ÇANKIRI KARATEKİN UNIVERSITY

GRADUATE SCHOOL OF APPLIED SCIENCES

MASTER'S THESIS

THE RELATIONSHIP BETWEEN GROWTH DIFFERENTIATION FACTOR-15 AND TESTOSTERONE HORMONE LEVEL IN PROSTATE CANCER PATIENTS

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The thesis **"The Relationship between Growth Differentiation Factor-15 and Testosterone hormone level in Prostate Cancer Patients"** prepared by **SAIF ABDULAZIZ METEAB BANIDAHIR** was unanimously accepted by the following jury as a MASTER THESIS in the Department of Biochemistry at Çankırı Karatekin University Institute of Science and Technology..

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Çankırı Karatekin Üniversitesi Fen Bilimleri Enstitüsü Lisansüstü Eğitim-Öğretim ve Sınav Yönetmenliğine göre hazırlamış olduğum "**Prostat Kanserli Hastalarda Büyüme Farklılaşma Faktörü-15 ile Testosteron Hormon Düzeyi Arasındaki İlişki**" konulu tezin bana ait, özgün bir çalışma olduğunu; çalışmamın hazırlık, veri toplama, analiz ve bilgilerin sunumu olmak üzere tüm aşamalarında bilimsel etik ilke ve kurallara uygun davrandığımı, tezin içerdiği yenilik ve sonuçları başka bir yerden almadığımı, tezde kullandığım eserleri usulüne göre kaynak olarak gösterdiğimi, tezin Çankırı Karatekin Üniversitesi Fen Bilimleri Enstitüsü'nden başka bir bilim kuruluna akademik amaç ve unvan almak amacıyla vermediğimi ve bu çalışmanın Çankırı Karatekin Üniversitesi tarafından kullanılan "Bilimsel İntihal Tespit Programıyla tarandığını, "intihal içermediğini" beyan ederim. Çalışmamla ilgili yaptığım bu beyana aykırı bir durumun saptanması halinde ortaya çıkacak tüm ahlaki ve hukuki sonuçlara razı olduğumu bildiririm. Çankırı Karatekin Üniversitesi Fen Bilimleri Enstitüsü Lisansüstü Eğitim-Öğretim ve Sınav Yönetmenliğine ilgili maddeleri uyarınca gereğinin yapılmasını arz ederim. (31/5/2021).

SAIF ABDALAZIZ METEAB BANIDAHIR

(imza)

ÖZET

Yüksek Lisans Tezi

Prostat Kanserli Hastalarda Büyüme Farklılaşma Faktörü-15 ile Testosteron Hormon Düzeyi Arasındaki İlişki

SAIF ABDALAZIZ METEAB BANIDAHIR

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Prostat kanseri (PCa), birçok ülkede, özellikle Asya'da sık sık erkeklerin ölümüne neden olan ve sürekli artış gösteren yaygın deri dışı kötü huylu bir kanser türüdür. Bu nedenle, hastalığın sonucunu ve tedavinin etkinliğini tahmin etmek için prognostik belirteçlere ihtiyaç artmaktadır. Çalışma kapsamında, hasta grubu olarak 70 erkek vakanın ve kontrol grubu olarak 30 erkek vakanın serum ve klinik verileri toplandı.. Serum büyüme farklılaşma faktörü 15 (GDF15), prostata özgü antijen (PSA), toplam serum testosteron, folikül uyarıcı hormon (FSH) ve C - reaktif protein (CRP) değerleri BioTek Elisa ve Roche Cobas c 311 kullanılarak test edildi. PCa hastaların serumlarında hastalık ilerlemesine bağlı olarak, GDF-15 seviyelerinde bir artış belirlendi. Ayrıca, PSA seviyelerinde önemli bir artış gözlenmekle birlikte, serum toplam testosteron ve FSH seviyelerinde küçük bir azalma eğilimi tespit edildi. Pearson Ölçeği ile ilgili olarak, prostat spesifik antijen (PSA) seviyeleri ile serum GDF-15 arasında pozitif anlamlı ($P < 0.0023$) korelasyon görüldü. Ek olarak, serumdaki C-reaktif protein (CRP) seviyesi ile hastalık süresi arasında pozitif ($P < 0.002$) bir korelasyon tespit edildi. Sonuç olarak, artan (GDF-15), PSA ve vücut kitle indeksi (VKİ) ile PCa riski arasında güçlü

bir ilişki görüldü. Ayrıca prostat kanserinin, FSH ve toplam testosteron seviyelerinin düşüşü ile ilişkili olduğu gözlendi. Bu çalışmada, PCa'da prognostik biyolojik belirteçler olarak GDF15 ve PSA incelemelerinin kullanılabilineceği tespit edildi. Ayrıca hastalığın ilerlemesini belirlemektedir.

2021, 71 sayfa

Anahtar Kelimeler: Prostat kanseri, GDF-15, Toplam Testosteron, PSA, S. C.R.P, FSH, Roche Cobas c 311, Prognostik belirteç

ABSTRACT

Master Thesis

The Relationship between Growth Differentiation Factor-15 and Testosterone hormone level in Prostate Cancer Patients

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Prostate cancer (PCa) is a common non-skin malignancy disease that frequently causes death males and the burden of this cancer continuously elevating in many countries and especially Asian. Hence the need to find prognostic markers to predict aggressiveness, patients' outcome, and efficacy of treatment are raising.We analyzed serum and clinical data from 100 case divided in to 70 men as patient group and 30 men as control group . Serum growth differentiation factor 15 (GDF15), prostate specific antigen (PSA), total serum testosterone, follicle-stimulating hormone (FSH) and C-reactive protein (CRP) were assayed by BioTek Elisa and Roche Cobas c 311. In Serum PCa patients with continuously disease progression were Levels of GDF-15 elevated.There was a significant increase PSA levels with prostate cancer patients with old injury but observed a trend to tiny depression in levels of serum total Testosterone and FSH. Concerning to the Pearson Scale there were positive significantly ($P < 0.0023$) correlated between serum GDF-15 with Prostate-specific antigen (PSA) levels. In addition, there were a positive significantly ($P < 0.002$) correlated to the duration of the disease with the level of C-reactive protein (CRP) in serum. In our conclusion, a strong

correlation was observed between increasing (GDF-15), PSA and body mass index (BMI) with PCa risk. Also, Prostate cancer patients was related to depress FSH and total testosterone levels. The present thesis reinforce the use of GDF15 and PSA examinations as prognostic biomarkers in PCa and in determining disease progression.

2021, 71 pages

Keywords: Prostate Cancer; GDF-15; Total Testosterone; PSA; CRP; FSH; Roche Cobas c 311; Prognostic marker

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SYMBOLS OF ICONS

-	Minus
%	Percent
/	Divide
+	Plus
°	Degree
µg	Microgram
I^-	Iodide
Kg	Kilogram
m^2	Square meters
ml	Millilitter
mm	Milimetre
ng	Nanogram
ºC	Degrees Celsius
OH	Phenolic Hydroxyl
WHO	World Health Organization

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1. INTRODUCTION

Cancer is a disease that affects the way cells in the body divided. Healthy tissue cells divide in an orderly fashion. However, this process may cause in the cells to form double tumor or malfunction in growth. The tumors are benign or malignant. Malignant tumors are cancerous and may spread to other areas of the body (Beilin et al., 2001).
Prostate cancer (PCa) is the most common form of non-skin malignancy diagnosed in many countries. Accounting for nearly 25% of the total number of male cancer diagnoses in 2014. It is estimated that by 2020 PCa will become the most common form of cancer (National Statistics Office 2014).

Worldwide, more than 913,000 men were diagnosed with PCa in 2008, with two-thirds of these male living in different world's locations. PCa is nowadays, the most common form of specific men cancer that has been diagnosed in the most Western European countries, USA, Australia, and New Zealand (Ferlay et al., 2010).

Prostate gland has important biological function of reproduction human being through secreting fluids which significant for swimming toward the ova and fertilize it, these secretions are responsible to save and nourish sperm, this sperm is a compound of glucose, protein-splitting enzymes, simple sugared fructose, zinc, and citric acid (Qi. et al., 2001).

If the cancer is not treated, the cells may spread to surrounding tissues and destroy them. If some cancerous cells leave the primary cancer and spread to other parts of the body, they may form another tumor known as secondary cancer. This process is called metastasis. Secondary cancers of the organ are not the same with primary cancers that affect this part of the body (Gray et al., 2000).

Previous studies revealed that Growth Differentiation Factor-15 could be a diagnostic marker for many diseases and various kinds of cancers. In determine disease severity

and disease risk layers should be established suitable reference ranges for GDF-15 to be as a clinically useful biomarker. GDF-15 can be used for routine clinical practice, clinical measurement and it can support therapeutic administration. The level of GDF-15 can provide diagnostic and diagnostic information; it can also be used to make clinical judgments about specific diseases. GDF-15 can be used as a single mark or multi-mark approach with another individual mark. There is currently insufficient information about the pathophysiology of GDF-15 in certain diseases that are highly likely to result in death, such as prostate cancer and other types of cancer. (Li et al., 2013).

GDF-15 is a cytokine that response to stress. It is highly expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells in both normal and pathological conditions. Additionally, the GDF-15 ratio increases in response to tissue injury and inflammation. (Adela and Banerjee, 2015).

From this point, The Aims of this study to provide new data and information about the relationship between GDF15, PSA, and Testosterone for finding new biomarkers and improve prognostic of prostatic cancer that will help in choose a suitable way of therapeutics and improve the management of prostate cancer patients in Iraq. This project will be useful as one of the few ones in this area.

2. LITERATURE REVIEW

2.1. Prostate Gland

The urologists have been increasingly interesting on the anatomical structures of the human prostate gland and its relationship to prostate cancer development and prognosis since radical prostatectomy resumed in a long time (*Hammerich* et al., 2009).

The creation of prostate gland starts during the third month of pregnancy only in male. The normal structure of the prostate requires 5α-dihydrotestosterone, which is made from fetal testosterone by the action of 5α-reductase. The weighs of the prostate gland in the adult man most probably about 20 grams and its volume is close to the size of a golf ball (4 × 2 × 3 cm) (*Ward A. D. Crukley* et al., 2012; Figure 2.1).

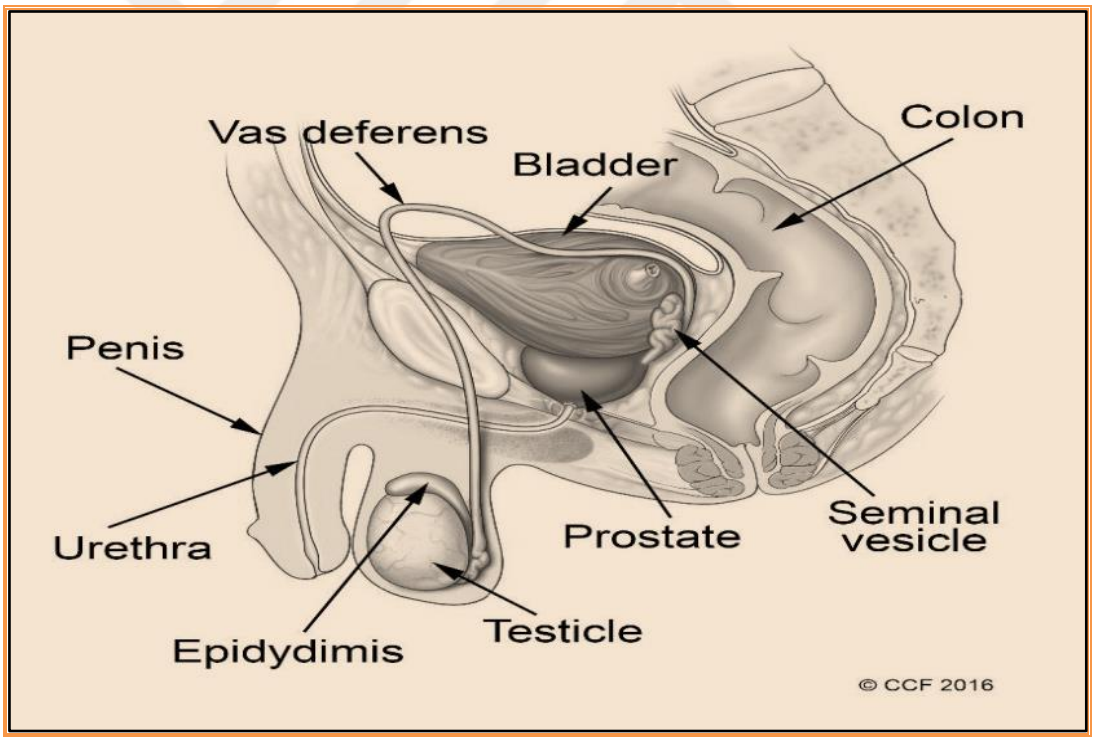


Figure 2.1. The localization of prostate gland and other surrounded organs in male system.(*Ward A. D. Crukley* et al.,2012)

The region's prostate anatomy is similar to a cone in adults male. The main struction or zones of prostate was illustrated as Figure 2.2.) (Shah and Zhou, 2019; The cone region accounts for 70% of the prostate gland by size. The scoop is the central region and accounts for 25% of the prostate gland in adults male. The remaining 5% of the prostate is made up of the transition region, which is composed of two small bulging tissues that form a horseshoe-like shape around the front and side of the proximal urethra. (Johnson et al., 2014).

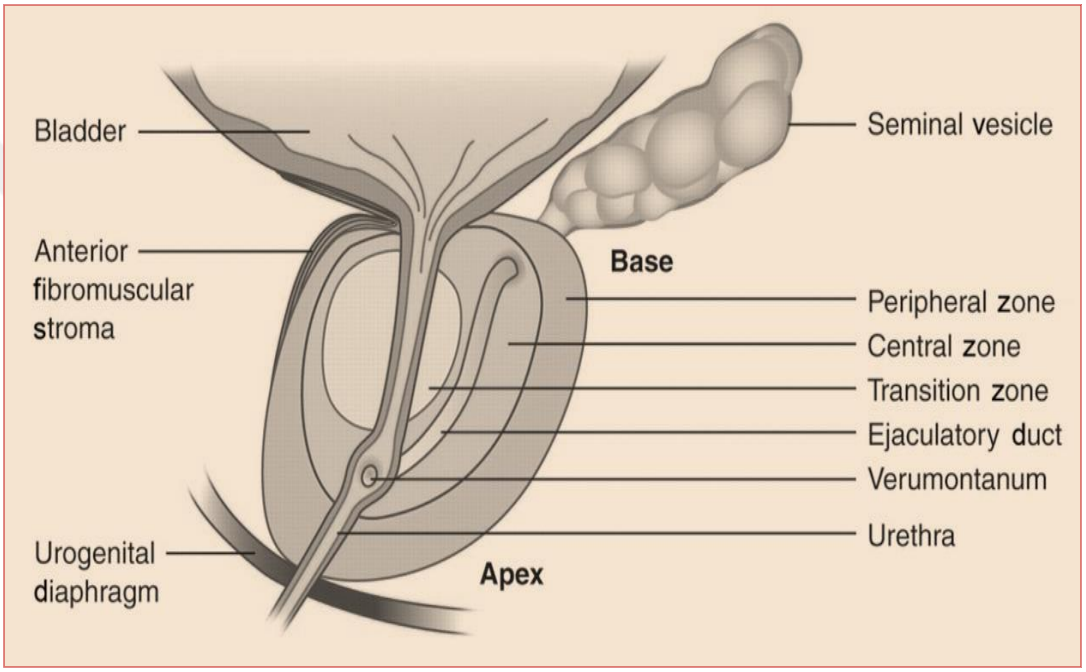


Figure 2.2. Graphical shown the normal zonal description of prostatic anatomy illustrates in a sagittal view (Verma and Rajesh,2011)

The prostate regions are histologically defined, so many prostate diseases have a logical distribution. Seventy percent of glandular tumors (adenocarcinomas) arise in the peripheral region and 20% of glandular tumors originate in the transition region, while only 10% of glandular tumors originate in the central region. Prostate adenocarcinomas

arise in the glandular components of the prostate gland (Thompson and Seay,1997; Figure 2.3).

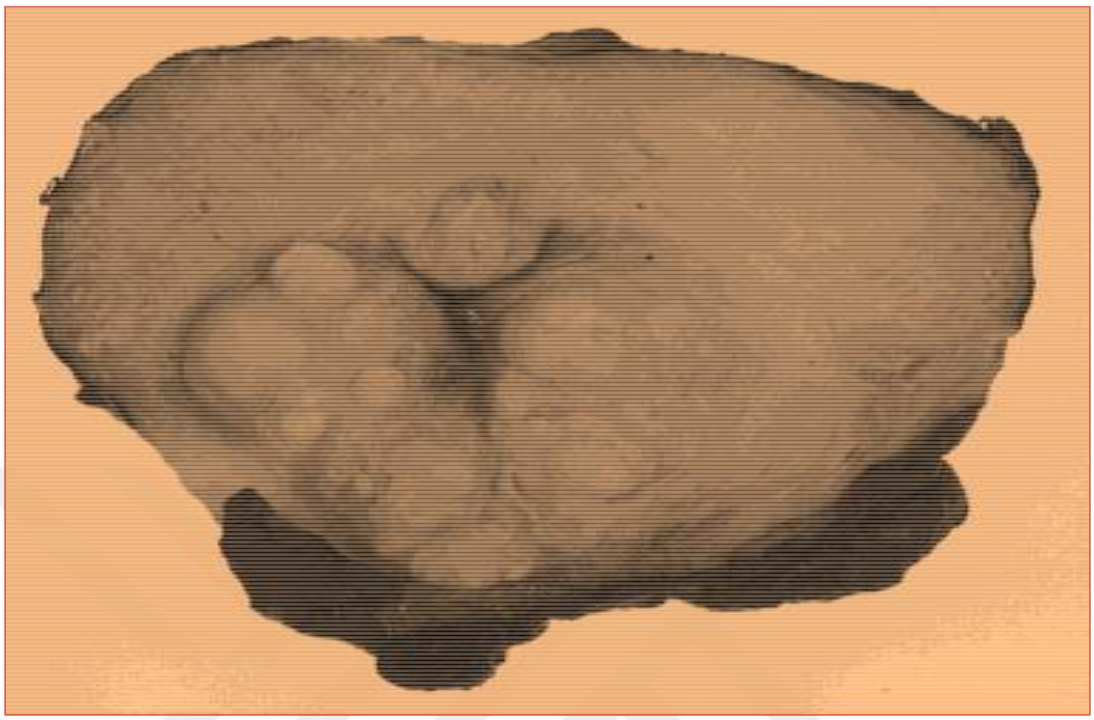


Figure 2.3. A cross-section of the prostate at the verumontanum level clearly illustration the central, peripheral, and urethral swelling of BPH. The lateral and posterior parts of the prostate are not affected by the nodular process (Foster,2000)

2.2. Prostate Cancer

Prostate cancer (PCa) is categorized as an adenocarcinoma, It begins whilst the cells of the prostate gland that secrete semen into abnormal cells (Tumor cells). The glandular tumor is the most common occur in the peripheral region in prostate gland. Initially, prostate cancer begin with small groups of cancer cells stay confined in the healthy prostate glands, While prostate cancer spread to the lymph system, bones, and other parts(Kyrianou, 1994).

In addition to, factors for example, hereditary, diet, inflammation, way of life, infectious agents, and so forth are largely expected agitators for the advancement of prostate malignancy. These causal elements may cooperate or consecutively to start or advance the improvement of disease (Coffey,2001; American Cancer Society 2010).

During the previous three decades, PCa rates in UK, for instance, has been tripled from 33 cases per 100,000 man in 1975 to 97 cases per 100,000 men in 1997, this pattern does not show any sign of undo, the most currently statistics monitored during the previous decade (1998-2008) reveal a 49% increase in diagnoses in England alone. Similar patterns exist in the majority of the western world (Cancer Research UK, 2010).

Prostate disorders are generally connected with maturing; as age is expanding, the probability of creating prostate issues is expanding as well, the dimensions of the prostate fluctuates with age, and the youthful prostate couldn't measure up to a more seasoned man. Anatomy is complicated, and the prostate isn't best, which may causes additionally problems in neighboring organs. At the same time, it eliminates the effects of urinary incontinence, impotence and the prostate's inability to store and secrete fluids. This is because of the anatomical changes to hypertrophy that generally movement with age. Disease of prostate divide in to three sorts Pca , prostatitis and kindhearted prostatic hyperplasia (Macleod et al., 2010, Gonzales and Riboli,2010).

Prostatitis: Prostatitis is an inflammation occur in the tissue of prostate gland. it's miles the most not unusual genitourinary diagnosis in patient humans (male) among the ages of 18-50 years, Also, it is possible for injuries to lower ages. In many prostate inflammation cases may be lead to some signs and symptoms like ejaculation and aching urination (Collins et al., 1998).

Benign prostatic hyperplasia: **BPH** is a very common disease in a male up to 58 years old and rarely a impendence to life. It refers to an enlarged a transitional region in prostate as a result of the non malignant tumors of epithelial cells and stromal as a

sequence to rise proliferation. Because of an enlarged prostate, Because enlarged in the prostate, the surrounding tissue layer prevents it from stretching, causing the prostate part of the urethra to be compressed. The transition region is the primary site for the development of benign prostatic hyperplasia, whilst the peripheral region is where the malignant tumors are situated (Zhong et al., 2015, Blennerhassett et al., 1966).

BPH causes to now are unknown, but it is believed that many potential factors contribute to BPH. Because, Benign prostatic tumors can't appear in the absence of androgen hormones and the application of additional androgens does not raise BPH symptoms. In particular, the dihydrotestosterone (DHT) hormone found in prostate and it derived from the testosterone, it may be play a important role in benign prostatic hyperplasia (Isaacs and Coffey,1989).

Until in older male, the DHT can still be produced in huge proportions and canbe stored in a professional state in the prostate. Benign prostate tumors is generally can treated with some drugs such as five α-reductase inhibitors that make to contract the prostate, then lead to slow its growth or eradicate through the urethra. (Schroder and Blom,1989).

In a normal functioning of prostate, cells grow, divide, and die regularly. During differentiation process for the cells in malignant growth will lead to inorrectly aggregation for lymphocytic cells (Nagle et al.,1987), During that process, the cells divide uncontrollably and grow due to either the cells are not subject to their systemic death of cells or divide very quickly. In some cases, cancer cells had the ability to transfer some to nearby organs such as the bladder, seminal vesicles and In advanced cases may move to different parts of the body. (Coffey and Pienta,1987).

2.2.1. Etiology and Risk Factors For Prostate Cancer

In addition to using DRE for early identification of prostate cancer and performing an appropriate PSA and TRUS examination, beside physicians should be also think about predisposing elements in the evolution of this malignant disease(National Comprehensive Cancer Network 2004).

2.2.1.1. Age

The age has been noted already is the most important factor to the occurrence of prostate cancer. previous study proved that < 1% of prostate cancer has been showed in men under 50 years of age, 16% has been detected in men between 50 and 64 years old, while the residual 83% has been showed in men > 64 (Miller et al., 1992).
Consequently, it is highly recommended to annual malignant tumour scans for 50 and more years old men . However, this Recommendation should be weighed against the affected individual's health and survival. (Haggstrom, 2013).

2.2.1.2. History Family

Recently, family history has been recognized as a significant danger factor for Pca. Around 20% of patients with prostate cancer have a family history of the disease, but it may develop not only due to genetics, but also due to similar exposure to certain environmental carcinogens and common lifestyle behaviors. (Carroll PR, 2002). In a case study and evidence, 691 men were more likely to develop 31 malignant prostate cases in patients with a first-degree relative with prostate cancer compared to people without a family history of Pca. Additionally, as the number of affected family members increases, the risk of disease increases as well, increasing the risk of PCa in men with two or three first-degree relatives by 5 to 11 times. (Steinberg et al., 1990). It turned out to be certain that the men with a family background of the diseaese must to go through

recognition endeavors similar with their high-risk condition given the current 10% age risk of developing prostate (Shen et al., 2020).

2.2.1.3. Hormones

The hormones are significant for the normal functioning of the Prostate during puberty, and are predominantly synthesized (> 95%) in interstitial cells also know as (Leydig cells) from the testicle, and the remaining 5% produce from the adrenal glands. Synthesis of androgen hormones begins in the hypothalamus. Androgen hormones such as lutein hormone, follicle-stimulating hormone, and other hormones are synthesized by releasing the gondotropin-releasing hormones in the hypothalamus, which in turn serves to stimulate the pituitary gland to secret these hormones. (Figure 2.4) (Tucci et al.,2009).

All methods of hormonal treatment of cancer metastasis are the final common pathway for reducing circulating testosterone. Symptoms of sexual dysfunction in male with an abnormally low level of testosterone include loss of desire for sex, difficulty achieving full erection, the need for prolonged stimulation to reach orgasm, low semen volume, and often reduction of the resulting pleasure during the foreplay of the penis and orgasm (Schover,1987).

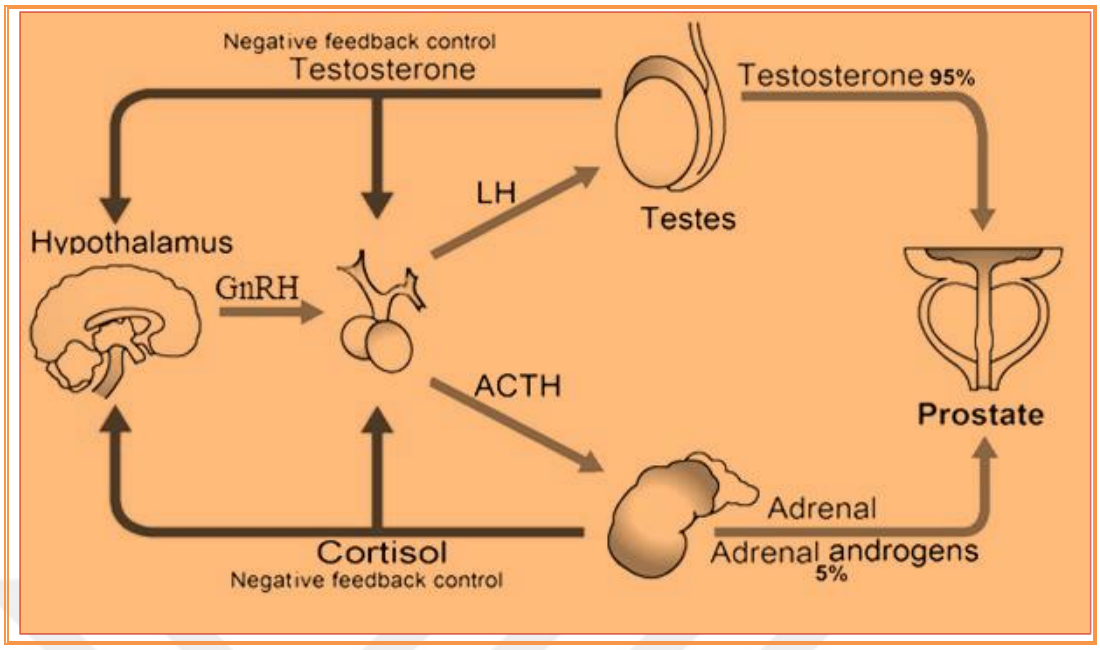


Figure 2.4. Testosterone production under super hypothalamic and pituitary control (Abouhamraa H. 2013)

2.2.1.4. Race

Available data on this subject are inconsistent, though it appears that race has no relationship to poor prostate cancer outcomes. However,the critical question, is whether factors such as grade, stage, and other clinical criteria can be used to demonstrate black males' poor outcomes. This is accomplished through the use of data from a comparable medical center, where disparities in care are minimized and detailed clinical characteristics can be obtained and explained. Even after adjusting for clinical characteristics, black males are more likely to have prostate-specific antigen (PSA) repetition following radical prostatectomy in an equal access environment; thus, these hypotheses supported that a black race is associated with prostate cancer aggression. (Gaines et al., 2014).

2.2.1.5. Diet

Although there have been some investigations into nutrients in the epidemiology of prostate cancer, little is currently known about their specific role. Overall, nutrients suggested from previous case and control studies with a possible role in prostate cancer had little effect on these data. No association was found between prostate cancer and lifestyle of eating, such as, consumption of meat, fish or milk. There was some evidence that men who consumed greater amounts of butter, margarine, and cheese had an inverse link with PCa (Severson et al., 1989).

2.2.2. Classifying and Staging of Prostate Cancer

The pathologist and physicians depend on the Gleason Scoring System. The scoring system was found in 1966 by Gleason for the diagnosis the stage of the prostate cancer. The scoring system was updated in the last century in the years 1974 and 1977, respectively. (Gleason, 1992).

The Gleason scoring system is separated the men's prostate cancer in five categorizations depending on the aggression. The aggression is categorized as a result of the morphological details that are shown on the needle-biopsy or samples get from the section of the prostate gland. According to the Gleason Grading system, there are five stages of aggression starting from level one until level five where level one is the minimum and level five is the maximum (Catalona,1982; Figure 2.5.).

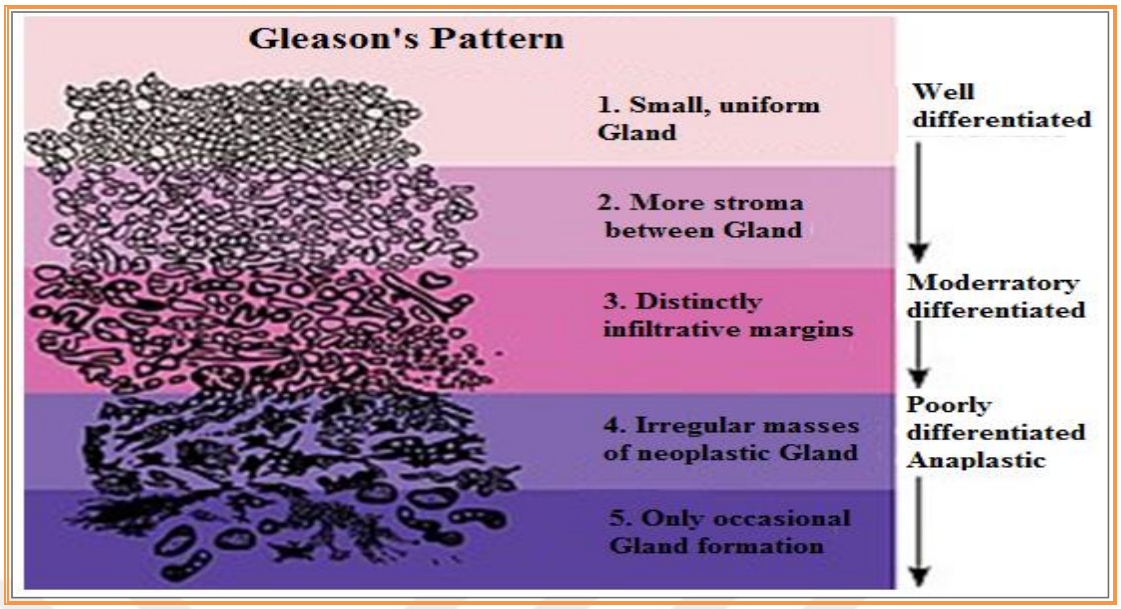


Figure 2.5. Gleason grades characteristics system (Arvanitiet al., 2018)

From Figure 2.5, it has been found that there are clear differences observed in gland structure between spots annotated by various Gleason pattern. The benign spots in the prostate gland showed that contained to a good configuration outer layer of basophils, these revealed no evidence of cytological atypia (Arvanitiet al., 2018).

The histological assessment of human tissues - based on the optical estimation based on microscopic examination of non-trivial cellular and morphological patterns is time-consuming and often suffers from restricted reproducibility. Therefor PCa in particular, it can be very difficult to identify Gleason patterns 3 and 4 related medium severity unequivocally (Arvaniti et al., 2018; Fuchs and Buhman.,2011).

- In the Gleason 3 patches, the prostate glands are variable, but therewith round and well created.
- In Gleason 4 patches, it is noted compact glands, which are small and irregular, In addition an implicit gametophyte.
- In Gleason 5 stains, it found mostly no gland formation and solid sheets of the tumor.

2.2.3. Prostate Cancer Diagnosis

Pca diagnosis depends upon asymmetry in the prostate gland that has shown by many pathways such as digital rectal examination , biopsy and prostate cancer level. DRE is a performed by urologist uses to check tangible changes When a relative enlargement of the prostate gland occurs (Selly et al.,, 1997).

Another constraint is that most tumors happen in zones that are not reachable by DRE. The Transrectal Ultra sonography (TRUS), computerized tomography (CT) and magnetic resonance imaging (MRI) may be used for the clinical diagnosis of prostate cancer. The imaging has extended from the portrayal of cutting edge or metastatic cancers to involve marking of a tumor inside and outside the prostate, including anatomical and logical anatomy that provides basic primary information (Cupp and Oesterling,1993).

A strong need remains for an effective methodology of not only detecting but providing an accurate prognosis for PCa. Although the yearly estimate of PCa does not need to start until the age of 50 in most men only but checking at an early age may be justified in men at high risk (Moskalik, 2001).

2.2.3.1. Physician Diagnosis

Over the past century, physicists have focused on continuous innovation in terms for imaging techniques that help radiologists to development of the diagnosis techniques of prostate cancer and other kinds of cancers. (Downer et al., 2017).

However, human diagnosis still suffers from faulty disclosure or explanations distortions during clinical decisions. There are two main reasons cause of these errors (Hambrock et al., 2013): First, Observe limitations (for example, restricted human visual perception, fatigue or distraction) and another reason, The complexity of the

clinical cases, for example, due to unbalanced data (normal cases are more than cancerous cases and normal cases are intertwined with cancerous structures.

2.2.3.1.1. Digital rectal examination (DRE)

The abbreviation (DRE) refers to the Digital rectal examination for diagnosis the prostate cancer, It is the traditional method for determining the presence of malignant disease in the prostate gland and is still recommended by the American Cancer Society as a yearly evaluation in men aged 40 years or older. (Goodman et al., 1995; Figure 2.6).

DRE for diagnosis the prostate cancer depends on determining the size of the prostate, its consistency, nodularity and asymmetry. It has been concluded that DRE is beneficial, an investigator proved the survival rates for 5 and 10 years were similar to those for identical control groups. Additionally, men diagnosed with PCa after the first year of examination had a higher rate of clinically localized disease than men diagnosed during the initial examination. (Thompson et al., 1987).

However, DRE has ability to reveal prostate cancer at an early phase restricted to organs in asymptomatic men was investigated. As previously stated, the stage of disease increased in nearly half in a men whose primary prognosis for topical prostate cancer was clinically determined by DRE following total prostatectomy. However, these remaining men who have prostate cancer still have a disease that is organ-specific, making them possible candidates for treatment. Additionally, previous studies that suggested a beneficial effect of DRE screening have been criticized for their lack of randomization and lead time bias. (Jenson et al., 1960). Moreover, without a randomized long-term, controlled trial, the question of whether DRE detects primarily clinically important cancers cannot be assessed. However, its ease of use, low cost, and lack of adverse effects, combined with the ability to detect some cases of potentially treatable prostate cancer, ensure its continued use. (Gilbertsen,1971).

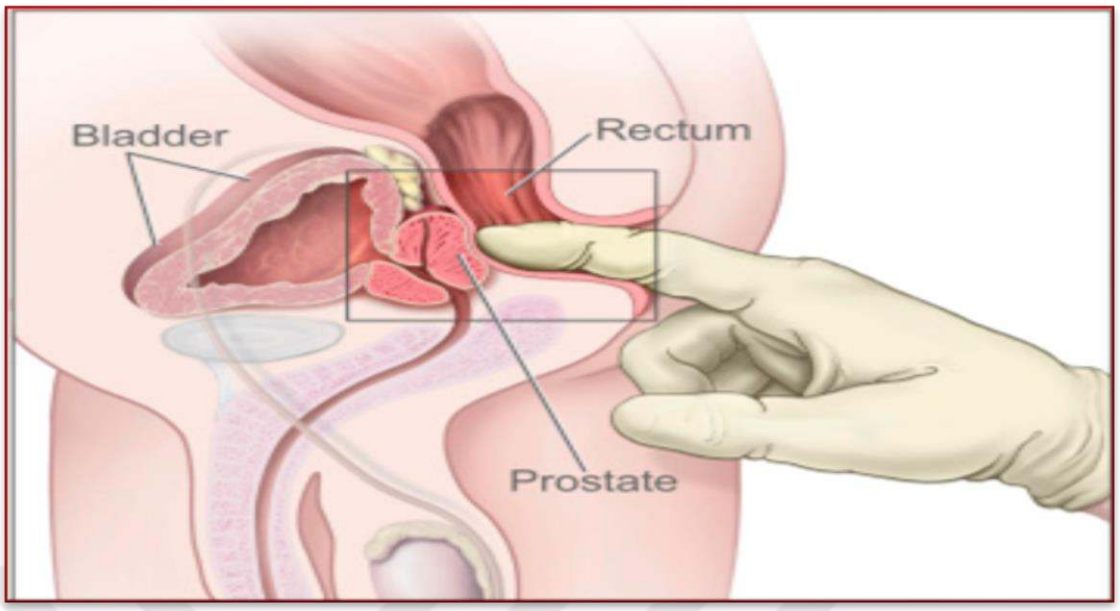


Figure 2.6. Digital rectal examination (DRE) is a normal piece of prostate cancer (PCa) screening and gives significant prognostic data (Okotie et al., 2017)

2.2.3.1.2. Imaging Methods

The Image Method (IM) allows a high-resolution dynamic contrast with quantitative and quantitative perfusion estimates and capillary surface and extra-vascular extracellular space. (Vos et al., 2007).

Functional imaging methods such as MR have improved dynamic contrast of materials MRI. Multi-parameter MRI demonstrated values in detection, localization, and separation (Kitajima et al., 2010).

Diffusion weighted imaging, which quantifies the restriction of free proton movement, has become more prevalent in prostate imaging, not only for detection and localization but also for assessing the degree of aggressiveness of the lesion. (Verma et al., 2010).
The apparent spread coefficient (ADC) values calculated from the weighted images enable more objective quantification of the microtissue environment. However, despite

being quantitative, the analysis of prostate cancer on multi-parameter MR images is a challenge, requiring advanced skill and being subject to observer variation. (Lim et al., 2009).

Computer aided diagnostic (CAD) techniques have been developed for radiological evaluation of various malignant tumors, such as breast cancer, lung cancer, and colorectal cancer. CAD appears to be especially beneficial for inexperienced radiologists, as it appears to improve their ability to detect tumors. (Figure 1.7.;de Hoop et al., 2010).

2.2.3.1.3. Prostate Ultrasound

Ultrasound of the prostate can be performed by scanning through the abdomen, perineum, transurethral or rectal. Trans rectal scanning provides the best image quality due to the prostate's close proximity and patient acceptance. Prostatic trans rectal imaging has become a routine and ultrasound scaling procedure in urology clinics today. Additionally, ultrasound guidance of a puncture biopsy has become a widely used needle. (Gratzke et al., 2015).

Although specificity and sensitivity have not been as prominent as they might be, still it is disputed if compacted lesions on the Trans rectal ultrasound can be used to diagnose malignancy. Depending on the size, location, and specification of the transducer, the appearance of the malignant lesions may vary on the image. (Giesen et al., 1995). On the other hand, not all lesions depicted on an image are cancerous. To differentiate malignant tumors from benign tissues, a comparison of the left and right lobes' symmetry and regularity, as well as the echo, may provide critical information. (Hill et al., 1991). However, transrectal ultrasound has limitations, as image interpretation is user-dependent (Melchior and Brawer,1996). Additionally, the interpretation is constrained by the limitations inherent in human visual perception. Ultrasound can also be used to assess prostate disease infections (Doble and Carter,1989).

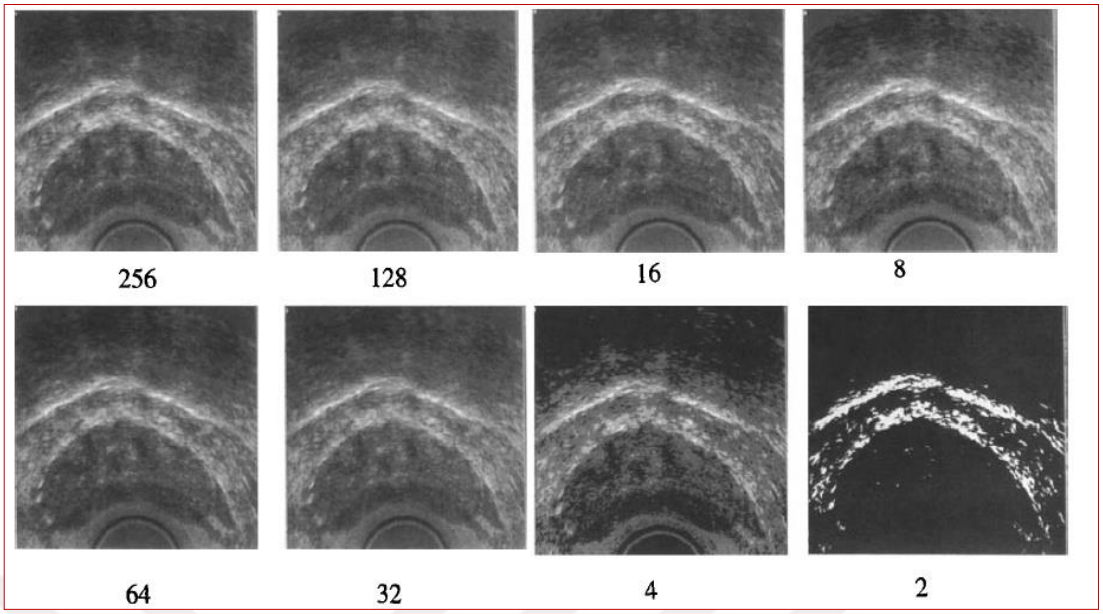


Figure 2.7. Ultrasound pictures of prostate at exclusive grey ranges. unique image with 265 grey values turned into used to get photos with reduced wide variety of gray (Aarnink et al., 1998)

2.2.3.2. Clinical Diagnosis of Laboratory

The three most common methods of treatment are radical prostatectomy cancer, final pelvic radiotherapy and different hormones, each cause having varying degrees of impaired organic sex. Of course, these treatments also harm fertility. But fortunately the vast majority of men have prostate cancer when they get older. Urologists must remain vigilant for a man who may be married to a younger woman. Over the years, many men have in keeping cold. But they were not seen this option before treating cancer. Because the doctor assumed that the patient was too older to be concerned about fertility (Walsh and Donker,1982).

2.2.3.2.1. PSA

The Prostate-specific antigen (PSA) offers a quick and cost-effective way to screen illnesses person (male) for a possible PCa with a single blood test. In many countries, the PSA examination is offering as a standard protocol for all illnesses person (male) over 50 years old (American Cancer Society 2010).

2.2.3.2.1.1. Definition

PSA examination for male without symptomatic, actual examination, is increasingly common in many developed countries. This increased use of the PSA examination resulted in an increase in the incidence of prostate cancer, which began in the 1990s and continues to be noticeable today. (Walter et al., 2006).

The majority of PSA tests begin in primary care. While other factors such as urologists' practice, local guidelines, user requirements, a man's social network, and the media all influence the frequency of PSA testing, GPs play a critical role in determining the population's testing levels and patterns. Thus, increasing understanding of PSA test triggers in asymptomatic men is critical, even more so if evidence-based practice in this area is possible. (Evans et al., 2007, Drummond et al., 2008).

2.2.3.2.1.2. PSA in PCa diagnosis

PSA is used to aid in the detection of prostate cancer and to monitor its treatment effects. While it is a fact that some non-malignant conditions, such as inflammation and benign prostatic hyperplasia (BPH), can cause PSA levels to exceed the current biopsy threshold, these conditions are very rare(Ruckle et al., 1994). To aid in detection, the overall PSA speed (tPSAv) is suggested as a predictor. Numerous studies have confirmed that increased tPSA speeds are associated with an increased risk of

developing prostate cancer. Numerous reports indicated that a rapid increase in PSA prior to treatment was indicative of aggressive disease (Hanks et al., 1996).

PSA concentrations are expressed in nanograms per milliliter (ng/mL). Although most physicians consider a PSA level of less than 4 ng/mL to be normal, some physicians believe anything greater than 2.5 is abnormal. (American Cancer Society, 2018).

2.2.3.2.1.3. Factors Affecting Serum PSA Level

PSA is the primary product of prostate secretion. The mean PSA concentration in the seminal plasma is 0.39-3 g /L (Wang et al., 1998). The PSA molecule is a glycoprotein that contains 240 amino acids. The molecular weight of PSA is 34 kDa. The PSA gene is located on the XIX chromosome, near the pancreatic and glandular kallikrein (hK1 and hK2). PSA synthesis is affected by testosterone, adrenal androgen and dihydrotestosterone (DHT) (Wang et al., 1981). The average PSA concentration in prostatic tissues is 15 mg per gram of tissue. However, the expression of PSA is the highest in the epithelial cells of BPH, mild in normal and inferior epithelial cells in PCa cells (Billis et al., 2007).

2.2.3.2.2. Testosterone Hormone

Testosterone is a steroid hormone whose levels can be used to diagnose hypogonadism; elevated testosterone levels are associated with a variety of diseases, including prostate cancer. Testosterone is thought to play a critical role during the neonatal period in determining spermatogenic capacity and phallus development. Testosterone levels rapidly increase during puberty. After the third decade of life, longitudinal studies indicate that testosterone levels in healthy men begin to decline by approximately 3.2 to 3.5ng/dL per year (Harman et al.,2001; Zmuda et al., 1997). Testosterone is thought to play a critical role during the neonatal period in determining spermatogenic capacity and phallus development. Testosterone levels rapidly increase during puberty. After the

third decade of life, longitudinal studies indicate that testosterone levels in healthy men begin to decline by approximately 3.2 to 3.5ng/dL per year (Wright et al.,1996). Due to testosterone's effect on prostate cell growth, it also nourishes prostate cancer cells and is required for the tumor mass to continue growing. As a result, it was determined that the increase in prostate tissue mass caused by the tumor's growth will be accompanied by an increase in testosterone levels. It is therefore presumed that the examination of a patient's testosterone levels is a useful way to determine whether or not they have prostate cancer, and how large it is. (Bostwick, et al., 1999).

2.2.3.2.3. FSH

FSH comprises two polypeptides (alpha and beta). FSH is part of the glycoprotein hormone family and resembles members of his family such as LH, thyroid-stimulating hormone, and chorionic gonadotropin in structure. There are 96 amino acids in the alpha subunit of these hormones, while the beta subunit varies in size. Participation in the biology activity is only available to the heterodimer. The FSH-β subunit contains 111 amino acids, and is the biological work and receptor interaction that account for the biological activities of FSH (Robboy,2009; Figure 2.8).

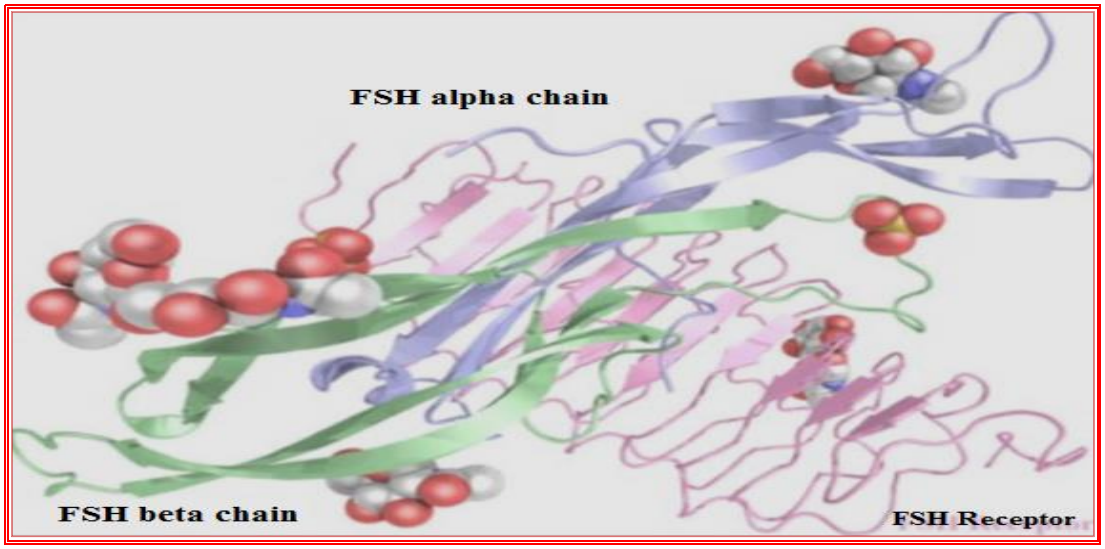


Figure 2.8. The form of FSH-alpha and beta-receiver follicle stimulating hormone (FSH) (K. W. (Ed.), 2012)

2.2.3.2.4. Growth Differentiation Factor-15

GDF-15 is robustly expressed in wide different types of human cancers such as PCA. However, its role in the pathophysiology of cancer to now remains mysterious. The relationship between the expression of GDF-15 and the phase of the tumor or cancers outcome is very useful, maybe GDF-15 an important biomarker for detection of prostate cancer in future (Vanňhara et al., 2012).

Consequently, the suppression of some groups of autoimmune cellular immune systems may be the primary role of development of GDF-15 and development of cancer. Recognized the primary GDF-15 as a factor meddles with macrophage activation. The role of GDF-15 in fetal evolution unresolved (Böttner et al., 1999).

2.2.3.2.4.1. Definition and secretion

The placenta and prostate both contain GDF-15 in a high ratio, but GDF-15 is less concentrated in the other organs such as the heart, pancreas, liver, and colon. Additionally, tissue injury, hypoxia, and inflammation responses to cytokines release macrophages, smooth muscle vascular cells, muscle cells, adipose cells, and endothelial cells. When GDF-15 is present in the bloodstream, it functions as a functional endocrine gland. (Dinget al., 2009). Serum GDF-15 concentrations are associated with a variety of different types of tumors, implying the importance of serum GDF-15 measurement in cancer diagnosis and management. An analysis of total serum levels of GDF-15 demonstrated an apparent estimated ability to such as PCa mortality and disease outcomes although there are many molecular shapes of GDF-15, which justify further future studies to choose GDF-15 as a clinical biomarker for PCa. In general, GDF-15 analysis may provide a robust sorting method For suspected patients (Franket al., 2008).

2.2.3.2.4.2. GDF-15 in Prostate Cancer

The more concentrations of GDF-15 is referring to increase mortality due to prostate cancer and other kinds of disease such as; cardiovascular and non-cardiovascular diseases, GDF-15 is critical in the evolution and progression of cardiovascular diseases such as heart failure, coronary artery disease, atrial fibrillation, diabetes, also cancer, and cognitive impairment. Increased GDF-15 expression is a characteristic of a variety of cancers; in addition to prostate cancer, it is expressed in breast, colon, and pancreas cancers. (Lindahl,2013, Wallentin et al., 2014).

Increased knowledge information of the signal routs induced by GDF-15 in the cancers cells produced or received will support to an understanding of the changes that make up the complex elements within the tumor micro-environment. Each GDF-15 protein has two parts, with properties and tumor inhibitors for both. Also, the cellular and histological effects of GDF-15 signals have been demonstrated in distinct experiment conditions, almost certainly, GDF15 is a functioning and significant part in creating PCa instead of onlooker instigated stress. Therefore, introducing GDF-15 in clinical students may be make to supply new possibilities to best understand the evolution of cancer and may be enhance diagnostic or treatment (Danielset al., 2011).

2.2.3.2.4.3. Role of GDF-15 in Pca

According to investigation data, there was little genetic variation in the GDF-15. Polymorphisms in individual nucleotides were not associated with PCa. The wild type function GDF-15 can be distinguished in the advanced PCa. The probability of the tumor evolution In the living tissue to the early stages of development are (Vaňhara et al., 2012):-

- An uncontrolled regeneration.
- A cellular reprogramming,
- Or a regression.

GDF-15 is expressed in the division of epithelium caused by urogenital sinuses and sprouts, then GDF-15 ratio decrease when progress cancer to reach the lobes of the prostate. GDF-15 is then reactivated during sexual maturation, and its expression is associated with differentiation signs. The information from the development of foetal and early postnatal indicate a distinct double function of GDF-15 in regulates the proliferation of urogenital sinus epithelial cells and their differentiation in the late stage of the formation of prostate lobular structure. According to the recorded information of the cancer, demonstrated clear differences in GDF-15 expression about (Noorali et al., 2007):-

- Advancement prostate.
- Prostatic hypergenesis.
- Intraepithelial neoplasms in prostate.

This is in stark contrast to the expression of GDF-15 in normal tissues (epithelial), where there are two distinct peaks. Reproducing in sprouts and segments, grown prostate recognition, it is accompanied by a lack of recognition signs in transgenic tissues that appear the development of an hyperplasia to prostate intrae-pithelialneoplasias epithelial (Kasper et al., 1998; Figure 2.9.).

Figure 2.9. GDF-15 plays a critical role in a variety of disease states. In Pca, cardiovascular, other cancers, and chronic diseases (Banerjee,2015)

Compared with the parental PC3, the resistance to docetaxel after chemotherapy increased the GDF-15 ratio in PC3 cells. An identical trend was noted in serum and plasma of Pca patients with docetaxel-resistant. Patient survival rate has a significant impact (Wang et al., 2012).

The GDF-15 is not significant for the normal development In mammals; Result Nevertheless, it may play a role in the fetal-maternal reaction in humans and may focus light on immune refusal in the uterus. Also there is growing proof of GDF15 being involved a regeneration in some tissue or reaction to various stress conditions. Severe impacts by GDF-15 frequently copy those saw by TGF-Generic, yet there are contrasts in reactions of the chose individual. It is particularly critical to explain the intracellular signs that range from the development of receptors to the connecting accomplices that moderate the impacts of GDF-15 (Costa et al., 2010).

Increased recorded data about the GDF-15 signaling pathways in cells produced or received contributes to an understanding of the events in the tumors micro-environment. Each GDF-15 protein has two parts, with properties and tumor inhibitors for both. it is high probably that GDF-15 proteinsis an important in developing PCa rather than bystander-induced stress. Therefore, studying GDF-15 in clinical discussions will give new data for understanding the progression of cancer.(Chen *et al.,* 2007; Figure 2.10.).

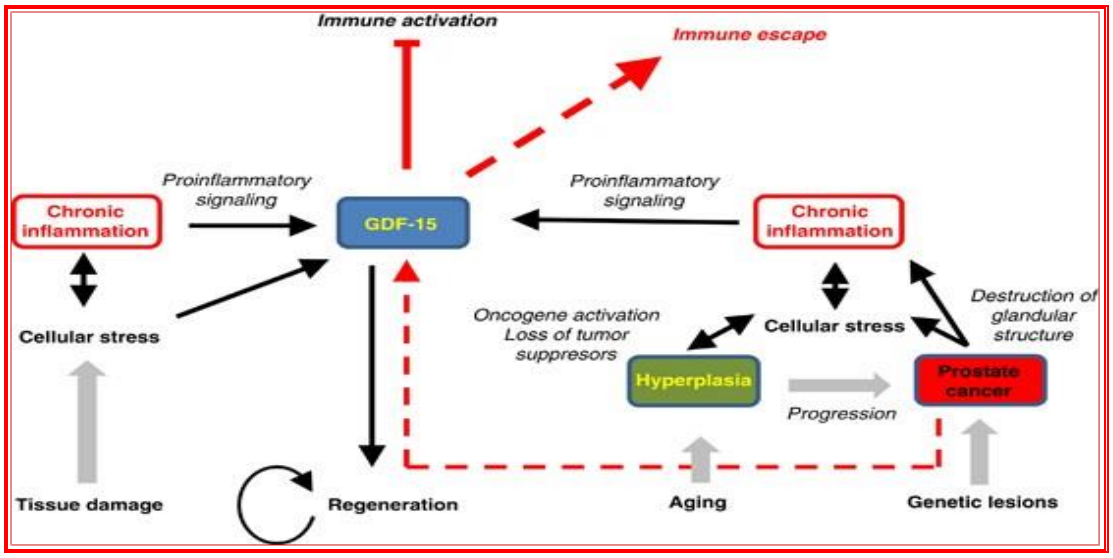


Figure 2.10. The role of GDF15 clear shown in the PCa stages (Vaňhara et al., 2012)

2.2.3.3. Prostate Biopsy

Today, the only final way to detect and confirm prostate cancer is by prostate biopsies. The current clinical scale is the action of prostate biopsies under the control of transrectal 2D ultrasound (TRUS). The US probe is equipped with a needle guide for transrectal prostate access. The guide aligns the needle path with the level of the American image of the end, making it possible to vision the path on the picture to monitor the position of the needle (Ahmed et al., 2007).

However, early and middle stage cancer is often isoechogenic, therefore, it is not apparent in US images, which makes it essential to take a sample of the gland according to a systematic style. The usual protocol includes the acquisition of 10–12 cores and nearly takes under consideration that most tumors (about 70%) develop inside the gland and especially the peripheral region (Andriole et al., 2007; Figure 2.11.).

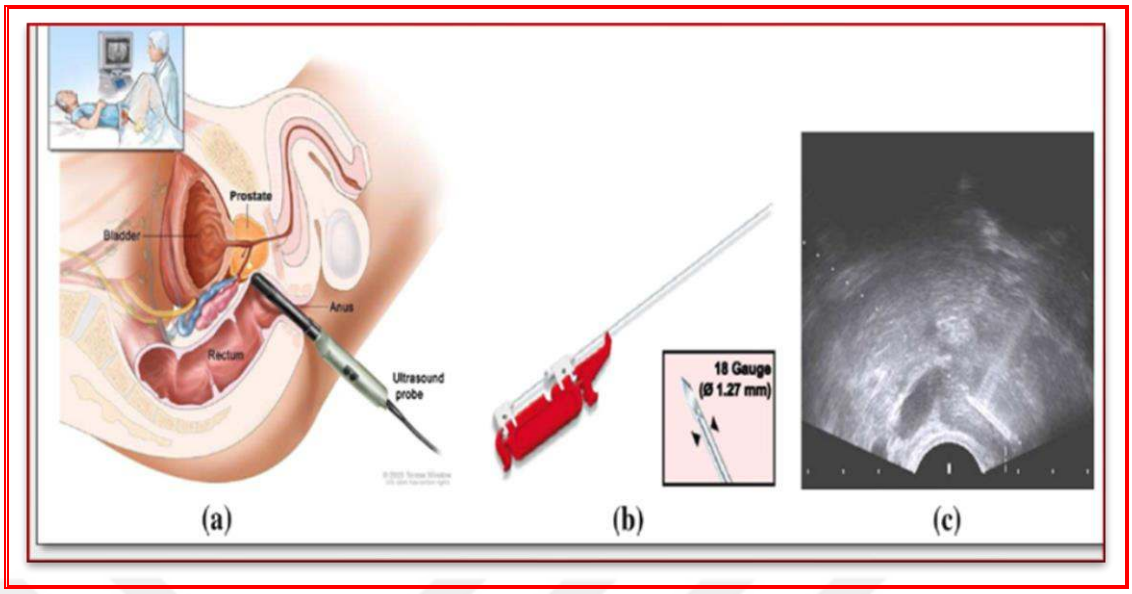


Figure 2.11 Directed 2D-TRUS prostate biopsy during decubitus the patient is in dorsal or lateral with local anesthesia. Be entered the TRUS 2D probe into the rectum of patient to place a needle. The strictly attached needle guide ensures that the hole path is located in the American plane. (B) Shows a pistol measuring 18 diameter (1.27 mm diameter). (C) Shows a two-dimensional TRUS image.

3. MATERIAL AND METHOD

3.2. Ethical Approval

December 2019 to November 2020, the subject were selected from Anbar teaching hospital, Anbar Cancer center and Haditha general hospital. Questionnaires were filled by participants and to get the agreement to participants in this study to collect the information of patients groups.

3.2.1. Design of Study

The study was take the analytical cross- sectional designed. The samples collected at the beginning of the study were 140 patients depending on the data of the study but 70 patients were selected after making sure of the preliminary analyzes in order service of researches aim.

Blood samples were collected from control and patients group in the morning at 08:00 a.m - 01:00 p.m. Using a disposable syringe, the blood was withdrawn from the vein (5 ml). The samples were put into disposable tubes with a gel that facilitated serum separation. Blood keep in the gel tubes allowed to clot at 37º C approximately at ten - fifteen minutes and then centerifuged at 2000 RPM for five - ten mintues then the serum was stored at (-20º C) untile analysis (Prostate Specific antigen, C-reactive protein, Growth differentiation factor-15 (GDF-15), Total Testosterone hormone and Follicle stimulating hormone.

3.2.2. Subject

The subjects of the study divided into two groups. All subjects are male and range in age from 35 - 90 year. The study was based one 100 Samples.

Patient group: Consist from 70 patient, these 70 people diagnosed with prostate cancer, All of them are of the gender of men, with ages ranging from 40 to 90 years. PSA values of most patients > 4 ng/ml in order service of researchers aim.

Control group: Consist from 30 healthy male with age range (35 - 90 years). The PSA values of most patients are < 4 ng/ml.

3.2.3. Exclusion criteria:

1. Any patient suffering from Urinary Tract Infection (UTI) that will lead to increase a PSA levels.
2. Any patient who had sexual intercourse or ejaculation in a recent period therefore consider abstaining from sexual activities that may result in ejaculation for 24 hours before the test.
3. Any procedure that causes temporary bruising or trauma to the groin can have an effect on PSA levels.
4. Any patient with cardiovascular disease (CVD) which affecting in increase GDF-15 levels.

3.2.4. Inclusion criteria :

All patient have got Prostate cancer (PCa).

3.3. Instruments Used in the Laboratory Analysis

Table 3.1 Apparatuses that are used and the companies supplied them and their origin

Apparatus	SN	Company	Origin
Elisa ELx800	257381	BioTek	U S A
Cobas c 311 Automated Chemistry Analyzer	19R0-09	Roche	Germany
Centrifuge C- 12000	6M 1810968	Xinkang	China

Water bath	L513.0977	Memmert	Germany
Refrigerator	100269	Bosch	Germany
Pipette	YE5E507726 YE5A491999 YE5E506188	Dragon	China

3.3.1. BioTek Elisa ELx800

3.3.1.1. Overview

This Elisa is intended for use in clinical, biotechnology, and pharmaceutical laboratories. It is a good solution for microplate-based biological assays due to its compact design and overall footprint. When used independently, the Elisa ELx800 on-board software supports a wide variety of qualitative and quantitative applications.

The reader's optical characteristics were excellent, with a dynamic range of up to 3.000 absorbance units in some read modes. The wavelength range is 400–750 nm. The term "ultraviolet" refers to devices with wavelengths ranging from 340 nm to 750 nm. With its massive curve fitting, cutoff calculation, data transformation, and validation capabilities, the onboard information discount outperforms many computer software applications. The BioTek Elisa ELx800 interfaces with Diagnostic Automation Data Analysis Software to enhance data analysis and reporting flexibility.

Figure 3.1. BioTek Elisa ELx800

3.3.1.2. Calibration Work and Result Reading

Biotek Elisa xl 800 fully automated therefore we insert calibration for kits in maps of instrument. Some kits continent four or five or more calibrations depending on each kits. Then insert standard in maps then choose numbers of sample then press on start for reading the results.

3.3.2. Roche Cobas c 311 Automated Chemistry Analyzer

3.3.2.1. Overview

Roche Diagnostics' cobas C311 analyzer is a fully automated, software-controlled clinical chemistry analyzer. It is intended for both quantitative and qualitative in vitro determinations, utilizing a broad range of analytical tests. The Cobas C311 analyzer

employs serum/plasma for photometric assays and ion-selective electrode measurements.

Figure 3.2. Roche Cobas c 311 Automated Chemistry Analyzer

Cobas c 311 is a stand-alone Clinical Chemistry analyzer. Consolidation of routine and special chemistry workloads is accomplished through the use of a flexible system. 45 tests can be performed on-board, with a throughput of up to 480 tests per hour. Serum, plasma, urine, cerebrospinal fluid (CSF), hemolysate, and whole blood are all analyzed using the analyzer.

3.3.2.2. Specifications

Table 3.2 Roche Cobas c 311 automated chemistry analyzer specification

System	Clinical Chemistry and Homogeneous Immunology full automated, random access analyzer (HIA).
Expected production yield	Up to 300 samples/hr
Throughput of test	300 photometry tests per hour 480 tests per hour for only ISE tests
Channel count Reagent	On the ISE module, there are 42 cassette slots and three channels.
Sample types	Slots for serum, plasma, urine, cerebrospinal fluid, and whole blood (HbA1c only).)
Volume of Sample	1.0 to 35 µl in 0.1 µl steps
Dilution	3 to 121 times, diluent 100 µl
Detection of sample clots	Available
Smallest quantity of material to be tested	700 µll Primary tubes 100 µl l sample cup 50 µl l micro cup
Calibrator/QC Input	On the sample disk, bar coded
Sample data base	10.0 utine / STAT samples

3.3.2.3. Calibration work and Result reading

The calibration procedure and read samples in cobas C 311 very is easily because of is cobas c 311 full automatic, The instrument only passed the serial number for solution through the red right light that read the bar code for this kit.Then put the calibration and QC in patient positions on the outer row with the barcodes facing out. The sample was

placed for reading its values it in suitable position, then from instrument monitor it choose the tests for this sample, then put start for reading.

3.3.3. Centrifuge C- 12000 (6M 1810968)

In this study, it has been used the centrifuge with 5000 RPM/min of Chinese origin. After collecting the blood, separated it in the device with a speed of 3000 spins for five minutes.

Figure 3.3 Centrifuge C- 12000

3.3.4. Memmert Water Bath (WNE 22) (L513.0977)

Memmert water baths are recommended for makes heating tasks in the standard laboratory. It is made from erosion,-resistant stainless steel and it characterized double protection against harmful over-heating. Memmert WNB water baths offer state-of-the-

art control technology, accurate heat control, and monitoring, and advanced safety protection.

3.4. Biochemical Kits

Table 3.3 Materials and Kits that used

Apparatus	Ref.	Company	Origin
Prostate Specific Antigen (PSA) kit	52030	Human Diagnostica mbH	Germany
Growth Differentiation Factor 15 (GDF15) kit	H0150F038	MyBioSource	USA
C reactive protein (CRP) kit	000495684219 0c501V11.0	Roche Diagnostics GmbH	Germany
Testosterone Hor. kit	S010	VEDALAB	France
Follicle stimulating Hormone (FSH) kit	100269	Monobid	USA

3.4.1. Prostate Specific Antigen

PSA, or prostate-specific antigen, is a protein produced by both normal and malignant cells of the prostate gland in men. The PSA test determines the PSA level in a man's blood. PSA levels in the blood are frequently elevated in men with prostate cancer. A blood sample (which must be serum-separated) is sent to a laboratory for analysis in this test. Typically, the results are expressed as nanograms of PSA per milliliter of blood (ng/mL). (Ilic et al., 2018).

3.4.1.1. PSA principle

The human PSA ELISA kit is intended for professional use. The ELISA for direct antigen utilizes highly specific monoclonal anti-PSA antibodies bound to microliter wells on the surface, which are covalently linked to the enzyme through an enzyme-coupled immunosorbent assay (ELISA). The first incubation step involves mixing the specimens, calibrators or controls, and antibody-enzyme conjugate to form the sandwich complex on the well surfaces. Excess conjugate and unbound antigens are washed out at the end of the incubation. When TMB/Substrate is added (step 2), a blue color develops that changes to yellow upon reaction termination. The color's intensity is proportional to the PSA concentration in the specimen.

3.4.1.2. Reagents and Contents

Table 3.4 Materials provided and storage for FSH hormones kits

Abbreviation	Reagent	Quantity
MIC	Microwell Assay plate 12*8 wells	1
CAL	Calibrators (A:white, B:yellow, C:green, D:red, E:blue, F:black)	1 vial of 6×2.0 ml
CON	Antibody-Enzyme Conjugate(white cap)	1 vial of 13 ml
WS20X	Wash soluthion (white cap)	1 vial of 50 ml
SUB	Substrate Reagent (black cap)	1 vial of 13 ml
STOP	Stop solution (red cap)	1 vial of 15 ml
Adhesive strip		1

3.4.1.3. Preparation of Reagent

Before use put all reagents in room with temperature (15 - 25ºc). but reagents that not use should be stored temperature (2 - 8ºc). While, working wash solution are prepared by dilution [WS20X] (1+19) with fresh deionized water, e.g. 50 ml from [WS20X] + 950 ml =1000 ml. Stability up to 60 days at 15 - 25ºc.

3.4.1.4. Wash Procedure

Washe one: Remove strips on plates, then aspirate off the contents in wells, add WASH after 30 sec. aspirate off content and repeat washing 4 times.

Wash two: If there are automatic washers only fill and prime with (WASH). Subsequently, five times wash strips.

Wash three: After washing, take off the remaining liquid by tapping the plate upside down on papers.

3.4.1.5. Procedure of PSA (REF 52030)

Table 3.5 Materials provided and storage for FSH hormones kits

Reagents and specimens should be at room temperature before use. Before use, all samples and kits reagents should be set at room temperature		
Step one	Wells [µl]	
	D2 Calibrator(A1)	Specimen(E2)
Calibrators (CAL)	25	….
Samples and Controls; in duplicate	…..	25
Antibody-Enzyme Conjugate (CON)	100	100
Mix and cover wells with Adhesive Strip		
Incubat eat 20 to 25°C for 30		

Wash 5 times as (W one(W1) – W three(W3)		
(WASH)	300	300
Step 2		
Substrate Reagent (SUB)	100	100
Incubat eat 20 to 25°C for 15		
Stop and mix carefully	100	100
As soon as possible or within 10 minutes after reaction termination, determine the absorbance at 450 nm.		

3.4.2. Human GDF15 (Growth Differentiation Factor 15)

It is a member of the superfamily of transforming growth factor beta proteins. GDF-15 is expressed at low levels in the majority of organs under normal conditions and becomes unregulated following injury to organs such as the liver, kidney, heart, and lung. Is a distant relative of the transforming growth factor (TGF-) superfamily and is found in a variety of mammalian tissues. Its expression is tightly regulated and is frequently induced in response to cellular stress conditions.

GDF-15 has been shown to be a significant prognostic factor in patients with a variety of diseases, including heart disease and cancer.

3.4.2.1. Principle

The technology used in this kit, sandwich enzyme-linked immune sorbent assay. All 96 wells were pre-coated with antibody and the biotin-conjugated antibody was used as indicator antibodies. subsequently, the specimens, standards and biotin conjugated detection antibody was added to wells in plates, then washed with wash solution. Then, after added HRP-Streptavidin to wells, unbound conjugates were washed away with wash solution. TMB substrate also was used to visualize HRP enzymatic reaction. The blue color was the product after added HRp that stimulated by TMB, then color

changed into yellow color after added stop solution. Read the optical density (O.D.) absorbance at wavelength 450 nm in a microplate reader, and then calculated the concentration of target.

3.4.2.2. Kit Components

Table 3.6 Materials provided and storage for FSH hormones kits

Item		Storage
ELISA Microplate (Dismountable)	8×6/8×12	4°C/-20°C
Lyophilized Standard	1 Vial/ 2vial	4°C/-20°C
Sample/Standard dilution buffer	10mi/20ml	4°C
Biotin-labeled Antibody (concentration)	60ul/120ul	4°C(Protect from light)
Antibody Dilution Buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate (SABC)	60ul/120ul	4°C(Protect from light)
SABC Dilution Buffer	5ml/10ml	4°C
TMB Substrate	5ml/10ml	4°C(Protect from light)
Stop Solution	5ml/10ml	4°C
Wash Buffer (25×)	15ml/30ml	4°C
Plate sealer	3/5pieces	
Product Description	1 copy	

3.4.2.3. Procedure

When dilution of the reagent and specimens is required. They must be thoroughly combined and distributed uniformly. Prior to adding to the wells, the TMB substrate

should be equilibrated for 30 minutes at 37°C. A standard curve should be plotted for each test.

1. Place the normal, test specimens (diluted at least 1/2 with Sample Dilution Buffer) and blank wells on the pre-coated plate, and then record their locations. It is recommended to conduct duplicate measurements of each standard and sample. Before adding the standard, sample, and control (blank) wells, wash the plate twice.
2. Add 100µl each of the zero tube, the first tube, the second tube, the third tube, the fourth tube, the fifth tube, and the sixth tube, as well as the Sample Dilution Buffer (BLANK).
3. Add 100µl of specimens into wells.
4. Put the cover on the plate and incubate for 90 min. at 37°C.
5. Place the cover aside and pour out the well contents. Next, wash the wells with the wash buffer two times.
6. In each well, add 100µl of the working solution (biotin-labeled antibody) (standard, test specimen and blank wells). and keep it in an incubator at 37 °C for 60 minutes.
7. Remove the cover and add three times a wash buffer to the wells so the wash buffer remains in the wells for 1-2 minutes.
8. Fill each well with 100µl of Working Solution (SABC), cover the plate, and incubate for 30 minutes at 37°C.
9. Remove the cover and keep the wash buffer one to two minutes in the wells.
10. An additional 90 µl was added (TMB) Carefully place the substrate into each well and cover the plate with a cover plate and incubate at 37°C in darkness for 10 to 20 minutes.
11. Adding 50µl of stop solution to each well will complete the reaction. The color will immediately turn yellow..
12. At last read the O.D. absorbance at 450 nm in microplate peruser following adding the stop solution.

3.4.2.4. Calculation

The O.D.450 of each well divided by the total number of wells yields the relative O.D. (the O.D.450nm blank well). The standard curve can be plotted as the Y O.D.450 relative to the standard solution concentration (X). We can approximate the target concentration by extrapolating from the standard curve. Professional software is recommended for these calculations.

3.4.3. CRP (0004956842190c501V11.0)

The CRP test looks at the amount of CRP in the blood. C-reactive protein is a protein made by the liver, which measures the amount of inflammation in the body. In response to inflammation, it is released into the bloodstream. Inflammation is the body's response to injury or infection; it helps to protect tissues.

A high CRP level in the blood is indicative of inflammation. A number of different conditions can cause it, from infection to cancer. Having a high CRP level may indicate an increase in the risk of heart attack because of the presence of inflammation in the arteries of the heart.

3.4.3.1. Principle

The immune turbidimetric assay was performed with a particle enhanced turbidimetric assay. Of latex particles coated with monoclonal anti-CRP antibodies, human CRP agglutinates. Turbidimetry is used to quantify the aggregates.

3.4.3.2. Working Solutions

Reagent 1: buffer with bovine serum albumin; preservatives.
Reagent 2 : Latex particles covered with antiCRP (mouse) in glycine buffer; immunoglobulins (mouse) ; preservatives.

3.4.3.3. Procedure

By Cobas C111 systems automatically calculate the results and the analytic concentration of each sample.

3.4.4. Testosterone Hormone

The level of testosterone in the blood can be used to diagnose and monitor conditions such as prostate cancer, which is associated with high levels of the hormone. Testes are producing about 95% of testosterone(Diagnostic Automation Inc. 2001). Because testosterone stimulates the growth of prostate cells, it also stimulates the growth of prostate cancer cells and is required for the tumor mass to continue growing. As a result, it was determined that the increase in prostate tissue mass caused by the tumor's growth will be accompanied by an increase in testosterone levels. Thus, if a patient is found to have an elevated level of testosterone, it can be assumed that a tumor is present and may indicate the tumor's size. (Bostwick D et al., 1999).

3.4.4.1. Principle of Assay

Testosterone Elisa works on the principle of competitive binding between Testosterone in the test specimen and the Testosterone – HRP conjugate in order to detect a constant amount of anti-Testosterone antibodies. HRP-labelled Testosterone competes with the endogenous Testosterone in the standard, sample, or control serum for a fixed number of binding sites of the specific Testosterone antibody while the amount of Testosterone

remaining in the standard, sample, or control serum is allowed to vary during the incubation.

Thus, as the concentration of Testosterone peroxidase conjugate is removed and the wells washed, the amount of Testosterone peroxidase conjugate immunologically bound to the well gradually decreases. Following that, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes. This results in the development of blue color, which changes to yellow, and the absorbance at 450 nm is measured spectrophotometrically.

Enzyme activity controls the intensity of the color, and testosterone concentration is inversely related to the intensity of the color. Absorbance is measured spectrophotometrically at 450 nm.

3.4.4.2. Specimen Collection

To avoid hemolysis, serum samples should be prepared from whole blood obtained using acceptable medical techniques. Only undiluted serum samples could be assayed. The specimens can be stored at +2ºC to +8ºC. If screening is performed within 24 hours, or they may be stored at -20 ° c temperature in a deep freezer. Steer clear of repeated freeze/thaw cycles.

Do not use contaminated, hyperlipaemic or hyperhaemolysed sera. Do not use plasma sample.

3.4.4.3. Procedure

1. Before assay, put all reagents solution in room at temperature (+18º C to +25º C)
2. Fill wells with 10 µL of standard, specimens, and controls.
3. In each well, add 100 µL of Testosterone HPR conjugate conjugate reagent.
4. Fill each well with 50 µL of rabbit anti-Testosterone reagents.
5. Gently mix for 30 seconds.

6. Incubate at 37 º C for 90 min.
7. Remove the contents of the plate and then wash and flick the wells five times with 300 L distilled water. (Please refrain from using tap water.)
8. Sharply strike the wells onto absorbent paper or paper towels to remove any remaining water droplets.
9. TMB reagent is dispense into each well. Mix gently for ten seconds.
10. Take in the dark for 20 minutes at room temperature
11. Stop the reaction by adding 100 µL of stop solution to each well.
12. Thirty seconds mix softly. It is critical to ensure that the entire blue color changes completely to yellow.
13. Within 15 minutes, determine the absorbance (optical density) at 450 nm using a microtiter plate reader.

3.4.4.4. Result calculation

1. Calculate the absorbance values (A 450) on an average basis for each set of reference standards, controls, and samples.
2. On linear graph paper, construct a standard curve by plotting the mean absorbance of each reference standard against its concentration in ng/ml. With absorbance values vertically or on the Y-axis and concentration horizontally or on the x-axis.
3. Determine the corresponding testosterone concentration in ng/ml from the standard curve using the mean absorbance value for each sample.

3.4.5. FSH

In our study, one of the important tests that we were researched that is FSH, Follicle-stimulating hormone is one of the hormones essential to pubertal development and the function of women's ovaries and men's testes. This hormone stimulates the growth of ovarian follicles in women prior to the release of an egg from a single follicle during ovulation. Additionally, it stimulates the production of oestradiol. Your pituitary gland,

a small gland located beneath the brain, produces FSH. It is important in the development and functioning of sexual organs. FSH regulates the menstrual cycle in women and stimulates the growth of eggs in the ovaries.

3.4.5.1. Principle

Immunoenzymometric assay: High affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen are necessary for an immunoenzymometric assay. The immobilization occurs at the surface of a microplate well during the assay due to the interaction of streptavidin-coated wells and exogenously inserted biotinylated monoclonal anti-FSH antibody. When a monoclonal biotinylated antibody, an enzyme-labeled antibody, and a serum containing the native antigen are mixed, a reaction between the native antigen and the antibodies occurs without competition or steric hindrance, resulting in the creation of a soluble sandwich equation.

Simultaneously, the complex is deposited in the well via a highly specific reaction between streptavidin and biotinylated antibody. To separate bound antibody from unbound antigen, decantation or aspiration can be used. When an antibody-bound enzyme fraction is isolated, the enzyme activity is directly proportional to the concentration of the native antigen. A dose response curve can be generated from several different serum references with known antigen values, from which the antigen concentration of an unknown can be determined.

3.4.5.2. Procedure

1. Prior to beginning the assay, all reagents, serum reference calibrators, and controls must be brought to room temperature (20-27°C).
2. The wells in the micro plate should be prepared to accommodate each serum reference calibrator, control, and patient specimen for assayed.

3. Pipette 0.050 ml (50µl) of the serum reference calibrator, control, or specimen into the designated well.
4. To each well, add 0.1 ml (100µl) of FSH-Enzyme Reagent solution..
5. Gently swirl the microplate for 20-30 seconds to combine and cover.
6. Incubate at room temperature for 60 minutes.
7. Discard the content of the micro plate by decantation or aspiration. If decanting, blot the dry with absorbent paper.
8. Add 350 µl wash buffer (see section on reagent preparation), decant (tap and blot), or aspirate. Repeat twice more for a total of three (3) washes.
9. To each well, add 0.1 ml (100 µl) of working substrate solution.
10. Incubate for fifteen (15) minutes at room temperature.
11. Fill each well with 0.05ml (50µl) of stop solution and gently mix for 15-20 seconds.
12. In a microplate reader, measure the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections).

3.5. Statistical Analysis

The Statistical Analysis System- Spss (2012) and GraphPad prism version7 program was used to detect the effect of difference factors in study parameters. Studied T. test was used to compare the significance of the differences between groups. Pearson's correlation was used to determine the association between studied biomarkers.

The statistical importance level was set as P value of less than 0.05. Descriptive statistics consist of mean, standard deviation (SD), T-test was calculated for each parameter.

4. RESULTS AND DISCUSSION

4.1. Preamble of Results

Patients were recruited from the Department of Oncology of AL-Ramadi Teaching Hospital and Haditha General Hospital of Al_Anbar - IRAQ. A total of 100 male patients were recruited from December 2019 to November 2020. All patients received after clinically and laboratory diagnoses. Patients was 70 person while the control groups 30 subject.

Table 4.1 Comparison of Clinical characteristic between patients and control in Age, BMI

Parameters	Mean ± SD	
	Age (year)	BMI (kg/m2)
Patients group	64.45 ±10.97	24.93 ±4.03
Control group	59.33 ±8.01	28.49 ±4.04
T-test	4.413 *	1.747 **
P-value	0.0233	0.0001
* (P≤0.05), ** (P≤0.01).		

4.2. Age

In the studies was results patients and controls for Age (64.4 ± 10.9 year; 49.3 ± 8.02 year) respectively, This results was a statistically significant (*P 0.011) at P < 0.05, as shown in Table 4.1 (Figure 4.1). The study concluded that prostate cancer gradually increased with age. In previous studies, Age is considered to be the biggest risk factor for prostate cancer. Understandably, this disease has become one of the biggest public health problems (Syrigoset al., 2005). This result is also in agreement with our study.

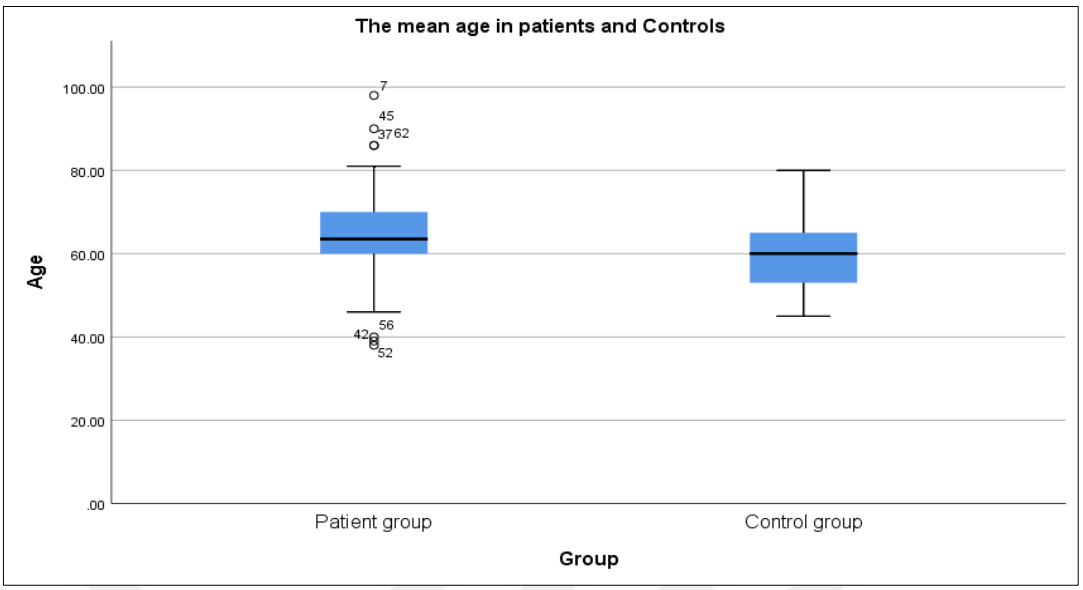


Figure 4.1 A chart showing the relationship between the means patient group and control group given as percentage in Age

4.3. BMI with Prostate Cancer

In the weight was patients and controls results (24.93 ±4.03 kg/m2; 28.49 ±4.04 kg/m2) respectively, this results was highly significant statistical (*P 0.0001) at P < 0.05, as shown in Table 4.1 (Figure 4.2). Obesity is a risk factor for the development of aggressive prostate cancer, according to the majority of published studies. (Choiet al., 2020). Some previous studies revealed after following up to 15 year for obese people that baseline obesity was associated with prostate volume and with the rate of change in PSA (Wallner et al., 2011). while other studies provide opposite results regarding local or overall prostate cancer risk (Gong et al., 2006). The reason for the inverse association of obesity with prostate cancer diagnosis may be at least partially due to detection bias, which is due to large prostate sizes and hemodilution in obese men (Wallner et al., 2011).

As well, Dickerman et al., 2017 supported our research. Weight loss and obesity were found to be associated with an increased risk of prostate cancer. The Dickerman et al., findings indicate that there is a positive correlation between long-term weight gain and the risk of fatal prostate cancer (2017) .

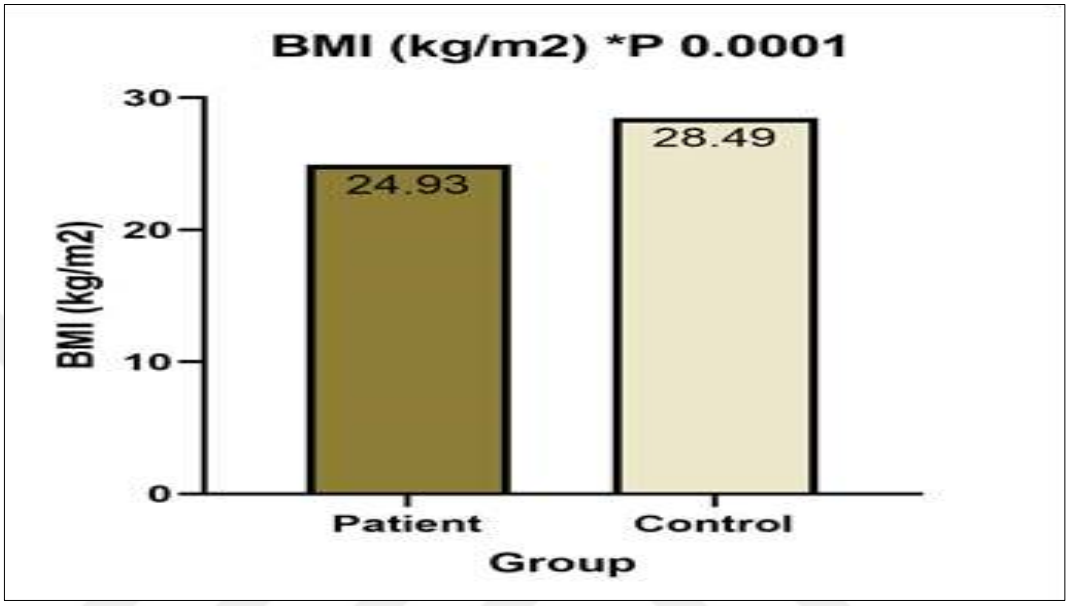
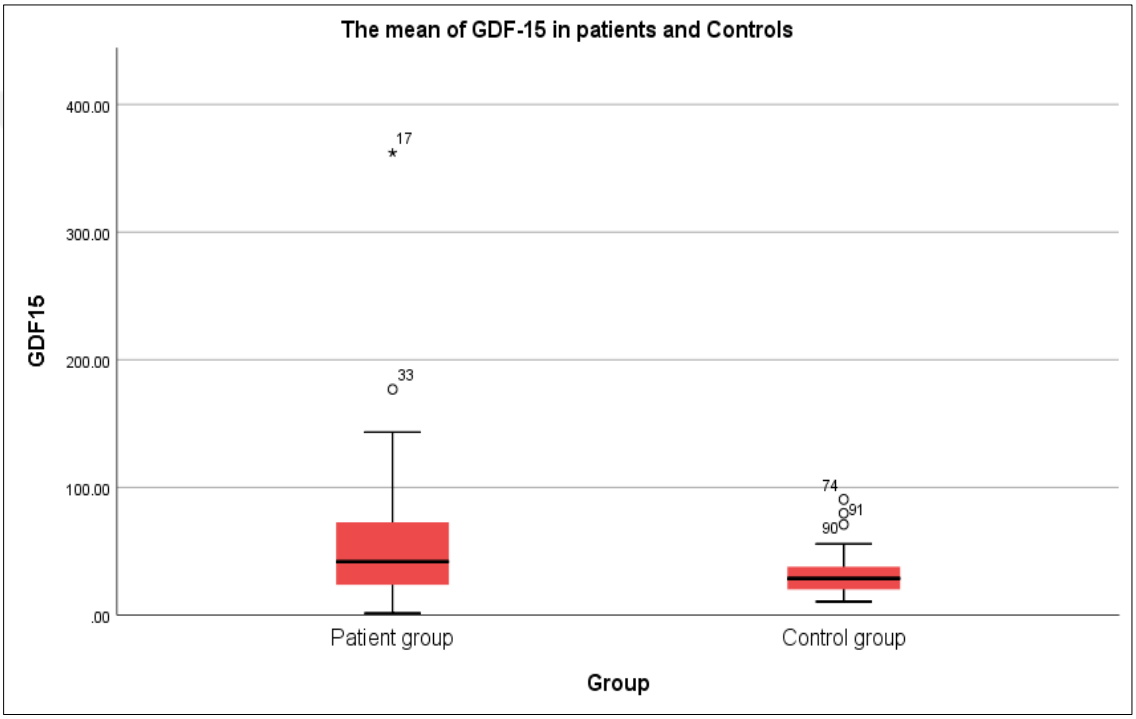


Figure 4.2 The percentage difference in BMI levels between the patient and control groups.

4.4. GDF15

In our study, serum GDF15 levels were significantly higher in the patient group than in the control group. The mean of GDF15 (mg/dl) was has a significant difference between groups, the results was (53.8 ± 50.8 mg/dl; 33.4 ± 19.3 mg/dl, respectively) (*P 0.0352) at $P < 0.05$, as shown in Table 4.2 (Figure 4.3). GDF-15 concentrations have frequently been found to be elevated during the progression of various types of cancer, including gastric, ovarian, prostate, and breast cancers. (Bauskin et al., 2006 and (Baek et al., 2009). Despite the fact that the GDF-15 expression profile has been well described in a variety of cancers, its specific role in prostate tumor development

requires extensive research. Our study supported with presented studies. The presented data indicated that serum GDF15 levels were significantly higher in patients with metastasized cancer (Pca) who had a rapid disease progression(Winand et al., 2014). Welsh et al. discovered that metastatic prostate cancer patients had significantly higher serum GDF15 levels than normal controls. (Welsh et al., 2003). In another previous study, analysis of gene expression data from over 1,000 primary and 200 metastatic prostate cancer samples revealed that metastatic prostate cancer expressed less GDF15 than primary prostate cancer. GDF15 in the primary tumor lower levels are associated



with prostate cancer patients (Zhanget al., 2019).

Figure 4.3 The percentage difference in GDF-15 levels between the patient and control groups.

Table 4.2: Comparison between patients and control in C.R.P, PSA and GDF15

Parameters	Mean ± SD		
	S.C.R.P (nmol/L)	S. PSA (ng/ml)	S.GDF15 (mg/dl)
Patients Group	92.57 ±79.30	38.92 ±33.21	53.85 ±50.88
Control Group	12.96 ±30.95	2.19 ±5.78	33.35 ±19.27
T-test	29.72 **	12.107 **	19.041 *
P-value	0.0001	0.0001	0.0352
* (P≤0.05), ** (P≤0.01).			

4.5. PSA

The PSA results of patients and controls were (38.9 ± 33.1 ng/ml; 2.19 ± 5.78 ng/ml , respectively) this results was a significant statistical (*P 0.0001) at P < 0.05, as shown in table 4.2 (figure 4.4). Other findings from a major multicenter study showed that the total serum PSA use for prostate biopsy is greater than 4 ng/mL (Catalona et al, 1994). While serum PSA is a sensitive indicator of prostate cancer early detection, its specificity is limited by elevated levels in benign prostate disease (Oesterlinget al., 1991). These studies supported our study. In another study, PSA levels decreased in prostate cancer (Catarinicchia and Crawford, 2016). PSA concentration in some patient's have come down due to receive some drugs such as abiraterone and degarelix and these treatment will reduces the production of androgens in the body that can promote the growth of a prostate gland tumor. Suggesting the influence of both testosterone and FSH on PSA levels (Catarinicchia et al 2016).

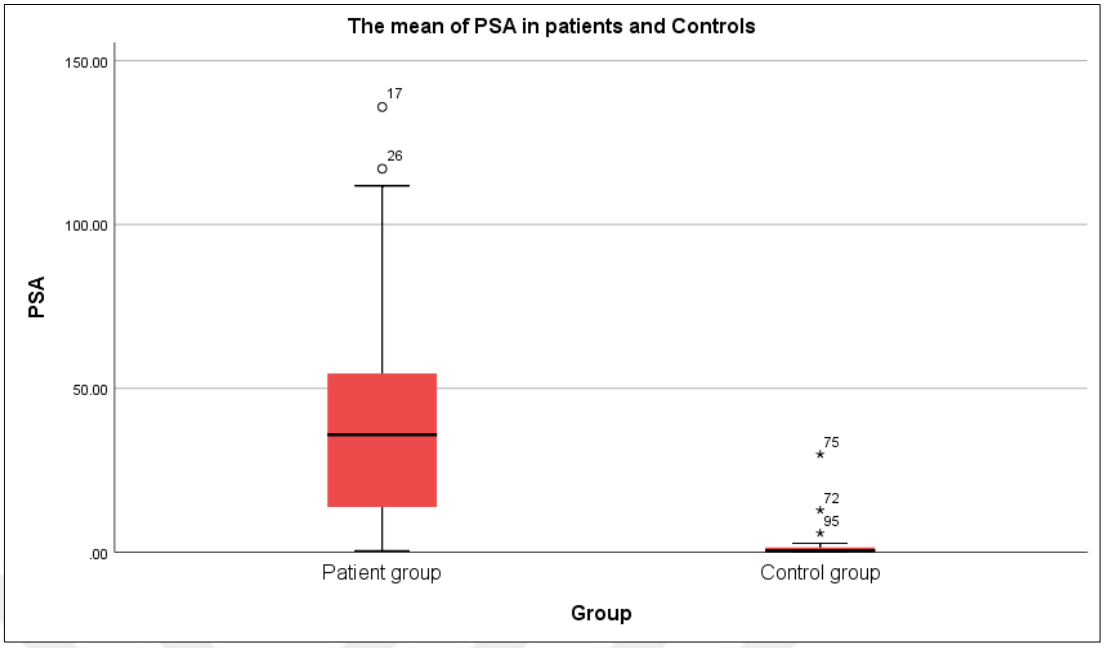


Figure 4.4 The percentage difference in PSA levels between the patient and control groups.

4.6. Serum CRP

The CRP results of patient and control were (92.6 ±79.3 nmol/L; 12.9 ±30.9 nmol/L) respectively. The results was a significant statistical (* P 0.001) at P <0.05, as shown in Table4.2 (Figure 4.5). Elevated serum CRP levels before treatment in PCa patients are closely related to bad prognosis (Wu et al., 2021). CRP levels decreased in prostate cancer patients that received treatment (Margel et al., 2020). CRP is a readily measurable biomarker that has the potential to detection the progress of the patient's condition deterioration or improvement (Prins et al., 2012).

As well as in our study showed a highly positive correlation between CRP with duration of disease (r = 0.421***, P < 0.002 respectively) as shown in Table 4.4 by used Pearson Correlation Analysis. This shows that there is a close relationship between CRP concentration and progression of disease in PCa patient. In some of the previous studies,

CRP levels have been shown to be related to worse PCa patient outcomes. (Beer et al., 2008).

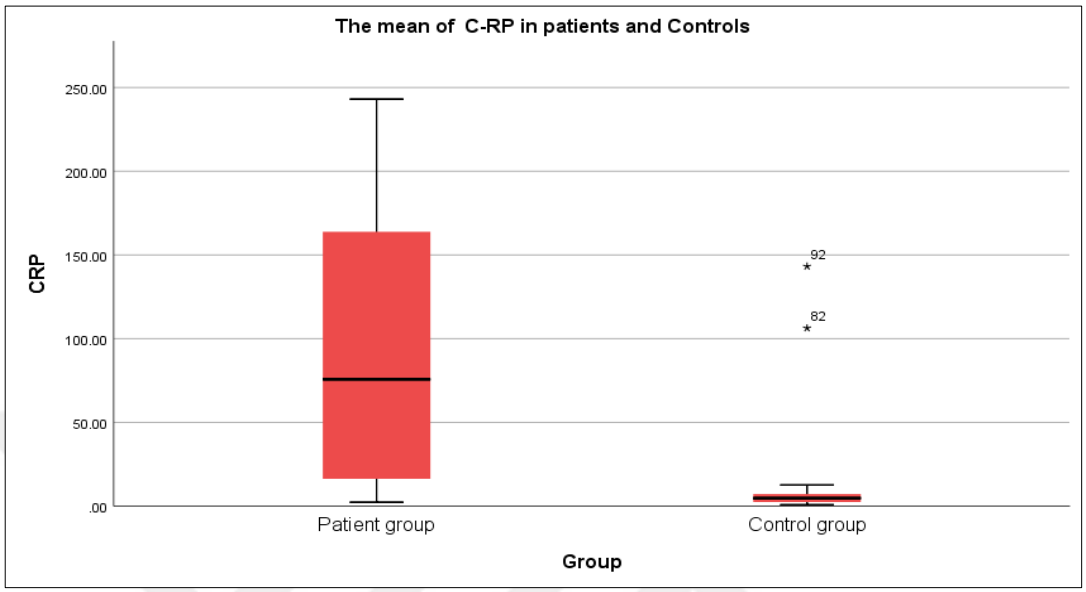


Figure 4.5 The percentage difference in CRP levels between the patient and control groups.

4.7. Serum Testosterone and Serum FSH with Prostate Cancer

The testosterone results of patients and controls were (5.72 ± 3.63 ng/ml; 4.37 ±2.28 ng/ml) respectively. The results were a non-significant statistical (* P 0.064) at P <0.05, as shown in table 4.3 (Figure 4.6). The studies are consistent with the report which reported that serum testosterone has not related with the incidence of prostate cancer (Chen et al., 2003). However, some studies contradicted our findings that high TT serum levels are associated with high-risk disease in PCa patients. Endogenous TT should be considered as a biological marker for assessing EAU PCA risk classes (Tafuri et al.,2020). Internal prostatic milieu levels are not accurately reflected by serum testosterone levels (Clapset al.,2018). On the other hand, if testosterone levels are to be

considered in the etiology of prostate cancer, they should be measured and interpreted chronically over a period of years using multiple measurements. (Loughlin, 2016).

While the FSH results was in patients and controls (4.79 ± 5.47 mIU/ml; 2.89 ±3.40 mIU/ml) respectively, this results was a non-significant statistical (* *P 0.082*) at P < 0.05, as shown in table 4.3 (Figure 4.7). Previously, researchers have discovered that Follicle Stimulating Hormone (FSH) is an important component in the natural history for progression of PCa (Catarinicchia., and Crawford, 2016). The result shown don't supported our study. Because, FSH concentration may be related to tumor size, revealing that there is a correlation before treatment. Most of the PCa patients in this study had undergone to different treatment methods, one of these methods used is medication which originally work to reduce some hormones that help in the growth of cancer cells may be the cause of this noticeable decrease in FSH and Testosterone hormone. Catarinicchia et al revealed in one of the experiment worked that some medication such as abiraterone and degarelix lead to decrease levels of FSH and Testosterone in PCa patient (Catarinicchia et al., 2016). Whereas a recent study established that postoperative FSH levels were significantly lower. Also, FSH levels decreased after radical prostatectomy (Choiet al., 2020). However, additional research is required to fully understand the differences in FSH concentration between patients who have received treatment and those who have not.

Table 4.3 Comparison between patients and control in Hormones level

Parameters	Mean ± SD	
	S. Testosterone (ng/ml)	S. FSH (mIU/ml)
Patients Group	5.72 ±3.63	4.79 ±5.47
Control Group	4.37 ±2.28	2.89 ±3.40
T-test	1.427 NS	2.146 NS
P-value	0.0641	0.0820
NS: Non-Significant.		

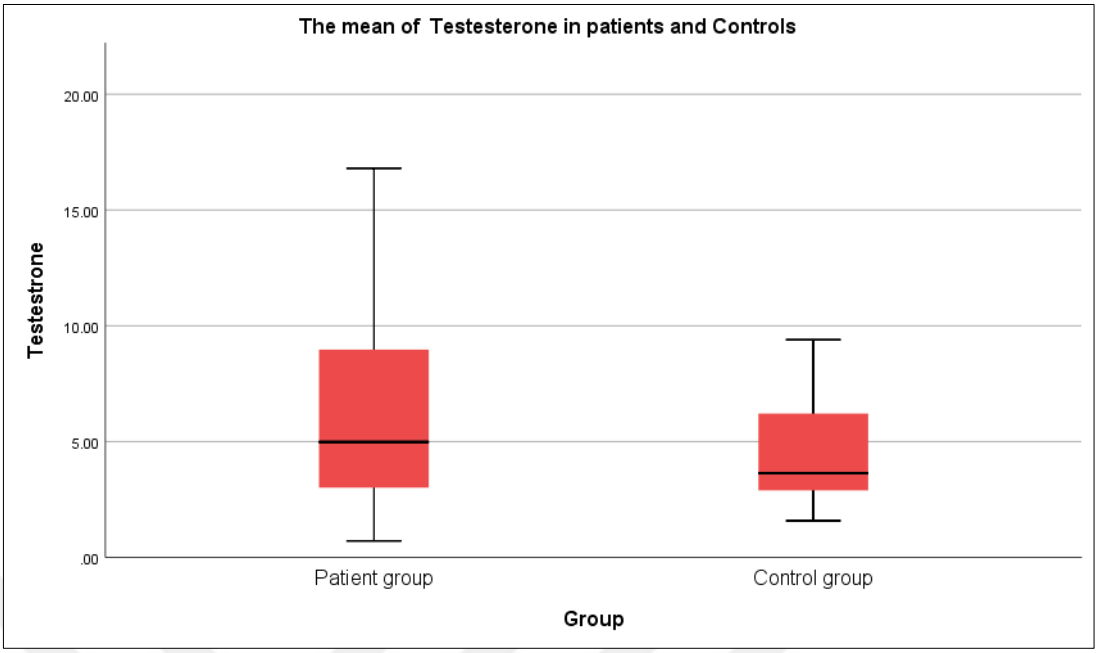


Figure 4.6 The percentage difference in Testosterone levels between the patient and control groups.

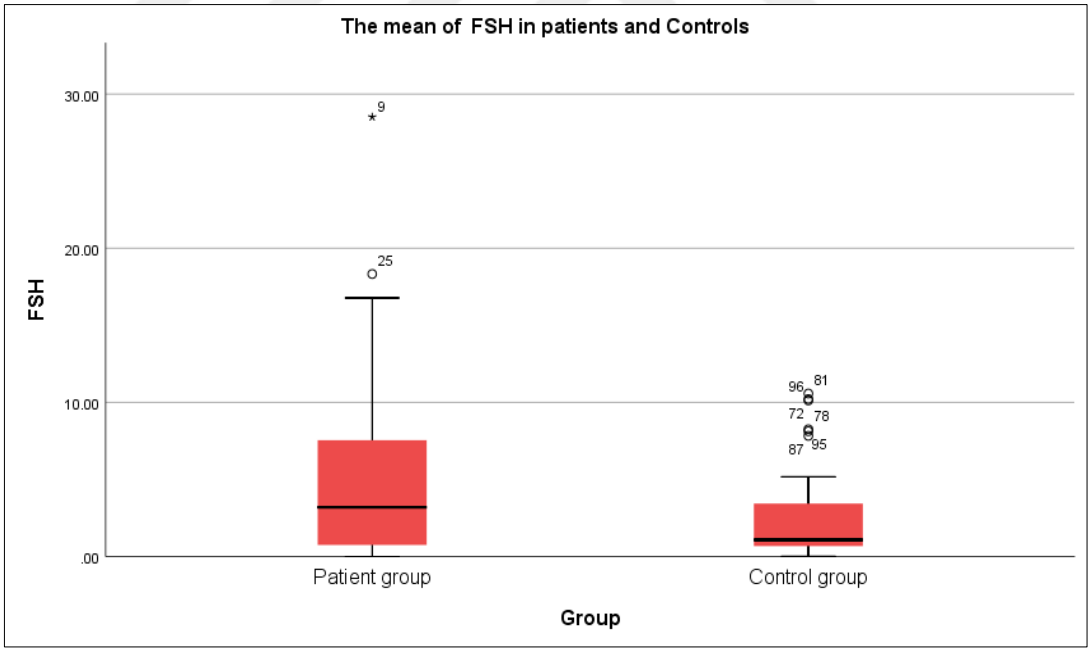


Figure 4.7 The percentage difference in FSH levels between the patient and control groups..

4.8. Pearson Correlation Analysis

4.8.1. Correlation between Studied Parameters

As shown in Table 4.4 there was a linear positive correlation between PSA with GDF15 (r = 0.30**, P < 0.002 respectively). Also, there was a linear positive correlation between PSA with FSH (r = 0.20, P < 0.047 respectively), CRP with PSA (r = 0.22 *, P < 0.023 respectively). While was negative correlation between GDF15 with CRP, Testosterone and FSH. Also, was negative correlation between PSA with Testosterone and FSH.

Table 4.4 Correlation between Studied Parameters in all Case of Prostate Cancer

Parameters	Correlation coefficient-r	P-value
S. CRP & PSA	0.22 *	0.023
S. CRP & GDF15	0.14 NS	0.160
PSA & GDF15	0.30 **	0.002
PSA & S. Testosterone	0.10 NS	0.314
PSA & S. FSH	0.20 *	0.047
GDF15 & S. Testosterone	0.06 NS	0.527
GDF15 & S. FSH	-0.07 NS	0.507
Duration of disease & S. CRP	0.421***	0.002
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.		

5. CONCLUSIONS

1. In this study have conclusion that men with raises of GDF-15 have a risk come to be capture prostatic diseases.Therefore, GDF-15 is a very important biomarker for diagnosis of prostate cancer and also very important for revelate a disease progression.
2. The values for serum PSA in Pca patients were elevated. Also results in the study indicated that serum Psa values is used as an non specific in the early diagnosis of prostate cancer. As well, there was to shown a direct positive correlation between PSA serum levels with GDF-15 in patients. It observed reduction in FSH and Testosterone hormones levels in Pca.
3. All men in the wide world are at risk of having prostate cancer. The thing that raises the odds of having prostate cancer the most is age .
4. The levels of CRP in a patient with PCa increased significantly, especially as the disease duration advanced. As well, there was to shown direct positive correlation between serum CRP levels with duration of disease.
5. For the BMI in this study was explained that fatness considered a risk factor for prostate cancer.
6. The prostate diseases are easily evaluated by used the PSA, GDF-15 and CRP test and Age.

5.1. Recomendation

1. We obtained elevated levels of GDF-15 and PSA in serum in prostate cancer patients even after taking the treatment. Therefore, we recommend a qualitative study of these parameters by dividing samples into groups before and after treatment.
2. To fully understand the variation in FSH concentration between treated and untreated patients, further research is required.
3. Study more samples of prostate cancer aiming to give more data about increase and decrease levels in some parameters such as Testosterone.

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