behaviours that have the potential to raise PSA levels (Andriole *et al.* 2006). After a year of treatment with 5 alpha-reductase inhibitors, PSA levels drop by roughly 50% (Gann *et al.* 1995). Some medical specialists suggest doubling the PSA results for people on chronic 5 alpha-

reductase inhibitors in order to correctly interpret them (Andriole *et al.* 2006). A PSA of more than > 4 ng/mL is often regarded as abnormal (Gann *et al.* 1995).

However, it is difficult to distinguish between BPH and prostate cancer using PSA readings between 4 and 10 ng/mL (Eggener *et al.* 2015). There are several ways to improve the specificity of PSA readings and reduce pointless biopsies. 4 Measuring the proportion of free PSA for males with PSA levels between 4 and 10 ng/mL should be taken into consideration. Free PSA readings more than >25percent most likely indicate BPH; no biopsy is necessary for these individuals (Partin *et al.* 1998, Cabarkapa *et al.* 2016).

Another strategy for improving specificity with a PSA level between 2 ng/mL and 10 ng/mL is PSA velocity, which is the pace at which PSA changes over time. For males whose PSA velocity value is more than 0.35 ng/mL per year, further testing, including a biopsy, should be seriously considered, according to the majority of specialists. An FDA-approved test called prostate cancer antigen 3 (PCA3) can help men over 50 who have previously had negative prostate biopsies decide whether they need another biopsy. Men with a PCA3 score more than 25 were 4.6 times more likely to have a positive biopsy in a prospective, multicenter study than men with a score lower than 25. 4 The use of DRE in prostate cancer screening is debatable in the era of PSA testing. The majority of organisations advise DRE as an additional test rather than a screening technique for males with increased blood PSA (Carter *et al.* 2013).

The gold standard for diagnosis in males with probable cancer is microscopic examination of prostate tissue obtained by needle biopsy. The goal of screening guidelines is to strike a balance between the benefits of early discovery of prostate cancer in reducing mortality and the risks of overdiagnosis and overtreatment of this sometimes slow-growing illness. The screening guidelines from several national medical organisations are compiled in Table 1. Despite minor variances, all organisations stress the significance of patient education regarding the advantages and disadvantages of screening and direct communication between the clinician and the patient during joint decision-making (Carter *et al.* 2013, Basch *et al.* 2012, Bibbins-Domingo *et al.* 2017). Most organisations advise

avoiding or stopping screening in individuals with a life expectancy of fewer than 10 years. This is because screening recommendations are frequently based on a patient's life expectancy (Carter *et al.* 2013, Bray *et al.* 2018, Luszczak *et al.* 2020).

Organization	Year updated	Screening age (y)	Screening of patients at high risk	Screening interval	PSA level for biopsy
US Preventive Services Task Force[14]	2018	Shared decision-making for patients 55-69	None specified	None specified	None specified
American Cancer Society[12]	2010	Begin at age 50 in those with life expectancy > 10 y	Begin at age 40 in those with life expectancy > 10 y	Annual if PSA > 2.5 ng/mL	Select patients if PSA > 2.5 ng/mL; most patients if PSA > 4 ng/mL
American Urological Association[13]	2013	55-69	40-69	Every 2 y	None specified
American College of Physicians[9]	2013	50-69	40-69	Annual if PSA > 2.5 ng/mL	None specified

PSA, prostate-specific antigen.

Figure 2.4 Prostate screening recommendations

Management

Whether or whether therapy is required is the first choice. Since low-grade forms frequently develop slowly in elderly men, no therapy is necessary. Treatment might not be required if a person has other serious health problems or isn't expected to live long enough for symptoms to appear. Strategies that postpone therapy are referred to as expectant management. The two strategies for anticipatory therapy are active surveillance, which also has a therapeutic goal (aims to prevent the cancer from progressing) and watchful waiting, which just seeks to alleviate symptoms. Watchful waiting is the less aggressive of the two strategies (Filson *et al.* 2015).

Depending on the disease stage, Gleason score, & PSA level, the best option will be determined. Age, general health, and an individual's opinions towards prospective therapies and their potential adverse effects are additional crucial variables. Due to the

potential severity of side effects from most therapies, including erectile dysfunction and urine incontinence, treatment conversations frequently centre on finding a balance between the advantages and disadvantages of therapy and lifestyle changes. More research that focuses on person-centered outcomes is required, according to a 2017 review, to help patients. Frequently, a mix of therapeutic modalities is advised. Estimating life expectancy is necessary for guidelines for particular clinical conditions. More people will live long enough for signs of their prostate cancer to manifest as a result of improvements in the treatment of other diseases that have increased average life expectancy (Picard *et al.* 2012, Mongiat-Artus *et al.* 2009, Mohan and Schellhammer 2011, Jayadevappa *et al.* 2017).

To ascertain if patients are adequately informed and comprehend their treatment options, an 18-item questionnaire was devised. In a 2015 research, fewer than half of the questions were properly answered by the majority of newly diagnosed participants. Almost all occurrences of cancer are still detected after the age of 65, while around 25% are detected beyond the age of 75 as a result of the extensive use of PSA screening in the US. Despite the US National Comprehensive Cancer Network's suggestion that life expectancy have been used to guide treatment decisions, many elderly people are given hormone therapy or watchful waiting rather than curative treatment alternatives like radical prostatectomy & radiation therapy (Cooper 1992, Fitzpatrick 2008, Mohan and Schellhammer 2011).

Active treatment

There are both surgical and nonsurgical therapies available, however they can be combined and therapy can be challenging. Prostatectomy, brachytherapy, external beam radiation therapy, cryosurgery, high-intensity focused ultrasound, & brachytherapy are some of the therapeutic options that are routinely made available to male patients whose cancer has advanced to the prostate. Chemotherapy and hormone treatment are frequently used to treat metastatic illness. For advanced cancers with little metastases, local or radiation-directed treatment are exceptions that may be employed (Sartor and De Bono 2018, Dhondt *et al.* 2019).

Some tumours in the early stages are treated with hormonal treatment. If the initial course of treatment is unsuccessful and the disease worsens, chemotherapy, hormone therapy, and cryotherapy (freezing the tumour) may be recommended. Sipuleucel-T, a cancer vaccine, was said to enhance life in metastatic prostate cancer patients by four months, however its marketing authorization was revoked on May 19, 2015. Although radical prostatectomy is a technically difficult procedure, it may be an option if radiation treatment is unsuccessful. Radiation therapy, however, may come with a number of risks following a failed operation. A little rise in bladder and colon cancer is linked to it. After five years, bowel, erectile, and urine function appear to be similar between radiotherapy and surgery (Mouraviev *et al.* 2006, Hammerstrom *et al.* 2011, Wallis *et al.* 2018).

Nonsurgical treatment

High-intensity focused ultrasound, radiation treatment, chemotherapy, hormone therapy, external beam radiation therapy, particle therapy, or a combination of these therapies, may all be utilised as non-surgical treatments. Castrate-resistant prostate cancer is the term used to describe prostate cancer that endures after hormonal treatment lowers testosterone levels (CRPC). Normal testosterone levels are necessary for the growth of many early-stage malignancies, but not CRPC. The name "CRPC" was coined because these tumours exhibit a dependence upon hormones, notably testosterone, for androgen receptor activation, and were formerly known as "hormone-refractory prostate cancer" and "androgen-independent prostate cancer" (Peyromaure *et al.* 2009, Hong *et al.* 2010, Seruga *et al.* 2011, Tran and McGill 2011).

Docetaxel, a cancer chemotherapy drug, has been used to treat CRPC, with a median survival benefit of two to three months. Cabazitaxel is a second-line chemotherapy drug. Bevacizumab, docetaxel, thalidomide, and prednisone tend to work well together to treat CRPC. Sipuleucel-T immunotherapy appears to extend life in patients with CRPC by four months (Petrylak 2003, Kantoff *et al.* 2010, De Bono *et al.* 2010).

On May 19, 2015, the sipuleucel-T marketing authorization was revoked. Abiraterone, a second-line hormonal treatment, extends life expectancy by roughly 4.6 months. Another

second-line hormonal treatment with a five-month survival benefit is enzalutamide. In people with CRPC who have not previously had chemotherapy, clinical studies are now being conducted with both abiraterone and enzalutamide. Not all individuals react well to medications that impede androgen signalling. A few cells that resemble stem cells continue to function normally.

As a consequence, to improve CRPC outcomes, greater doses or combination therapies with drugs that inhibit several androgen-signaling pathways were utilised. However, stem-like cells that do not display androgen signalling will not be impacted by even these combinations. Doctors utilise a range of bone-modifying medications on patients who have metastatic prostate cancer that has migrated to their bones in an effort to avoid skeletal problems and encourage the development of new bone mass. Denosumab, a RANK-ligand inhibitor, and zoledronic acid, a bisphosphonate, both appear to be effective treatments but are linked to more frequent and severe side effects (Rane *et al.* 2012, Jakob *et al.* 2020).

Surgery

The prostate, seminal vesicles, and surrounding lymph nodes are all removed during a radical prostatectomy, which is thought to be the standard surgical therapy for prostate cancer. Either a laparoscopic procedure or an open approach (a skin incision in the lower abdomen) can be used. The most popular open surgical procedure is radical retropubic prostatectomy. It is currently common practise to perform a prostatectomy using robotic assistance. Open radical prostatectomy may result in longer hospital stays and more blood transfusions, whereas laparoscopic radical prostatectomy and robotic-assisted radical prostatectomy could result in shorter hospital stays & fewer transfusions for men with localised prostate cancer (Ilic *et al.* 2017, Jakob *et al.* 2020).

It is uncertain how these therapies stack up in terms of overall survival or recurrence-free survival. The typical surgical procedure for benign prostate enlargement is transurethral excision of the prostate. This technique is not used to treat prostate cancer itself; rather, it is used to address the symptoms of urine retention brought on by a big prostate tumour.

A resectoscope is introduced within the penis during the surgery, and excess prostatic tissue is removed to provide room for the passage of urine (Ilic *et al.* 2017, Zhou 2018).

Complications

Erectile dysfunction and stress-type urine incontinence are the two primary side effects following prostatectomy and prostate radiation. The majority of men regain continence within 6 to 12 months of surgery, thus medical professionals often wait at least a year before turning to invasive procedures. Urinary incontinence under stress typically develops during radiation therapy or prostate surgery as a result of variables such nerve and tissue damage to the urethral sphincter and surrounding tissues. The urethra, a muscular tube that seals the urine bladder, is encircled by the prostate. Any of the aforementioned causes might result in the urethra's ineffective closure and consequent incontinence. Kegel exercises, lifestyle modifications, bladder training, and the use of incontinence pads are all part of the first therapy (Ilic *et al.* 2017).

A mechanical device known as a urethral sling & artificial urinary sphincter is used to replicate the function of the urethral sphincter. Through a button that is surgically inserted in the patient's scrotum, the patient can manually activate this gadget. This type of surgery is considered to be a more invasive alternative to traditional urinary diversion surgery. Patients who have moderate or severe stress urine incontinence are typically candidates for this latter treatment, which is regarded the gold standard (Singla and Singla 2014).

Nearly all men who get prostate cancer treatment, such as radiation or surgery, experience erectile dysfunction to varying degrees; however, most of them see improvement within a year. This development might not occur if nerves are injured. PDE-5 inhibitors like viagra or cialis, as well as injectable intracavernous medications administered directly into the penis, are examples of pharmacological treatments (prostaglandin E1 and vasoactive drug mixtures). Penile implants and vacuum constriction devices are examples of further non-pharmacological treatment (Singla and Singla 2014).

Transforming growth factor b (TGF-b)

In order to guard against foreign invaders and tolerize self-antigens, the immune system in vertebrates must be constantly regulated. A variety of regulatory elements operate to limit the immune system in order to maintain this vital equilibrium. These elements include specialised cell types like regulatory T (Treg) cells that regulate immune effector cell growth, checkpoint molecules like CTLA-4 and PD-1 that balance antigen receptor signalling, and immunosuppressive cytokines, the most notable of which is transforming growth factor b (TGF-b) (Li and Flavell 2008). Numerous immune cell types' production and effector activities are regulated by TGF-b (Sanjabi *et al.* 2017).

It does this by vigorously encouraging the growth of regulatory T cells (Tregs) while actively suppressing the maturation and function of effector T cells & antigen-presenting dendritic cells. This allows it to exert control over adaptive immunity (DCs). Natural killer (NK) cells can be inhibited, and the complicated linkages between neutrophils and macrophages can be regulated, thanks to this treatment. TGF-b similarly controls the innate immune system to produce a web of antagonistic immune regulatory inputs. TGF-impact b's on immune regulation is significantly outweighed by the role it plays in development, homeostasis, & tissue regeneration. Congenital abnormalities, fibrotic illnesses, immunological dysregulation, and cancer are all brought on by malfunctions of this route (Oshimori and Fuchs 2012).

TGF-b affects cell proliferation, differentiation, adhesion, adhesion, motility, metabolism, communication, and death in the majority of adult mammalian cell types. This is particularly interesting since TGF-b inhibits pre-malignant cells' ability to proliferate and causes them to die, acting as a powerful tumour suppressor. These cells become fully malignant because to mutations that disrupt the TGF-b pathway or decouple it from apoptosis, but they can also employ TGF-b to produce additional stromal modifiers that promote tumour development and metastasis and an immune-suppressive tumour microenvironment (TME). The TGF-b family's significant involvement in development, homeostasis, and illnesses, including cancer, as well as the structure and

function of the TGF-b signalling pathway, have all been covered in-depth elsewhere (David and Massagué 2018, Oshimori and Fuchs 2012).

The TGF-b Signaling Pathway

A typical membrane-to-nucleus signalling route, Direct receptor-mediated activation of SMAD transcription factors is a component of the TGF-b pathway. Activated SMAD proteins bind to multiple loci across the genome under the control of partner transcription factors, whose availability in a particular biological setting determines this cell's response to TGF-b (Figure 2.5). Only a brief summary of the pathway's components and rationale is provided here because they have recently been examined elsewhere (David and Massagué 2018). TGF-b family members communicate via type I and type II receptors, which are paired transmembrane serine/threonine protein kinases (Figure 2.5). Eight SMAD proteins, five type II receptors, and seven type I receptors are all encoded in mammalian genomes. TGFBR1 and TGFBR2, TGF-b receptors, also known as TbR-I, ALK5, TbR-I, and TbR-II, play a role in TGF-b signalling. The TGF-b1, TGF-b2, and TGF-b3 proteins are the only ligands that are capable of binding to the TGFBR1 and TGFBR2 receptors. Because SMAD2 and SMAD3 is substrates for TGF-b subfamily receptors and SMAD1, 5, and 8 are substrates for BMP subfamily receptors, these SMAD proteins are referred to as receptorregulated SMADs, or R-SMADs for short. SMAD4 does not function as a receptor substrate; rather, it forms heterotrimeric transcriptional complexes by interacting with R-SMADs that have been activated. Chemicals that inhibit SMAD6 and 7 put a stop to signalling activity between receptors and SMADs. TGF-b receptor degradation is controlled by SMURF1/2 ubiquitin ligases that are recruited by SMAD7 and ubiquitin-specific peptidases that compete with them (USP11, USP15). Induction of SMAD6 and 7 by TGF-b & BMP family members leads to the production of negative feedback loops in the system. The globular MH1 and MH2 domains, which are located at the N-termini and C-termini of SMAD proteins, respectively, are connected to one another by a linker region. Although the MH2 domains of other SMADs, working transcription factors, chromatin readers, and modifying factors also bind DNA, the MH1 domain of R-SMADs and SMAD4 is the only one that is responsible for the binding of DNA. The components of the TGF-b-SMAD pathway are substantially conserved, and a

good number of the structural foundations underlying its interactions and activities are understood (Macias *et al.* 2015).

When it is released from latent complexes, TGF-b receptor type III (TGFBR3), an accessory co-receptor, or directly to the receptors are both efficient ways of binding (Figure 2.5). Betaglycan is particularly important for TGF-b2 binding. In driven receptor complex of TGF-b-, SMAD2 and 3 are phosphorylated at two C-terminal serine residues by TGFBR2 after activating and phosphorylating TGFBR1. Following that, SMAD4 joins with receptor-phosphorylated versions of SMAD2 and 3 to create heterotrimeric complexes. With the help of other transcription factors, activated SMAD complexes bind to hundreds of regulatory sites in the nucleus (Mullen *et al.* 2011, Trompouki *et al.* 2011, Wang *et al.* 2017). There are other interactions that take place between co-activators and co-repressors that have an effect on the outcome of the transcriptional process. Within these complexes, RNA polymerase II kinases CDK8 and CDK9 phosphorylate R-SMADs, therefore producing sites that allow for the recruitment of other co-factors. In addition, when glycogen synthase kinase 3b (GSK3b) is activated by CDK8/9-mediated phosphorylation, SMADs become susceptible to polyubiquitination by the HECT-domain ubiquitin ligases NEDD4L & SMURF1, which leads to SMAD deactivation. This occurs when glycogen synthase kinase 3b (GSK3b) is activated. On the other hand, R-SMADs undergo dephosphorylation and subsequent dissociation from DNA in preparation for future signalling cycles (Mullen *et al.* 2011, Trompouki *et al.* 2011, Wang *et al.* 2017).

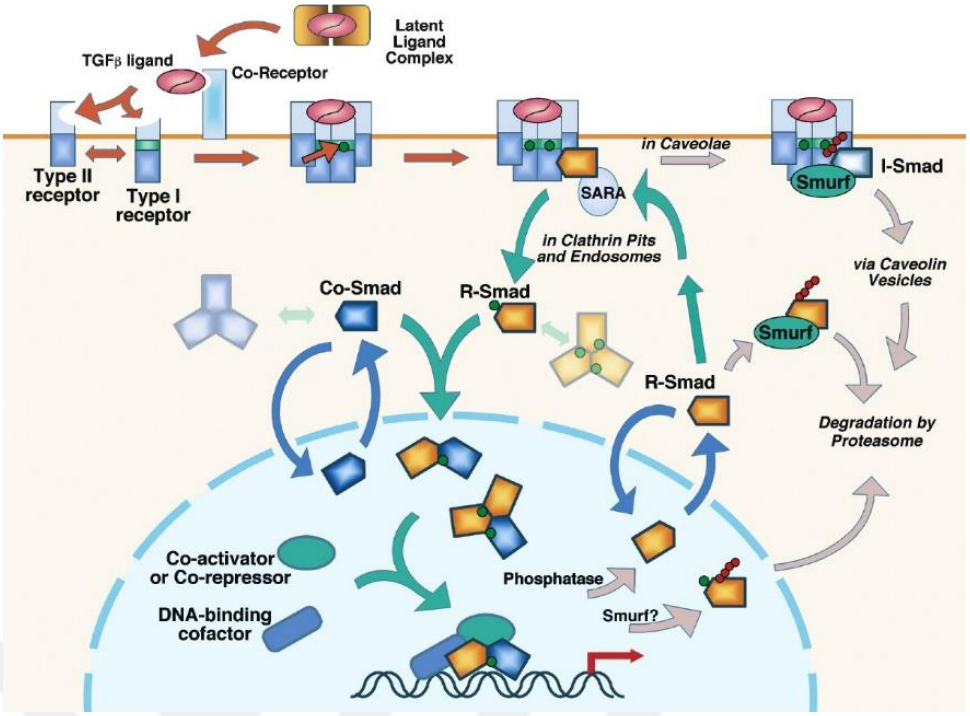

Figure 2.5 The TGF-b signaling pathway

3 MATERIALS AND METHODS

3.1 Materials

The tools that were utilised in this investigation are detailed in Table 3.1

Table 3.1 The study utilised a variety of different instruments.

Items such as spectacles and instruments	Companies	Countries
Refrigerator	BEKO	Turkey
Spectrophotometer	Biobase	India
Different-glasses	-----	China
Pipette	BioSan	Germany
Sysmex device	Sysmax	Japan
Shaking water bath	Memmert	Germany
Oven	Memmert	Germany
Light-microscope	Olympus	Japan
Centrifuge	Memmert	Germany
Spectrophotometer	Biobase	India
ELISA	Labon	China

3.2 Patients

Participating in this study were a total of eighty (80) men who were diagnosed with prostate cancer. The patients were between the ages of 25 and 70. Each patient's case information sheet was compiled with information on their name, age, sex, marital status, place of residence, history of chronic illnesses, history of drug use, history of smoking, and kind of surgery. These patients were hospitalized to Kirkuk Kirkuk oncology center between January and March (2022). 50 healthy individuals were also included in this study as a control group.

3.3 Blood Samples Collection

Each patient had blood drawn by skilled nurses. On whether or not it is necessary to get fresh blood samples, several scientists differ. As a result, it is no longer required to use

recently obtained blood. Each subject had their five millilitres of venous blood separated into an EDTA tube (1.0 mL) and a plain vacutainer tube (4.0 mL).

Clear serum samples were obtained by centrifuging blood that had been held in plain Vacutainer tubes for a brief length of time to allow the blood to clot, and then doing so at a speed of 4000 revolutions per minute for 10 min. The serum was divided into five straightforward tubes, sealed, and kept at -20°C until the analysis was finished. Before use, the frozen serum samples were gently mixed at room temperature after thawing at 4–8°C.

3.4 Kidney Functions

3.4.1 Creatinine

In an alkaline environment, picric acid and creatinine combine to produce an orange-colored complex with the alkaline picrate. The amount of creatinine in the sample directly correlates to the intensity of the colour generated.

3.4.1.1 Procedure

The working reagent, specimens, and the standard were all incubated at the reaction temperature of 37°C. After then, the enhancements were carried out in line with the Table 3.2.

Table 3.2 Steps of creatinine procedure

Addition sequence	S	T
Working reagent	1 mL	1 mL
Standard	50 µL	-
Sample	-	50 µL
After exactly 30 seconds have passed, you should now read the initial absorbance (A) for both the standard and one of the tests. Test again one minute and twenty seconds after reading the second absorbance (A) of the two standards. Find out the difference in absorbance, A, between the test sample and the reference.		

3.4.1.2 Calculation

The following Equation (3.1) is used to determine the creatinine level:

Results= A2-A1 (sample)/A2-A1 (standard) x C Standard = mg/dL (3.1)

total creatinine a standard.

3.4.2 Urea

The breakdown of urea by urease results in the production of ammonia as well as carbon dioxide. When the ammonia that was produced reacts with sodium salicylate, alkaline hypochlorite, & sodium nitroprusside, a green cromophore is produced. The presence of sodium nitroprusside as a coupling agent is required for this reaction to take place. There is a one-to-one relationship between the amount of urea present in the specimen and the degree of coloration.

3.4.2.1 Procedure

At the temperature of the reaction (37°C), the working reagent, samples, and standard were all incubated. After then, the additions were carried out in accordance with the Table 3.3

Table 3.3 Steps of urea procedure

Addition sequence	Blank	Sample	CAL standard
Working reagent	1 mL	1 mL	1 mL
Sample	-	10 µL	-
CAL standard	-	-	10 µL
After combining, incubate for five minutes at 37°C or ten minutes at room temperature (16–25°C).			
R3	1 mL	1 mL	1 mL
After thoroughly mixing, place the tubes in an incubator for 10 minutes at room temperature (16–25°C) or 5 minutes at 37°C. Compare the absorbance (A) of the samples, standard, and reagent blank at 600 nm.			

3.4.2.2 Calculation

(Sample/standard) x C Standar

If a sample's concentration is more over 300 mg/dL (50 mmol/L), it should be diluted using saline at a 1:5 ratio before being resubmitted for analysis. Multiply the answer by 5 to get the final result.

3.5 Liver Enzymes

3.5.1 Determination of ALT enzyme

Spectrophotometry is used to measure ALT, as shown in Table 3.4

Table 3.4 Steps of ALT procedure

Sample	50 µL
Reagent R	1000 µL
Combine and incubate for a period of two minutes. Every minute for a period of three minutes, there is a change in the absorbance measured at 340 nm and 37 C. Determine the standard deviation of the change in absorbance each minute. (ΔA/min).	

The activity of ALT is calculated according to the Equation (3.2):

(ΔA/min) x Factor (3.2)

3.5.2 Determination of AST enzyme

Spectrophotometry is used to measure AST, as shown in Table 3.5

Table 3.5 Steps of AST procedure

Sample	50 µL
Reagent R	1000 µL
Combine and incubate for a period of two minutes. Every minute for a period of three minutes, there is a change in the absorbance measured at 340 nm and 37C. Determine the standard deviation of the change in absorbance each minute. (ΔA/min).	

The activity of AST is calculated according to the Equation (3.3):

$$(\Delta A/min) \times Factor \quad (3.3)$$

3.6 Genetic Study

The materials used in the PCR study are shown in Table 3.6

Table 3.6 Materials that used in PCR study

	Materials	**Company**
1.	Agarose	Conda/ USA
2.	Red safe staining solution	Intron/ Korea
3.	6X loading dye	Intron/ Korea
4.	Ladder 100 bp	Intron/ Korea
5.	Pre mix pcr	Intron/ Korea
6.	TBE buffer 10X	Conda/ USA
7.	Primer	Intrgrated DNA technology/ USA
8.	i-genomic BYE DNA extraction Mini kit	Intron biotechnology/ Korea

3.7 Protocol

- Add 0.75 µL of inner forward primer
- Add 0.75 µL of inner forward primer
- Add 0.75 µL of the outer forward primer.
- Add 0.75 µL of the outer reverse primer.
- Add 5 µL of DNA
- Add 12 µL of water without nuclease.

From 1-6 were added into master mix tube, which was then placed in an exispin device before being placed in a PCR.

3.8 Electrophoresis

In order to identify the size of the PCR interaction's outcome bundle on the agarose gel, electrophoresis has been used to determine DNA fragments after the extraction procedure or to detect the result of the PCR interaction when standard DNA is present.

3.9 Prepare of the Agarose Gel

The agarose gel was created by melting 1.5 grammes of agarose with 100 millilitres of previously prepared TBE Buffer. This resulted in a 1.5% condensation. After heating the agarose to the a full & rolling boil, it was allowed to cool to 45 to 50 degrees Celsius. After securing the comb in order to create openings for the samples, the plate of agarose support that was being used was finally completed, and the gel was then poured onto it. After being carefully poured to prevent the formation of air bubbles, the gel was allowed to cool for a period of thirty minutes. The comb has been separated from the solid agarose in a meticulous manner. The plate has been fastened to its support within the horizontal electrophoresis unit, which is symbolised by the electrophoresis tank. The surface of the gel in the tank has been coated with TBE buffer, which was introduced earlier (Sambrook *et al.* 1989).

3.10 The Sample Preparation Step

Once 5 μL of supposed DNA for electrophoresis (Loading Dye) and 3 μL of the processor's loading buffer (Intron/Korea) have been mixed together, the loading procedure into the gel's holes has already begun. A 70-volt-per-centimeter-square electric current was allowed to flow through the gel for one to two hours until the tincture reached the opposite side of the gel. Following immersion in a pool containing 500 mL of distilled water and 30 L of a red-safe nucleic acid solution, the gel was put under the scrutiny of an ultraviolet light source with a wavelength of 336 nm.

3.11 DNA Isolation and Genotyping

In order to extract DNA, we needed two millilitres (mL) of venous blood from each participant or subject, and this blood was collected in an EDTA tube. In order to separate DNA, salting out was done (Lahiri *et al.* 1992). The red blood cells (RBC) needed to be lysed before the pellet could be obtained, thus the whole blood sample was treated with equal volumes of RBC lysis buffer containing Triton-X. After the pellet was lysed using WBC lysis solution containing 10% SDS, significant molar concentrations of NaCl were repeatedly added in order to separate the protein fraction. This was done in order to achieve this goal. After that, the DNA was separated out, resuspended in TE buffer, and stored at a temperature of -200 degrees Celsius until the PCR reaction was performed. In the end, ethanol that was frozen solid was added. The polymorphism in TGF-B1 at location +869 C/T was analysed by utilising the amplification refractory mutation method of the polymerase chain reaction (PCR) (ARMS PCR). In each reaction, a universal antisense primer was utilised, which, in essence, was the same primer. In addition, one of the two allele-specific sense primers, which will be discussed further below, You should use the sequence 5'- GGGCTGCGGCTGCTTCT-3 for the "T" allele, and for the "C" allele, you should use the sequence 5'- AGCAGCGGTAGCAGCATCG-3. In order to evaluate the results of the PCR amplification, an internal control of 306 base pairs was generated by employing a specific set of primers: 5'-GAGGACCTCAGCTTTCCCTCG-3' and 5'- CCGCAGCTTGGACAGGATCT-3'. In a total volume of 20 litres, the PCR incubation solution had 0.8 mM of each primer, 0.5 units of Taq polymerase, 10 mM Tris-

HCl with a pH of 9.0, 0.1% gelatin, and 40 ng of genomic DNA. Additionally, the solution had 0.1% gelatin.

Diagnosis of gene

The mix of interaction specific for the diagnostic gene is shown in Table 3.7

Table 3.7 The diagnosis gene by using specific interaction

Components	**Concentration**
Taq PCR PreMix	5 µL
Forward primer	10 pmol/ µL
Reverse primer	10 pmol/ µL
DNA	1.5 µL
Distill water	16.5 µL
Final volume	25 µL

Following a series of tests to determine the optimal conditions for first step of denaturation and annealing, temperature was adjusted across all samples by means of a gradient Pcr to determine the optimal statuations. Additionally, the concentration of the template of DNA was varied between 1.5 and 2 µL throughout the course of the experiment. Where are these two extremely important factors taken into consideration during annealing of the primer with the complement (Table 3.8).

Table 3.8 The optimum condition of steps of detection

Phase	**Tm (°C)**	**Time**	**No. of cycle**
Initial denaturation	94	3 min	1 cycle
Denaturation-2	94	45 sec	35 cycle
Annealing	56	1 min	
Extension-1	72	45 sec	
Extension-2	72	7 min	1 cycle

3.12 Statistical Analysis

Statistical analysis was done using Minitab and Excel. Standard deviation and mean were reported. The Dunkin' multiple test & ANOVA test were used to compare the experimental groups' arithmetic averages for statistical significance.

If the probability level was less than 0.5, the results indicated substantial differences; if it was greater than 0.5, they did not. The letters indicated whether two objects were significantly different or similar.

4 RESULTS AND DISCUSSION

4.1 Kidney Functions

4.1.1 Creatinine

The current findings showed that the investigated groups differed significantly. However, as indicated in the Table 4.1 and Figure 4.1, the patients' creatinine level (2.17±0.35) increased significantly (P≤0.05) when compared to the control group (0.93±0.28).

Table 4.1 The levels of Creatinine (mg/dL) in the groups that were examined

Parameter	Healthy group	Cancer group	P-Value
Cr	0.93±0.28	2.17±0.35*	0.0001

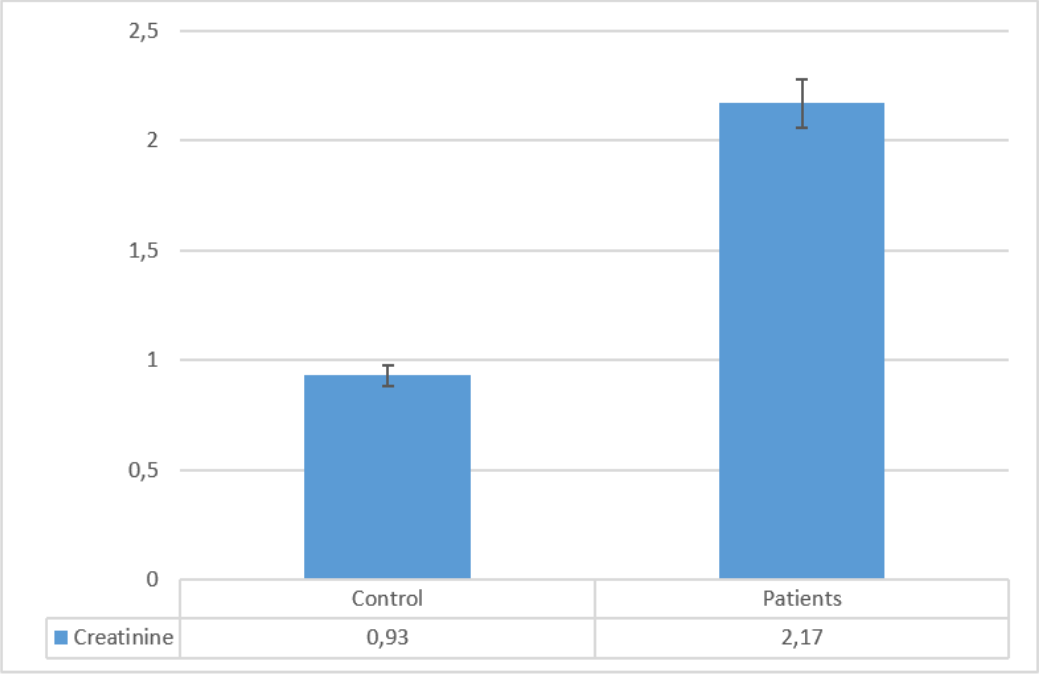

Figure 4.1 The levels of Creatinine (mg/dL) in the groups that were examined

4.1.2 Urea levels

The findings of the current investigation showed that the analysed groups differed significantly. In contrast, the urea level in the sick group (52.91±4.57) increased significantly (P≤0.05) when compared to the control group (21.52±1.84), as indicated in Table 4.2 and Figure 4.2.

Table 4.2 The levels of Urea (mg/dL) in the groups that were examined

Parameter	Healthy group	Cancer group	P-Value
Urea level	21.52±1.84	52.91±4.57*	0.0029

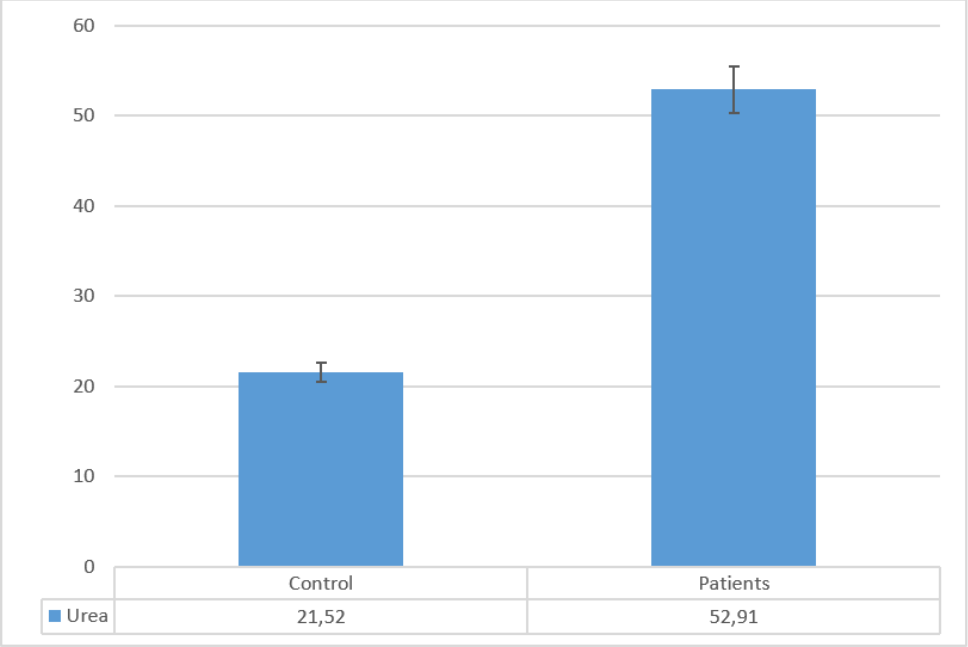

Figure 4.2 The levels of Urea (mg/dL) in the groups that were examined

In this prospective analysis, greater baseline serum creatinine concentrations were significantly associated with an increased risk of prostate cancer. When compared to patients whose serum creatinine levels were 1.02 mg/dL, we found that those whose blood creatinine concentrations were greater than > 1.19 mg/dL were at a risk that was twice as

high. This relationship was substantial when seen on a continuous scale, and it appeared to be dose-dependent. Serum creatinine is a measurement of renal function; however, because it is also affected by other variables, when it is found to be within normal limits, it is neither a sensitive nor a specific sign of renal illness (Levey *et al.* 1988, Perrone *et al.* 1992). Age, sex, muscle mass, food consumption and absorption of the meat-derived nutrients creatine and creatinine, as well as the glomerular filtration rate, all have an impact on creatinine concentrations (Perrone *et al.* 1992).

Additional documented correlates of serum creatinine include serum triglycerides & total cholesterol, blood pressure, ethnicity, weight, body mass index, lean mass, upper arm circumference, illnesses including diabetes, hypertension, and usage of statins, cimetidine, or diuretics (Salive *et al.* 1995, Culleton *et al.* 1999, Baxmann *et al.* 2008). It's interesting to note that one cohort study discovered that heavy smokers had lower blood creatinine levels (Vikse *et al.* 2004).

Additionally, it is a significant contributor to the risk of death from cardiovascular disease (Schillaci *et al.* 2001, Walsh *et al.* 2002, Praught and Shlipak 2005). As reported for males (mean age, 53 years) in the Framingham Heart Study (mean, 1.2 mg/dL), the median blood creatinine concentrations in the U.S. National Health and Nutrition Survey III were 1.11 mg/dL for men ages 40 to 59 and 1.18 mg/dL for men age ≥ 60 (Jones *et al.* 1998). Men aged 71 to 74 years in the Hordaland Health Study in Norway had median serum creatinine levels of 1.13 mg/dL (Vikse *et al.* 2004).

Various criteria have been used to define creatininemia, largely dependent on the methodologies employed in the laboratory assays, although they are frequently in the 1.5–2.0 mg/dL range (Jones *et al.* 1998, Coresh *et al.* 2001). Only 1% of the individuals in the current research had creatinine levels greater than 1.5 mg/dL, with the majority falling within the normal range. Serum creatinine has been the subject of several clinical studies as a possible staging and prognostic marker for prostate cancer (Johansson *et al.* 1991).

In one research (Vesalainen *et al.* 1995), for instance, creatinine concentrations predicted advanced prostate cancer and lower survival and were greater in individuals presenting

with high PSA and locally progressed or metastatic illness, as opposed to those with low initial PSA (Chiong *et al.* 2005). In a group of men with hormone-resistant prostate cancer, elevated serum creatinine was also linked to a worse rate of survival (Fossa *et al.* 1992). Other studies discovered that when other factors (like age, stage, race, or PSA) were taken into account, the significant relationships between elevated creatinine, disease stage and other disordres eliminated (Johansson *et al.* 1991, Merseburger *et al.* 2001); or they were either not associated (Sandhu et al. 1992).

It is doubtful that underlying renal illness accounts for the prospective relationships we found here, notwithstanding the possibility that compromised renal function may explain the link between high creatinine levels and a bad prognosis in prostate cancer patients. Serum PSA and serum creatinine showed a marginally positive correlation (r=0.05; P=0.02) in a study of Korean males, but this correlation disappeared after age was taken into account in a subsequent investigation (Ku *et al.* 2003).

In our investigation, baseline serum PSA was assessed for 118 cases and 7 controls, although there was no correlation between serum PSA and serum creatinine (r=-0.0007; P=0.99). It is uncertain whether or how a group who receives frequent PSA serology screenings would have a different relationship between serum creatinine and prostate cancer risk. Serum creatinine and serum homocysteine were associated in the current study, as well as in two earlier ones (Jacques *et al.* 2001, Elshorbagy *et al.* 2007). A methyl group from S-adenosylmethionine, which is then transformed via adenosylhomocysteine to homocysteine, is necessary for the production of creatine, which is turned to creatinine in muscle cells (Stead *et al.* 2006).

Therefore, it is feasible that creatinine may be used as a gauge for the presence of homocysteine. Homocysteine, along with other one-carbon indicators, were not linked to prostate cancer in this group, according to (Weinstein *et al.* 2003), hence adjusting for homocysteine had no effect on the results of the current study. Lower levels of creatinine are linked to reduced meat consumption (Levey *et al.* 1988), but neither meat nor protein intake were linked to serum creatinine or prostate cancer in our investigation, and they did not muddle the risk connection.

4.2 Liver Functions

4.2.1 ALT activity

The findings of the present investigation showed that there were significant differences between the tested groups. The ALT level in the patients (43.42±8.29) was significantly (P≤0.05) higher than that of the control group (16.03±1.64), as shown in Table 4.3 and Figure 4.3

Table 4.3 The levels of ALT (U/L) in the groups that were examined

Parameter	Healthy group	Cancer group	P-Value
ALT (U/L)	16.03±1.64	43.42±8.29*	0.0041

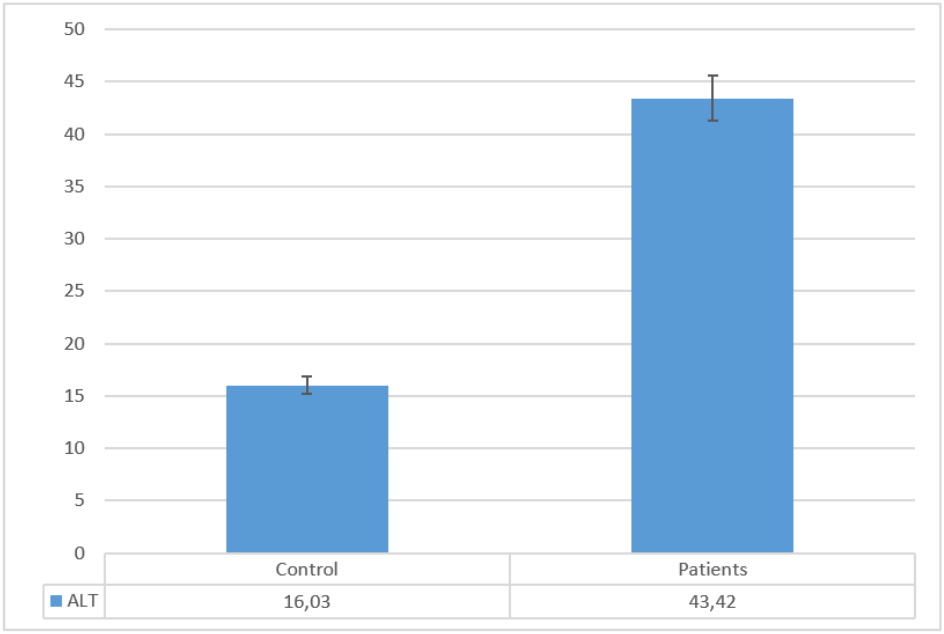

Figure 4.3 The levels of ALT (U/L) in the groups that were examined

4.2.2 AST activity

Significant disparities between the analysed groups were shown by the study's findings. A substantial (P≤0.05) rise in AST level was seen in the patient group (49.72±6.53) compared to the control group (20.42±2.54), as indicated in Table 4.4 and Figure 4.4

Table 4.4 The levels of AST (U/L) in the groups that were examined

Parameter	Healthy group	Cancer group	P-Value
AST (U/L)	20.42±2.54	49.72±6.53*	0.002

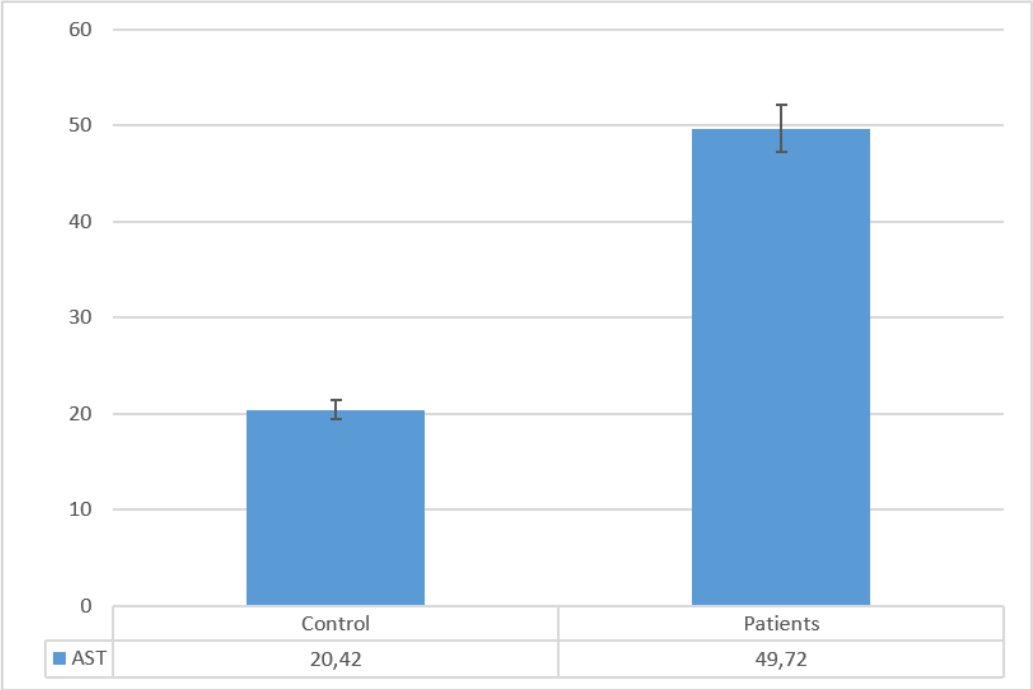

Figure 4.4 The levels of AST (U/L) in the groups that were examined

The primary circulating enzymes in blood, mostly in liver cells, are AST and ALT. AST and ALT are released from cells into the blood when hepatocytes are damaged or die, increasing blood AST and ALT levels (Pratt and Kaplan 2004). In their 2010 study report, (Bañez *et al.* 2010) found a statistically significant correlation between ALT and pathological Gleason total ≥ 7(4+3) malignancy. Additionally, they discovered that there

was no connection between AST or ALT and other harmful clinical characteristics or biochemical recurrence following radical prostatectomy (Bañez *et al.* 2010).

The authors of the study came to the conclusion that, while biochemical recurrence was not linked to serum AST and ALT levels, they could be with a higher Gleason score. After learning about this phenomena, we hypothesised that liver cell injury influences androgen metabolism in the liver indirectly, changing the amount of androgen in the blood and perhaps triggering the onset and progression of PCa (Bañez *et al.* 2010).

However, our findings indicated that the malignancy of PCa was not correlated with AST and ALT levels. The lack of participants, differences in race, or living conditions might be the cause of this paradox. Recent research on upper tract urothelial carcinoma (Zhao *et al.* 2020, Gao *et al.* 2017) and renal cell carcinoma (Lee *et al.* 2017) showed a substantial predictive effect for the AST/ALT ratio in urological malignancy. In contrast to ALT, which is mostly expressed in hepatocytes and has tissue specificity, AST is shown by (Mona *et al.* 2006) to be expressed in several tissue types throughout the body (Botros and Sikaris 2013).

We anticipated that AST levels may fluctuate at various levels, causing an alteration in the AST/ALT ratio, when certain organs or tissues of the body were aberrant or injured. According to (Zoppini *et al.* 2016), a high AST/ALT ratio may be a sign of systemic changes. These changes could imply that tumour cells are causing the AST/ALT ratio to rise. In PCa patients who had radical prostatectomy, (Wang *et al.* 2017) demonstrated that the AST/ALT ratio was a standalone risk factor for biochemical recurrence-free survival. Additionally, they believed that a higher AST/ALT ratio was linked to a higher Gleason Score. Although there is still debate over the AST/ALT ratio's ability to predict PCa prognosis, recent clinical studies have discovered that the ratio's alteration is in fact connected to the pathophysiology of PCa.

The most common manifestation of hepatic dysfunction in patients undergoing chemotherapy is an elevation in the levels of bi-lirubin, alkaline phosphatase (AP), & gamma glu-tamyl transferase (GT), with or without abnormal levels of aspartate

aminotransferase (AST) and/or oralanine aminotransferase. This indicates chronic cholestasis (ALT). The majority of patients appear with clinical manifestations that are analogous to acute icteric hepatitis or cholestatic illness. The progression of hepatotoxicity is brought on by a dynamic interaction between the harmful potential of the drug or its metabolites or the susceptibility of the patient, which is influenced by both genetic (i.e. extensive or poor metabolizer) and environmental factors (Kaplowitz 2007).

4.3 Molecular Study

It was demonstrated in TGF-β (+869 C/T) PCR optimised for +869 C/T detection (Figure 4.1). 62°C was the annealing temperature. The emergence of the thickest DNA fragment at the predetermined size, while reducing unspecific fragment, revealed these ideal conditions. Prostate cancer is the second biggest cause of cancer-related mortality among males worldwide, according to a study by (Siegel *et al.* 2018). TGF-β signalling has been shown by (Ying *et al.* 2019) to have two roles in the growth of prostate cancer tumours. Early on, TGF-signaling prevents the formation and growth of human prostate cancers. On the other hand, TGF- functions as an oncogenic signal in advanced cancer.

(Shi and Massagué 2003) demonstrated that the TGF- pathway plays a role in a number of cellular processes, such as differentiation, migration, and death. Dysregulation has been associated to genetic and vascular illnesses, fibrosis, and cancer, according to (Massagué *et al.* 2000). According to the research of (Dai *et al.* 2019), the constitutive activation of the TGF- pathway has been associated with bone metastasis in a number of cancers, including prostate cancer. Treatments that target TGF- can greatly reduce the spread of tumour cells to the bone (Hu *et al.* 2012).

A study by (Fournier *et al.* 2015) found that the specific TGF-β signalling inhibitor SD 208 reduced prostate cancer by preventing the communication between cancer cells. TGF-signaling looks to be a viable prostate cancer bone metastasis prevention target. Therefore, locating a crucial functional element that blocks TGF-β signalling may aid in the elimination of bone metastases in men with prostate cancer (Massagué *et al.* 2000, Shi and Massagué 2003, Dai *et al.* 2019).

According to the research of (Poniatowski *et al.* 2015), TGF-β is a versatile cytokine that controls a variety of biological processes, including angiogenesis, immune system modulation, cell migration, differentiation, healing, and bone formation. (Kingsley 1994) revealed that this cytokine is a member of the TGF-β superfamily, which also contains the cytokines BMP, GDF, activins, inhibins, AMH & TGF-alpha.

TGF-1 is the most prevalent and widely expressed of the three homologous isoforms of TGF that have been discovered in mammals (together with TGF-2 and TGF-3), according to (Lichtman *et al.* 2016). However, the research by (Morikawa *et al.* 2016) showed that TGF-β has a role in the development of the ovary, embryo implantation, gonad and secondary sex organs, placenta, pregnancy immune regulation, and spermatogenesis, among other things. TGF-β is also present in semen and is generated by the decidua and embryo, indicating that it may contribute to the development and maintenance of the conceptus's immunotolerant environment (Ni and Li 2017).

TGF-1 synthesis is controlled on four levels, according to (Meng *et al.* 2014): transcription, translation, secretion, and post-translational modification to an active form in the extracellular environment. The effect of genetic variations on TGF-1 expression was the main topic of (Sachidanandam *et al.* 2001)'s study. The most prevalent type of polymorphism is called a single nucleotide polymorphism (SNP). They can be present anywhere in the gene, But since protein-coding genes are crucial for gene expression, they are more typically discovered there. SNPs may have an impact on a variety of processes, including alternative splicing, protein trafficking to ER, stability of mRNA, expression of gene, microRNA target sequence, and protein function via amino acid modifications (Cargill *et al.* 1999).

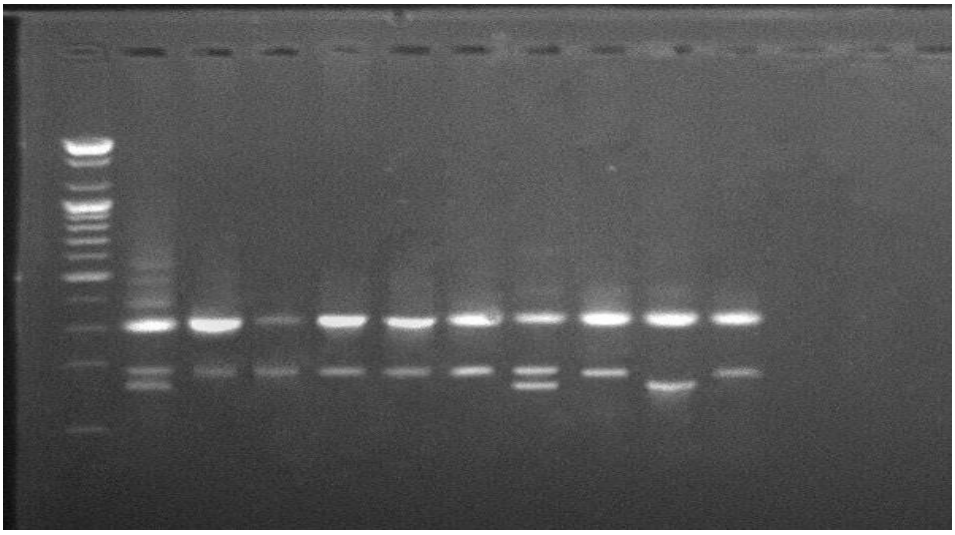

Figure 4.5 Agarose gel of the TGF- gene to the (+869 C/T) alleles C and T in individuals with infection

Table 4.5 The frequency of the TGF-(+869 C/T) gene genotypes in the infected individuals and the healthy control

	Genotype	**Infected samples (80)**	**Control samples (20)**	**(95%CI) OR**	**P value**
TGF-β (+869 C/T)	CC	25 (31.25%)	10 (50%)	2.00	0.081 NS
	CT	26 (32.5%)	6 (30%)	1.18 (0.64-1.33)	0.0082**
	TT	29 (36.25%)	3 (15%)	0.702 (0.45-1.13)	0.045*
*(P≤0.05), ** (P≤0.01)					

In conclusion, this study reveals a mechanism that is responsible for the constitutive activation of TGF-β signaling in prostate cancer. Also the SNPs were linked to the signal peptide's secondary structure, the mRNA's secondary structure, and the mRNA's resultant instability.

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- Patients' levels of urea and creatinine increased significantly ($P < 0.05$) when compared to the control group.
- Patients' ALT & AST activity increased significantly ($P \leq 0.05$) as compared to the control group.
- This study reveals a mechanism that is responsible for the constitutive activation of TGF-β signaling in prostate cancer. Also the SNPs were linked to the signal peptide's secondary structure, the mRNA's secondary structure, and the mRNA's resultant instability.

5.2 Recommendations

- Estimate the concentrations of sexual hormones in the serum of patients with prostate cancer.
- Estimate the concentrations of interleukins and immunoglobulin in the serum of patients with prostate cancer.
- Estimate the counts of blood cells in the blood of patients with prostate cancer.

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